Book of Abstracts

ADVANCES IN MICROBIOLOGY OF TABLE OLIVES

International Congress

Campus di Via Lanera, Matera

September 1-2, 2025

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UNIVERSITÀ DEGLI STUDI DELLA BASILICATA

Advances in the microbiology of table olives

September 1-2, 2025

Aula Magna, Università degli Studi della Basilicata,

Campus di Via Lanera

Matera, Italy

https://web.unibas.it/parente/?page_id=2088

Foreword.

Table olives are among the most ancient fermented foods. Although their production is concentrated around the Mediterranean basin, climatic change may lead to a significant growth in other continents, like the Americas.

The study of the ecology of this fermentation process, of the functionality of the microorganisms involved (lactic acid bacteria, yeasts, other halophiles) and the development of control strategies is challenging for several reasons:

- as for many other vegetable fermentations, no heat treatment is used
- the variety of raw materials (in terms of olive cultivars and ripeness of the fruit) and the variety of trade preparations strongly affects the initial contamination and the ecological factors affecting the dynamics of the fermentation
- development and use of starters and functional cultures is difficult and the choice is limited

The use of -omics approaches is providing and increasing body of knowledge which, however, is poorly integrated.

We therefore feel that, in the era of open data and open science, it is the right time for a meeting of the scientists involved in research in this area in order to:

- share state of the art knowledge on the microbiology of the process
- share data
- foster the development of standardized protocols
- foster the creation of collaboration networks

The meeting will be organized as part of the communication activity of the METAOlive project, co-funded by the Italian Ministry of Education, and by the European Union Next GenerationEU program.

Scientific Committee

Prof. Eugenio Parente, Prof. Giuseppe Montanaro, Università degli Studi della Basilicata
Prof.ssa Francesca De Filippis, Università degli Studi di Napoli "Federico II"
Dr. Francisco Noé Arroyo López, IG-CSIC, Seville, Spain
Prof. Efstathios Panagou, Agricultural University Athens, Greece

Organizing Committee

Prof. Eugenio Parente, Prof.ssa Annamaria Ricciardi, Dott.ssa Rocchina Pietrafesa, Università degli Studi della Basilicata

Program.

	Session	time slot	Subject/title	Speaker	Speaker organization
September 1, 2025	Welcome	10:00 - 10:30	Welcome addresses		
	S1 Facing the challenges of climate change. Chairperson: Prof. E. Parente	10:30 - 10:50	The challenges of olive grove cultivation in the Anthropocene	Prof. Giuseppe Montanaro	Università degli Studi della Basilicata, Italy
		10:50 - 11:10	Climatic changes and its influence on table olive fermentations.	Dr. Antonio Benítez Cabello	Instituto de la Grasa - CSIC, Spain
		11:10 - 11:40	Coffee break, poster viewing		
	S2 Table olives: diversity and microbiology . Chairperson: Dr. A. Benitez-Cabello	11:40 - 12:00	An overview of microbial communities of table olive varieties produced in Italy: the METAOlive project	Prof. Eugenio Parente	Università degli Studi della Basilicata, Italy
		12:00 - 12:20	Spanish-style table olive fermentations and its microbiology: general aspects	Dr. Eduardo Medina Pradas	Instituto de la Grasa - CSIC, Spain
		12:20 - 12:40	Greek natural black olive processing: Perspectives and challenges	Prof. Efstathios Panagou	Agricultural University of Athens, Greece
		12:40 - 13:00	Table olive ferments with probiotic potential.	Dr. Francisco Noé Arroyo López	Instituto de la Grasa - CSIC, Spain
		13:00 - 13:30	Closing remarks, session 1-2		
		13:30 - 15:00	Buffet lunch, poster viewing		
	S3 Microbiome, flavour, aroma, functionality of table olives. Chairperson: Prof. E. Panagou	15:00 - 15:20	Metagenome and volatilome unveil the functional potential of table olive microbiome	Dr. Vincenzo Valentino, Dr. Andrea Balivo	Università degli Studi di Napoli "Federico II", Italy
		15:20 - 15:40	Sensory evaluation and spoilage of table olives	Dr Konstantinos Tertivanidis	Region of Central Macedonia, Greece
		15:40 - 16:00	Table olives: from fermentation to taste	Mr. Kostas Zoukas	Panhellenic Association of Table Olive Processors, Packers and Exporters, Greece
		16:00 - 16:20	An integrated metagenomic and volatilomic approach to advance research in Spanish-style cv. Chalkidiki green table olives	Prof. Fani Mantzouridou	Aristotle University of Thessaloniki, Greece
		16:20 - 17:00	Coffee break, poster viewing		
	S4 Advances in research on table olives. Chairperson: Dr. F. N. Arroyo López	17:00 - 17:20	Innovative Strategies for Enhancing the Shelf Life and Safety of Naturally Fermented Table Olives	Prof. Hayriye Ünal	Sabanci University SUNUM Nanotechnology Research Center, Turkey
		17:20 - 17:40	Microorganisms drive a virtuous way to ferment table olives with improved safety, nutritional and sensorial traits. A story born in lab, moved to producing context and reaching the consumer's table	Dr. Gianluca Bleve	Consiglio Nazionale delle Ricerche - Istituto di Scienze delle Produzioni Alimentari, Italy
		17:40 - 18:00	How machine learning and microbial metataxonomic data can help to classify table olives.	Dr. Elio López-García	Instituto de la Grasa (CSIC), Spain
September 2, 2025	S5 Advances in	9:00 - 9:15	Opening remarks, Session 5	Prof. Eugenio Parente	Università degli Studi della Basilicata, Italy

	research on table olives. Chairperson: Dr. E. Medina	9:15 - 9:30	Functional table Olives: Fortification with probiotic *Lactiplantibacillus pentosus*	Prof. Hayriye Sebnem Harsa	Izmir Institute of Technology, Turkey
		9:30 - 9:45	Phenolic compounds content in cv. Chalkidiki olives as a critical factor for Spanish style processing conditions	Prof. Maria Z. Tsimidou	Aristotle University of Thessaloniki, School of Chemistry, Greece
		9:45 - 10:00	A new fermentation system for the production of Spanish-style green table olives	Mr. Lekocaj Gjergj Marko	Oleica start-up, Spain
		10:00 - 10:15	Microbial innovation in table olive fermentation: a decade of research on *Lactiplantibacillus pentosus* OM13	Prof. Nicola Francesca	University of Palermo, Italy
		10:15 - 10:30	A new generation of biological debittered olive patè enriched with beneficial *Lactiplantibacillus plantarum*	Dr. Roberta Prete	University of Teramo, Teramo, Italy
		10:30 - 10:45	Characterization of Lactic Acid Bacteria isolated from spontaneous fermentation brines of Green Olives for their potential use as starter cultures	Dr. Marisa S. Garro	Centro de Referencia para Lactobacilos (CERELA) – CONICET – FML – FECIC, Argentina.
		10:45 - 11:00	Development and validation of fast blue bb assay for the time-/cost-effective determination of phenolic compounds in extra virgin olive oil	Dr. Gianluca Picariello	Istituto di Scienze dell'Alimentazione, Consiglio Nazionale delle Ricerche, Avellino, Italy
		11:00 - 12:00	Poster viewing, light lunch		
		12:00 - 13:00	Concluding remarks: developing a collaboration network for research in table olives microbiology		

Sponsors and supporting organizations.

The meeting is being organized within the framework of the PRIN 2022 Project METAOlive project, grant 2022NN28ZZ and received funding from the European Union Next-GenerationEU, CUP C53D23005460006 (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1048 14/07/2023).
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Logistics and some aspects of the organization were covered by Allmeetings srl, Matera

The organization of the workshop is under the patronage of:

- Società Italiana di Microbiologia Agroalimentare ed ambientale
- Dipartimento di Agraria, Università degli Studi di Napoli “Federico II”, Portici (NA), Italy
- Agricultural University Athens, Athens, Greece
- International Olive Council, Madrid, Spain

Invited & Contributed Talks

The challenges of olive grove cultivation in the Anthropocene

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Background.

Global climate change is mainly driven by increasingly elevated atmospheric carbon dioxide (CO_2) concentration along with other greenhouse gases (GHG) (CH_4, N_2O) which results in increasing air temperature. To date, anthropogenic activity increased the average yearly temperature by ~1.0°C over preindustrial yearly temperature levels, and if current GHG emission rates continue, IPCC scenarios estimated to increase by 1.5 °C in the following three decades (10.1017/9781009157940.001). The agricultural sector (namely AFOLU) shares about 13-21% of global GHG emissions (doi.org/10.1017/9781009157988.004). In addition, emissions from cropland are predicted to increase because of the intensification and extensification due to agricultural land expansion and production growth in some countries (e.g., Africa and Latin America) (10.1073/pnas.091421610).

The olive tree (*Olea europaea* L.) was domesticated more than 3,000 years ago in the Mediterranean area and represents an integral component (as fruit and oil) of the Mediterranean diet for millennia to the extent that a coevolution between Mediterranean inhabitants and olives has been proposed (10.1017/S1368980007668530, 10.7314/apjcp.2015.16.6.2119). The specific drought tolerance mechanisms developed by olives as Mediterranean native species have greatly contributed to their longstanding success in dry and warm areas (10.1071/AR05169). Following this, it is expected that olive tree would be capable to adapt to increasing environmental limitations triggered by climate changes. However, the rate of climate change and increasing occurrence of extreme events seem to be faster than time for adaptation.

The recognition of olive products as functional food has renewed the interest for their consumption (10.1002/9781119135340) and in turn strengthened the socio-economic role of olive industry for Mediterranean economy. Olive fruit development (from the flower to the mature fruit) has been recently reviewed focusing mechanisms regulating the different phases of fruit development (10.3389/fpls.2023.1276178). However, a link with changing climate has not been adequately explored. On this background, the present contribution will summarize the main physiological, ecological and productive challenges posed to olive flower/fruit processes in relation to key climatic variables.

Rising air temperatures.

Some specific phenological stages in olive (from budbreak to flowering including beginning of flowering) are strictly correlated to air temperature to the extent that they can be accurately predicted (R^2 0.93-0.96) employing air temperature as independent variable (10.3390/plants10061115). Winter chilling (e.g., hours <7.2 °C) is considered relevant flowering factor in tree crops. The increasing air temperature due to climate change is resulting in a significant reduction of chilling unit accumulation in winter as recently documented in a ~75-year time series analysis in Southern Italy (10.6092/unibo/amsacta/7302). Insufficient chilling units in Southern olive growing areas will be further exacerbated by future warming further reducing flowering intensity (DOI 10.1007/s00704-013-0892-2). Although olive cultivars have a variable mean chilling requirement of about 400 chilling units (10.3390/f11080835), it has been demonstrated that olive cultivars have contrasting chilling requirement or even no-chilling (10.1300/J492v05n03_04) suggesting that genetic diversity might help to cope with warming winter (10.1016/j.scienta.2019.108759). Flowering time is also influenced by climate change. A recent 40-year in Central Italy, reports an about 10 days earlier start blooming in response to higher spring temperatures during 2020s compared to 1985s (10.1016/j.eja.2024.127435). This result is in line with model-based prediction of advanced blowing in Spain (10.1016/j.agrformet.2004.02.009). Advanced blooming might influence fruit-set in those varieties with self-sterility (e.g., Arbequina, Itrana, Leccino) causing potential yield reduction.

The vegetative season in olive is temperature dependent starting at the end of winter (>10°C), stops in summer when air temperature is >35°C. A second growing flush occurs after summer in case of raining events in case of rainfed groves. Increasing air temperature promotes high water demand because (i) of the increased *VPD* and (ii) leaf area growth to

the extent that summer pruning might positively help to control the occurrence of water stress (10.1016/j.scienta.2023.112612). Increasing temperature would also be detrimental for photosynthetic apparatus. Hence, using clay-based or kaolin-like particle film reducing leaf temperature might help to tolerate heat stress (10.3390/agronomy11091884). Flowering processes are also influenced by temperature. Although the percentage of perfect flower is mainly under genetic control (10.17660/ActaHortic.2012.932.2) explaining the cultivar-to-cultivar variability, rising air temperature reduces that percentage as well as fruit set (10.1016/j.scienta.2018.06.054). In an artificially heated field-grown olive, it has been documented that increasing air temperature +4°C across the whole year, olive flowering would be about 20 days advanced and last 5-10 days longer, significantly increase pistil abortion leading to a reduction in fruit set (10.1016/j.scienta.2018.06.054). As a consequence, a 40-50% and 30% reduction in yield and oil (FW) was recorded, respectively (10.1016/j.scienta.2019.01.046).

Early and late drought.

Climate is becoming increasingly drier in Europe not only during summer time. An increasing frequency in drought during winter (Dec-Feb) and spring (Mar-May) has been detected over the 1950–2015 period in regions of Mediterranean countries (e.g., Spain, Italy) (10.1016/j.gloplacha.2016.11.013). Water deficit during winter dormancy (Nov-March) would not impact fruiting parameter; in contrast with this a water deficit (e.g., 25% irrigation restriction) during the inflorescence formation (Mar-April) the number of flower is significantly reduced along with (10.1016/j.envexpbot.2011.11.021). In addition, the number of perfect (hermaphrodite) flower is also reduced by spring drought because reduced water status or by reduced assimilate supply caused by drought-induced low photosynthesis. In case of a drought event occurring at floral development (end Apr-May) likely there is no impact on flowering parameters but fruit set might be reduced by 30-40% (10.1016/j.envexpbot.2011.11.021).

Fruit quality.

Fruit quality and landscape are both key traits of olive production even their operational scale greatly differ. Quality traits in olive oil and table olive differ: while in olive oil high extractable fat content is prioritized, in table olives quality must meet certain criteria for human sensory acceptance and post-harvest processing (see 10.1016/j.scienta.2017.12.034 for review). For olive oil cultivar the main criticism related to climate change is the impact of drought and heat stress on fruit size development determining yield and oil harvest. Early fruit development depends on cell expansion and water availability (10.3389/fpls.2023.1276178); hence drought occurrence at this stage is impaired leading to fruit with smaller mesocarp and in turn low oil accumulation. Moreover, olive fruit development has a second growth after the pit hardening determined by the increase in cell size (10.3389/fpls.2023.1276178). Occurrence of drought in this period is detrimental for fruit size, too. A study using a latitudinal gradient with a range of altitudes inferred information on the effect of temperature on fruit growth and development. That is, 10.3390/horticulturae10121339 reports that pit hardening occurred earlier in warmer locations, resulting in an earlier onset of oil accumulation and a low final fruit weight despite fruit size is still under genetic control. Oil concentration in fruit is also dependent on cultivar but it slightly by 1-5% under high temperature up to 50° (10.3390/agronomy11081492).

For table olive, first perception of quality by the consumer relies on size, shape, color and the absence of damage (10.1016/j.scienta.2017.12.034). Size and flesh-to-stone ratio are influenced by drought (). Hence, table olives are highly vulnerable to increasing frequency of drought events. In addition, intrinsic quality of fruit at harvest might be influenced by climate change and in turn relevant post-harvest process. Briefly, warmer temperature and drought might advance (excess) fruit maturity characterized by a high-fat content but a low sugar content (10.3390/foods12193712) hampering subsequent lactic fermentation which is a key step for natural conservation of fruit (10.1016/j.scienta.2017.12.034). Following this, an early harvesting time is demanded to prevent fruit overripening. However, early harvesting may lead to the collection of fruit with high phenolic compounds (10.34133/plantphenomics.0061) and in turn high antioxidant (antimicrobial) activity (10.1155/2016/9589763) hampering a natural fermentation.

Landscape.

About 80-90% of the global olive plantations are located in Mediterranean countries (FAO, 2022) producing about 95% of global olive oil (10.1002/ejlt.201800135). These plantations are almost entirely under traditional systems (up to 300 trees per ha) (10.1061/(ASCE)IR.1943-4774.00006) largely contributing to the landscape of these regions. Olive groves landscape is increasingly recognized a tangible ecosystem service to the extent that it is considered a driver for rural economy (10.1016/j.landusepol.2017.03.017).

However, climate change is challenging the cultural ecosystem service offered by olive landscape. That is, it has been predicted that an increase in 0.8-2.5 °C in temperature 0.8 to 2.3°C along with an annual rainfall decrease of around 200 mm over the Mediterranean region for 2050, will likely "relocate" olive growing areas towards northern regions (10.3390/agriculture10110509). In line with this, in Italy, olive groves surface in the northern regions increased by 50% from 2000 to 2020 although a total of 15% national reduction (www.istat.it). The introduction of irrigation at traditional plantations might have contributed to intensification of cropping systems with potential negative impact on biodiversity (10.1016/j.landurbplan.2022.104429). In addition, irrigation has also been introduced, likely increasing substrate availability and accelerating the oxidation of carbon substrates by microorganisms as per the combination with high soil temperatures likely accelerating soil carbon depletion via increased soil respiration (10.1016/j.eja.2023.126815). Hence, irrigation could be a hidden challenge because it would be detrimental on carbon level in a long-term perspective. Therefore, irrigated olive groves demand organic input to prevent decreasing in soil carbon.

Conclusions.

The present contribution highlights that Anthropocene-driven pressures (mainly drought and increasing temperature) pose new challenges. Although olive trees are indigenous Mediterranean crop, nowadays are facing environmental constraints overcoming their adaptive capacity. The increasing temperatures during winter time is reducing chill accumulation and in turn flowering intensity and shifting phenological stages. This collectively reduces fruit set and yield. Climate changes pose relatively new challenges such as spring droughts which reduce inflorescence development and perfect flower formation. Heat stress also reduces fruit set, impacting productivity and quality. Taking all this together, the current transformation of olive industry towards high- and super-high density plantations (which have high water demand and is implemented with a few number of suitable cultivars) requires genetic and technological innovations to accelerate its adaptation to climate changes.

Table Olives in a Changing Climate: Implications for Microbial Diversity and Fermentation Stability

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Background.

Climate change poses a serious global threat, with significant implications for agriculture in Mediterranean regions (IPCC, 2023, https://www.ipcc.ch/report/sixth-assessment-reportcycle/). Among the affected crops are olive cultivars, which serve as the basis for both olive oil and table olive production. Table olives represent the most widely consumed fermented vegetables in the Mediterranean basin, with global production surpassing 3 million tons annually (IOC, 2024, https://www.internationaloliveoil.org/what-we-do/economic-affairs-promotion-unit/#figures). Their fermentation is primarily driven by lactic acid bacteria and yeasts, whose diversity and population dynamics are shaped by various factors, including the levels of sugars and phenolic compounds in the fruit (Garrido-Fernández et al., 1997). These intrinsic factors, in turn, are strongly influenced by environmental conditions such as temperature, rainfall, solar radiation, and soil mineral content, all of which can affect the final quality and safety of the product.

Methods.

In this work, we analyse and discuss the effect of climate change on the microbial diversity of table olives, with special emphasis on Spanish cultivars.

Results.

The alterations expected to occur in climate change scenario include changes in the microbial populations, their succession, diversity, and growth kinetics, which may impact in the evolution of the fermentation and the safety and quality of the table olives.

Conclusion.

Mitigation and adaptation measures are proposed to safeguard the authenticity and sensorial features of this valuable fermented food while ensuring food safety requirements.

References

Fernández, A. G., Adams, M. R., Fernandez-Diez, M. J. (1997). Table olives: production and processing. Springer Science & Business Media.

An overview of microbial communities of table olive varieties produced in Italy: the METAOlive project

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Background.

Table olives are among the most ancient fermented foods and their long history of production has resulted in a variety of products (10.3390/foods9020178). Five main categories are recognized by the International Olive Oil Council, including alkali treated olives, naturally fermented olives and dried/shrivelled olives and olives darkened by oxidation both of which are preserved by methods other than fermentation but may be spoiled by microorganisms. Several table olive varieties have received Protected Denomination of Origin denomination. In Italy alone four varieties (Oliva di Gaeta, Nocellara del Belìce, Bella di Daunia, Oliva Ascolana del Piceno) are included among PDO foods, and one (Oliva Taggiasca Ligure) among PGI, but many more included in the Italian List of Traditional Agricultural Products (https://www.gazzettaufficiale.it/eli/gu/2024/03/13/61/sg/pdf).

Sensory quality, safety and stability of table olives is affected by many factors, including olive ripeness, technological factors and, pre-eminently, microorganisms, either as natural contaminants from fruits, salt, brines, contact surfaces or added as starter cultures (10.3389/fmicb.2021.797295). The bacterial and fungal microbiota of table olives is complex, usually due to the lack of control dispersal and selection and includes groups which are usually considered beneficial, like lactic acid bacteria and yeasts, and other halophilic microorganisms, but also microorganisms associated with spoilage. The composition of microbial community of table olives and the factors affecting its dynamics have been reviewed multiple times in recent years (10.3389/fmicb.2021.797295; 10.3390/foods12203783). We have recently reviewed metataxonomic studies on table olives and found that a core microbiota of approximately 30 genera is characteristic of table olive fermentations, with wide variations among trade preparations and varieties (10.1101/2025.03.16.643505). With a few exceptions, studies on table olives microbiota are relatively small (<50 samples) and often focus on a limited number of varieties (10.3390/foods12203783). This, and the variety of sampling plans and experimental approaches used complicated the interpretation and the integration of these data to obtain a comprehensive view of the factors affecting table olive microbial communities assembly and dynamics.

We therefore carried out a large survey of olives belonging to a wide array of varieties and trade preparations from Italy and compared them with samples obtained from Greece, Spain, Cyprus, Peru, and Egypt, in order to obtain a comprehensive view of the bacterial and fungal microbiota of olives in Italian varieties and an understanding of variability both within and between different producing plants.

This also allowed us to compare, for black Itrana olives, samples having PDO and non PDO status, thus gaining information which may be important in authenticity studies.

Methods.

Table olive samples (374) were obtained from 39 producers in 6 countries. The majority of the samples were obtained directly from producers with the brine used for fermentation and prior to packaging. All were dispatched in insulated containers with ice packs. pH was measured using a digital pH meter; NaCl concentration was estimated from the measurement of chlorides using a selective electrode. Titratable acidity was measured in brines using NaOH 0,1 mol/L. Microbial counts were performed on a 1:1 mixture of olives and brine after homogenization in peptone water. Lactic acid bacteria were enumerated in modified MRS agar with 0.2% sodium azide (25°C, 72 h); yeasts and molds on Glucose Yeast Extract agar with 100 mg/L chloramphenicol (25°C, 5 days); halophilic microorganisms on PCA with 5% NaCl (w/v) (30°C, 48 h); contaminants on Pseudomonas agar base (25°C, 48 h); *Enterobacteriaceae* in Violet red Bile Glucose Agar

(30°C, 24 h).
Genomic DNA extraction and purification was performed from a 1:1 mixture of olives and brines using DNeasy PowerFood Microbial Kit. Library preparation (V3-V4 region of the 16S RNA gene for bacteria and ITS2 for fungi) and sequencing was performed by Novogene (Cambridge, UK). Raw sequences were processed using a pipeline based on DADA2. The results from different sequencing batches were assembled in a phyloseq object (McMurdie and Holmes, 2013) which was then used for further analysis using a combination of R packages.

Results.

Given the diversity of our dataset, which included green, turning colour and black olives from 38 varieties (23 of which from Italy, including all 4 DOP table olive varieties) most of the results reported in this contribution will be aggregated using a combination of olive ripeness and trade preparation. However, due to the rich metadata structure (which included information on style, use of starters, spoilage, duration of fermentation, firm, etc.) for the varieties for which more samples are available comparisons will be extended down to the producing firm level.
All chemical and physicochemical variables and microbial counts showed substantial variability within each combination of olive ripeness and trade preparation. Counts of microbial groups were even more variable with IQR > 2.5 for most microbial groups and olive groups. Halophilic microorganisms, yeasts and/or lactic acid bacteria were below the detection limit of the method (2.5 log(cfu/g)) for several samples, but high (>10^7) LAB and yeast counts were not infrequent. Contaminants and *Enterobacteriaceae* were detected at high counts in some groups, especially those which had been fermented in water or not fermented at all. Counts, chemical and physico-chemical data generally lacked a correlation structure. At least three components of a PCA were necessary to explain 55% of the variance. Although different ripeness/trade preparation groups tended to occupy different areas of the score plot for the first 2 components they largely overlapped. On the other hand, within some individual groups (Picholine, green naturally fermented olives), a separation between different varieties and firms was evident. More data are possibly needed to discriminate between varieties and firms.
Metataxonomic data for bacterial communities were available for only 336 samples. Alpha diversity was highly variable, with Chao1 between 8 and 135. Significant differences (WSRT, $p < 0.05$) were found between different groups and, within groups and varieties between varieties and firms respectively. The highest median diversity was found for green alkali treated and turning colour naturally fermented olives, but higher diversities were found in individual samples of black naturally fermented olives. The diversity of specialties was much lower. Taxonomic agglomeration and filtering was performed at the genus level and, after using a prevalence (0.01) and abundance filter (0.005), 135 out of 534 genera were retained. Prevalence and abundance of genera varied among different groups. Overall, 54 genera (mainly *Lactobacillales* and *Enterobacterales*), having a prevalence >=0.1 and a maximum abundance >0.01 could represent the core microbiota of olives. These genera overlap with those found in the OliveFMBNdataset. Only a few genera, had a prevalence >0.5 with *Lactiplantibacillus, Pediococcus, Secundilactobacillus, Lentilactobacillus,* and *Loigolactobacillus* having a prevalence > 0.75. This might be due to an unequal distribution of samples (with an unbalanced number of samples belonging to the Itrana variety) but it is conceivable that a bootstrap procedure could provide a more representative distribution.
Ordination using non metric Multidimensional scaling (MDS) of the Bray-Curtis distance matrix was rather ineffective in separating the different groups, with the exception of a Picholine variety, Nocellara del Belìce which clearly stood apart. However, when individual plots were produced for different subgroups, differences between varieties were more evident. Within-firms variability was not constant: for some firms all samples from different fermentation vessels were close among them, for others the reverse was true. MDS is a form of unconstrained ordination and the results may be dominated by random variability.
Constrained ordination using environmental variables was rather ineffective. Inferential analysis with PERMANOVA using adonis2 confirmed that significant differences existed between communities of the main ripeness/trade preparation groups and, within these, between varieties and firms. Results for differential abundance are too detailed to be shown here but a clear signature of alkali treated olives was higher abundance of halophilic and alkalophilic genera, including HALAB but also halophilic *Pseudomonadota*. Surprisingly, several *Pseudomonadales* and *Enterobacterales* were more abundant in naturally fermented olives: although they are not often found in final stages of fermentation, their DNA may persist under the relatively less selective conditions in black natural olives. A diverse range of

Lactobacillaceae was characteristic of all fermented table olives.
Data on fungi confirmed the wide variability of the microbiota. Fungal diversity was much lower than bacterial diversity, with median Chao1 values <20 for most groups. The largest variability was found in Picholines, with Chao1>100 for some samples, possibly due to lower selectivity. In fact, significantly higher diversity was found for alkali treated olives compared to naturally fermented olives. Significant differences between varieties for the same group and for firms within the same variety were also found confirming what was found for bacteria. Abundance and prevalence filtering at the species level using the same thresholds used for bacteria retained 119 taxa out of 795, with 99.6% of the initial sequences. A core micobiome of 26 species was found using the criteria used for bacteria and 5 species (*Pichia membranifaciens* and *P. manshurica, Candida boidinii, Wickerhamomyces anomalus* and *Saccharomyces bayanus*) showed a prevalence > 50% and a maximum abundance close to 100%, confirming results from the literature. A large diversity was found in core species abundance for each group, although some patterns were evident. Taxonomic agglomeration was performed at the genus level and the same analytical steps used for bacteria were used. Unconstrained ordination failed at clearly separating different olive groups except Nocellara del Belìce. On the other hand, PERMANOVA showed that the differences between different olive groups were significant, and so were those among firms for some varieties. DESeq2 also pointed out to several differentially abundant genera. Several of these had low prevalence and abundance and differences may due more to contamination patterns than to growth and colonization. The most prevalent genera often were differentially abundant, but their log2fold change was low, while some other genera, like *Kluyveromyces,* appeared to be reliably associated to alkali treated olives. Overall, bacterial communities were more discriminant at all levels compared to fungal communities.

Conclusions.

This work represents the largest survey of microbial communities of table olives in Italy, and provides a snapshot of the diversity of microbiota of many different varieties, with a special focus on Oliva di Gaeta, an important DOP variety, and provides detailed information on the large number of olive varieties from Italy and other major producing countries. One of the main observations is related to the huge variability which can be found within the same olive varieties and, in some cases, within the same producing firms. This points out to poor control of the process, which is characteristic in cases were small (240-280 L) fermentation vessels in open patios are used, and to larger impact of dispersal compared to selection.
These results can be easily combined with the OliveFMBN dataset, which shares the same metadata structure thus providing a combined dataset of >800 samples, which can be used by researchers and stakeholders to design improved classification schemes, provide support to authenticity studies on DOP varieties, and improve our understanding of the microbiology of all varieties of table olives. The diversity of beneficial microorganisms goes much beyond those commonly used as starters in olives. In fact, two species commonly used as starters, like *Lpl. pentosus* and *W. anomalus*, while prevalent, are not necessarily the most abundant ones. A large variety of LAB is usually present in olives undergoing fermentation (including Spanish style ones) and their role in quality should be explored more systematically. We have also confirmed that HALAB provide a clear signature of an alkali treatment. Their dynamics and interactions with other microorganisms also need more study.

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Spanish-style table olive fermentation and its microbiology: general aspects

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Background.
Table olives, particularly Spanish-style fermented green olives, constitute an emblematic product of the Mediterranean diet. Their importance transcends gastronomy: they represent a millennia-old tradition transformed through microbial biotechnology, where a naturally bitter and inedible fruit (*Olea europaea*) is transformed into a safe, stable food with an exquisite sensory profile, appreciated worldwide.

Spanish-Style Green Olive Elaboration Process.
The traditional process for Spanish-style green olives consists of well-defined stages (Kailis & Harris, 2007). Olives are harvested at the green-yellow stage, before full ripening. Damaged fruits, leaves, and twigs are removed, followed by washing with water to eliminate residues and size grading. Subsequently, olives are immersed in a sodium hydroxide solution (1.7–2.5% w/v) for 4–12 hours until the lye penetrates two-thirds of the distance from the skin to the pit. This step is critical for hydrolyzing oleuropein, permeabilizing the fruit's epidermis, and facilitating nutrient diffusion into the brine (10.1002/(SICI)1097-0010(199807)77:3<353::AID-JSFA50>3.0.CO;2-G). Afterward, one or several water washes are performed, and olives are placed in fermenters with an initial brine (10–12% NaCl w/v).
The fermentation stage involves a succession of microbes. During the initial phase, Gram-negative bacteria and yeasts dominate, consuming sugars and partially reducing the pH. Later, lactic acid bacteria (LAB), primarily *Lactiplantibacillus pentosus* and *L. plantarum*, become dominant and ferment available sugars into lactic acid, drastically acidifying the medium (10.1016/j.fm.2014.03.020). The pH drops to 4.0–4.5. When fermentable matter is depleted, metabolic activity decreases, and the preservation phase begins. Target pH and acidity are achieved, preventing unwanted secondary fermentations. Before final packaging (in cans, glass jars, or plastic bags), operations such as sizing, pitting, stuffing, or adding acidifiers/preservatives and/or pasteurization treatments may occur.

Factors Influencing Fermentation.
The success of Spanish-style green olive fermentation critically depends on controlling physicochemical factors modulating microbial growth. Brine salt concentration (NaCl) is decisive. Optimal levels of 5–6% favor homofermentative LAB growth while inhibiting Gram-negative bacteria, spoilage yeasts, and spore-formers (10.1016/j.fm.2009.05.010). Low salt concentrations promote butyric bacteria and enterobacteria growth. pH is also crucial. After lye treatment, the pH exceeds 10. Rinses and rapid acidification by LAB to a final pH below 4.5 are essential for food safety, inhibiting pathogens and spoilage agents.
The optimal temperature range is 25-30°C. Nutrient availability, especially soluble sugars, influences the acidification efficiency; insufficient levels due to excessive washing or inadequate fruit maturity limit lactic acid production. Oxygen presence must be minimized by complete tank filling, as it favours harmful yeasts and aerobic bacteria (10.1016/j.ijfoodmicro.2012.08.003). Finally, NaOH treatment requires balance. Insufficient treatment leaves residual bitterness from unhydrolyzed oleuropein, while excessive treatment damages fruit texture.

Microbial Spoilage
Key microbial alterations include gas pocket formation due to gas release by microorganisms (*Enterobacteriaceae* and/or *Celerinatantimonas* spp.) (10.1016/j.foodcont.2022.108868).
Putrid fermentations, caused by *Clostridium* species, produce butyric acid, leading to rancid odors/flavors (10.3390/metabo8040073). Both originate during early fermentation stages. Additionally, the "zapateria" spoilage,

identified by undesirable odors, is associated with propionic bacteria and clostridia during storage (Montaño et al., 1996). Lastly, the gas production causes package bulging due to CO_2 accumulation, primarily driven by fermentative yeasts or gas-forming bacteria favoured by residual sugars, inadequate pH, low salinity, and high temperatures (10.1016/j.ijfoodmicro.2012.08.003). Rigorous control of the described parameters, particularly salinity, final pH, and temperature, is crucial for ensuring efficient lactic fermentation and preventing defects that compromise olive quality and safety.

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Greek natural black table olive processing: perspectives and challenges

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Natural black olives processing.

Greece holds a longstanding tradition in the production of natural black olives in brine, based on established local practices and tradition. Consequently, this specific commercial preparation is widely recognized as "Greek-style" olives. Olive drupes destined for processing are harvested at full ripeness or slightly earlier, when around 75% of the mesocarp has developed its black colour. However, harvesting must be completed prior to the onset of the first winter frost, as freezing temperatures can result in irreversible damage to olives. In addition, mechanical damage, such as bruising or scratching, should be avoided since black olives exhibit a softer texture compared to green olives, which makes the raw material more susceptible to quality deterioration (Kailis and Kiritsakis, 2017).

Fermentation of natural black olives is based on the traditional anaerobic processing method, mainly practiced by olive growers and small-scale processors, and involves the direct immersion of olives in brine containing 10–14% (w/v) NaCl or higher. Under these conditions, fermentation proceeds spontaneously through the activity of a complex microbiota comprising Gram-negative bacteria, lactic acid bacteria (LAB), and yeasts. At high salt levels, yeasts typically dominate resulting in alcoholic fermentation, with lactic acid fermentation playing a secondary role. The final product exhibits relatively mild organoleptic properties and reduced intrinsic preservation potential. Specifically, in these fermentations, titratable acidity remains around 0.3–0.5% (w/v, expressed as lactic acid), and pH values are maintained between 4.5 and 4.8. These parameters are insufficient for long-term microbiological stability, and thus recent scientific advancements have prompted the industry to reassess and refine traditional methodologies to enhance product quality and safety.

Currently, the Greek table olive industry applies reduced salt concentrations (6–7%), resulting in final products with lower pH values (3.8–4.0) and higher titratable acidity (0.8– 1.0%), improving microbiological stability during storage. The influence of different salt concentrations on microbial dynamics, pH changes, and titratable acidity has been reported for Conservolea natural black olives (10.1006/fmic.2002.0480). Olives were fermented in brines containing 4%, 6%, and 8% NaCl (w/v) at two temperatures (18°C and 25°C) for six months. At both temperatures, LAB and yeasts emerged as the predominant microbial groups, with their relative abundance affected by brine salinity. Specifically, at 25°C and in brines with 4% and 6% NaCl, LAB exhibited a competitive advantage over yeasts, leading to a predominantly lactic fermentation characterized by enhanced preservative properties (pH 3.8–3.9; acidity 0.7% lactic acid). LAB populations exceeded 7.0 log CFU/mL, whereas yeast counts remained 2–3 log cycles lower. Gram-negative bacteria, primarily *Enterobacteriaceae* and *Pseudomonas* spp., were initially detected at elevated levels (4.0–5.0 log CFU/mL) but were rapidly eliminated within the first 20 days of fermentation. Similar trends were observed at 18°C; however, in 8% salt brines, LAB growth was retarded, regardless of temperature, allowing yeasts to predominate. This led to a mixed alcoholic/lactic fermentation, resulting in final products with higher final pH values (4.6–4.8) and lower acidity (0.37%, lactic acid). Optimal fermentation outcomes were observed at 25°C with 6% salt, producing the highest titratable acidity (1.2%, lactic acid) and lowest final pH (3.8). These findings indicate that salt concentrations exceeding 8% are inhibitory to LAB, whereas moderate salt levels (6–7%) facilitate LAB predominance, favouring a more lactic-type fermentation and improving self- preservation.

Biofilm development on natural black olives.

Another important aspect in natural black olive fermentation is the microbial colonization of olive drupes throughout the fermentation process. Scanning electron microscopy (SEM) studies have confirmed the presence of both yeasts and lactic acid bacteria (LAB) as biofilms adhering on the surface of the olives, including stomatal apertures and sub-stomatal cavities (Panagou et al., 2020). The olive skin is coated with a layer of epicuticular wax, which renders the epidermis largely impermeable to water and gases. This structural feature facilitates the adhesion and persistence of microbial cells, particularly yeasts and bacteria, on the olive surface. Each stomatal opening has been observed to be colonized by mixed microbial communities comprising both yeasts and LAB. This microbial aggregation in stomatal regions is likely attributed to the absence of a prior alkaline treatment in natural black olive processing, which leaves the cuticle intact. As a result, the exchange of nutrients between the mesocarp and the surrounding brine is restricted, permitting microorganisms to localize around stomatal openings where nutrients leach from the olive interior. The formation of such structured biofilms on olive drupes may lead to an underestimation of microbial populations, if enumeration is restricted to the brine, neglecting the microbial load adhered to the olive tissues themselves. Besides, the same microorganism may be present in the brine in the form of planktonic cells and at the same time it can be attached on the surface of olives as restricted or immobilized colonies, exhibiting different metabolic behavior. This realization has led to a shift in scientific focus towards investigating the microbial ecology directly on the olives, particularly emphasizing the development and structure of mixed-species biofilms composed of LAB and yeasts.

Use of starter cultures in natural black olive fermentation.

The fermentation of natural black olives is primarily driven by the indigenous microbiota of olives, which varies depending on the quality of the raw olives, harvesting practices, and post-harvest handling. The microbial community involved in such spontaneous fermentations can display a broad range of metabolic capabilities, which may differ not only between species but also among strains. These differences include growth kinetics, substrate utilization, antimicrobial activity, flavour development, and interspecies competitiveness within mixed microbial ecosystems. As a result, natural fermentations are inherently variable and often lack predictability and control. Consequently, there is a growing need within the table olive industry to implement controlled fermentations using selected starter cultures. Such inoculation strategies aim to enhance process consistency, improve product safety, and mitigate the risk of spoilage, particularly during the initial stages of fermentation when microbial competition for nutrients is most intense. Despite its potential advantages, the application of starter cultures in natural black olive fermentation has received limited attention. In a prior investigation, the use of two LAB starters, *Lactiplantibacillus pentosus* (commercial strain) and *Lact. plantarum* BFE 6709 (originally isolated from fermented cassava), was evaluated during the fermentation of Conservolea black olives (10.1016/j.fm.2007.10.005). These strains were inoculated at the onset of fermentation, and microbial dynamics alongside biochemical parameters were monitored over a 30-day period. Both LAB strains successfully colonized the brine environment, achieving populations exceeding 7.0 log CFU/mL. Although yeasts were also detected, they remained at lower levels (4.0–5.0 log CFU/mL) and did not dominate the process. Moreover, the inoculated fermentations exhibited a faster inhibition of Gram-negative bacteria, which were undetectable after 7 days, compared to the spontaneous fermentation in which these bacteria persisted up to 12 days. In addition, the presence of LAB starters was associated with increased acidification, as determined by HPLC, with final lactic acid concentrations of 113.5 mM and 117.6 mM for *Lact. plantarum* and *Lact. pentosus,* respectively. However, as the initial salt level was 6% in all fermentations, the control (uninoculated) treatment also supported vigorous lactic acid production, leading to convergence of LAB populations between treatments (i.e., inoculated vs. control) within the first 5–7 days. This suggests that the beneficial effects of starter cultures are most pronounced during the early phase of fermentation, when the risk of spoilage is increased.

Texture improvement of natural black olives.

Natural black olives, harvested at full ripeness, often exhibit softer texture compared to other commercial preparations. The degree of ripening is critical for achieving a final product with satisfactory firmness, as reductions in texture may

compromise the quality of olives during processing and storage. A common strategy to enhance firmness is the incorporation of divalent cation salts, such as calcium (either as calcium chloride or calcium lactate), into the brines. Calcium is known for its texture-enhancing properties and is widely used in the fermentation and preservation of olives and other vegetables. Specifically, the addition of 0.5% (w/v) $CaCl_2$ at the onset of Conservolea black olive fermentation has been shown to improve texture without altering microbiological or sensory attributes (10.1002/jsfa.2823). To optimize calcium uptake, olives were soaked in a $CaCl_2$ solution for 36 hours prior to salt addition. This pre-treatment allowed sufficient calcium absorption before adjusting brine salinity to 4%, 6%, and 8% NaCl. Results demonstrated that while $CaCl_2$ and NaCl concentrations had no impact on skin strength, the addition of calcium significantly enhanced mesocarp firmness—most notably at 4% NaCl, where flesh strength increased threefold relative to untreated samples. Scanning electron microscopy (SEM) revealed structural differences in the mesocarp, as treated olives displayed more extensive zones of cell fracture, indicative of improved tissue cohesion due to calcium. However, the firmness-enhancing effect of $CaCl_2$ diminished at higher NaCl concentrations (8%), suggesting a possible antagonistic interaction between sodium and calcium or other competing ions (10.1016/j.lwt.2010.06.027).

Reduced-salt natural black olives

Greek natural black olives have been traditionally processed in high salt concentrations (8– 14%). However, excessive dietary salt intake is a public health concern, due to its link with hypertension and stroke. Processed foods contribute approximately 75% of total sodium intake and consequently initiatives aim to reduce sodium consumption. In response, the food industry is increasingly developing low-sodium products through partial or total substitution of NaCl. In vegetable fermentations, replacing NaCl with alternative chloride salts such as KCl, $CaCl_2$, and $MgCl_2$ has shown promising perspectives. In Conservolea natural black olive fermentation, five brine formulations with varying NaCl, KCl, and $CaCl_2$ levels were tested to reduce sodium content (10.1016/j.fm.2011.05.008). All treatments led to successful lactic fermentations, with pH values ranging from 3.9–4.2 and titratable acidity between 0.70–0.86 g lactic acid/100 mL brine. LAB populations remained stable (6.0–7.0 log CFU/mL), while yeasts were present at lower levels (2.5–4.5 log CFU/mL). Substituting 50% of NaCl with KCl or $CaCl_2$ resulted in the highest acidity values (0.86 and 0.83 g/100 mL, respectively). Despite promising microbial and physicochemical parameters, sensory quality proved critical. High $CaCl_2$ concentrations or its combination with KCl increased bitterness, reducing consumer acceptability. Only the brine containing 4% NaCl and 4% KCl produced olives with acceptable taste and reduced sodium content. Although KCl can induce bitterness and metallic off-flavors (10.1016/j.tifs.2012.08.005), at a 50% substitution level, NaCl appears to mask such defects.

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Table olive ferments with probiotic potential

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Olive Fermentations.

During course of table olive fermentations, the microorganisms transform the fruit's components, developing unique texture and flavours. This aspect determines the quality and safety of the final product. Diverse yeasts and lactic acid bacteria (LAB) species are among the most relevant microorganisms responsible for fermentation. The LAB (mainly *Lactiplantibacillus pentosus* and *Lactiplantibacillus plantarum*) acidify the brines by consuming the sugars provided by the fruit, producing lactic acid, which inhibits the growth of undesirable microorganisms (10.1016/j.fm.2012.01.006). The yeasts (mainly species of the genera *Wickerhamomyces, Pichia, Candida,* and *Saccharomyces*) improve the organoleptic profile of fermentation by producing a diverse array of aromatic compounds, as well as by promoting the growth of LAB through the production of vitamins or the degradation of phenolic compounds (10.1016/j.ijfoodmicro.2008.08.018).

Table olives are a stable and safe food product due to their low pH (< 4.4), high salt concentrations (6 - 8% NaCl in equilibrium), and the presence of antimicrobial compounds (polyphenols) that are formed during fermentation. However, under uncontrolled fermentation conditions or poor manufacturing practices, the growth of pathogenic of spoilage microorganisms, such as *Propionibacterium, Pseudomonas, Staphylococcus*, *Clostridium, Vibrio,* or *Celerinatantimonas*, is also typical and can compromise the stability and quality of the final product (10.3389/fmicb.2013.00091; https://doi.org/10.3389/fmicb.2015.00873).

Olive Biofilms.

In the last years, researchers have focused on studying LAB and yeast species with beneficial features isolated from table olive fermentations (10.1016/j.tifs.2012.01.006; 10.1016/j.fm.2012.09.003). Diverse studies have demonstrated that the ability of fruits to act as carriers of these beneficial microorganisms is due to the formation of a biofilm on the surface of olives (10.1016/j.ijfoodmicro.2012.05.011). This biofilm is a poly-microbial ecosystem, consisting essentially of lactobacilli and yeasts, which attach to the epidermis of the fruits, forming a community characterised by the excretion of a protective adhesive extracellular matrix, mainly composed of exopolysaccharides. The study of mixed biofilms between yeast and LAB on the olive surface is a recent issue, with direct applications in starter development, as it could transform this fermented vegetable into an excellent vehicle for beneficial microorganisms to reach final consumers, achieving population levels on the olives of over 10 million CFU/g. This issue could turn table olives into an alternative to dairy products, delivering probiotic microorganisms to consumers, especially those who are lactose intolerant.

Olive probiotic ferments.

According to the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO), probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health Benefit on the host". Bontasou et al., 2017 (10.3390/microorganisms5020030) reviewed various studies on the probiotic features of microorganisms isolated from table olives, identifying more than seven yeasts and 67 LAB strains with probiotic potential. Then, new papers have appeared demonstrating the high probiotic activities of microorganisms isolated from table olive fermentations (10.3389/fmicb.2018.00595; 10.1016/j.fm.2017.07.010; 10.1111/jam.15065; 10.1007/s12602-019-09604-y; 10.3389/fmicb.2019.00836; 10.3390/foods11193050). It is also interesting to highlight the role exhibited by some strains of yeasts, which even present greater gastric and pancreatic digestion resistance, phytase activity, and degradation of cholesterol levels than bacteria (10.1016/j.fm.2017.07.010).

López-García et al., 2023 (10.3390/nu15081931) recently conducted the only clinical trial with humans to demonstrate the probiotic properties of *Lact. pentosus* LPG1, a naturally occurring strain isolated from the biofilms of Spanish-style Gordal olive fermentations. They found that daily oral intake of *L. pentosus* LPG1 for 30 days improved gut microbiota regulation, primarily by conserving its biodiversity and promoting the growth of beneficial taxa, such as *Parabacteroides* and *Agathobacter*. Previously, Benítez-Cabello et al., 2020 (10.1007/s12602-019-09604-y) demonstrated this bacterium's anti-inflammatory and immunomodulatory properties in a murine model of induced chronic colitis. Surely, new strains isolated from table olives with probiotic potential and the ability to improve human health will appear in the coming years.

Metagenome and volatilome unveil the functional potential of Mediterranean table olive microbiome

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Background.
The microbiota of table olives is usually dominated by lactic acid bacteria (mostly *Lactiplantibacillus* spp.) and yeasts (e.g., *Saccharomyces cerevisiae, Candida boidinii, Wickerhamomyces anomalus*). While several studies characterized its taxonomic composition, the functional potential is still unexplored. Moreover, the impact of microbiome dynamics on the volatilome also deserves further investigation.

Methods.
A total of 270 samples from different Mediterranean countries (Italy, Spain and Greece) were collected. Shotgun metagenomics was carried out by Illumina sequencing. Volatilome was analysed through Solid Phase Micro-Extraction (SPME) and Gas Chromatography Mass Spectrometry (GC-MS) analysis.

Results.
Lactiplantibacillus pentosus was the most abundant taxon in both natural and alkali-treated olives, while *Saccharomyces cerevisiae* was significantly lower in Spanish-style olives. The length of fermentation strongly influenced the community assembly by increasing the diversity of Lactic Acid Bacteria and reducing the presence of potentially spoilage microbes, which in turn reflected on the metabolic potential of the communities. In addition, strain-level analyses of LAB species identified markers of specific olive cultivar and processes. Natural and alkali-treated green olives showed higher ketone levels, while acids, esters, and alcohols were present at concentrations similar to those in Spanish-style olives. However, fermentation time significantly influenced the concentration of these volatile compounds, as observed in black natural olive samples.

Conclusions.
These findings advance our understanding of microbial markers and the volatilome in table olive production and may assist stakeholders in developing high-quality products.

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Sensory evaluation and spoilage of table olives

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Introduction.

According to the International Olive Council (IOC), table olives are defined as the sound fruit of specific cultivars of the cultivated olive tree (*Olea europaea* L.), harvested at the appropriate stage of maturity and quality, and subjected to suitable processing to yield an edible and well-preserved product. The main quality attributes of table olives relate to the safety of the final product, as well as to its nutritional and sensory characteristics. Although the importance that consumers assign to these quality traits may vary significantly by region, product safety remains a top priority that must always be secured. Sensory characteristics constitute a major factor influencing consumer choice, based on the assessment of appearance, aroma, taste, and texture through the human senses. Despite systematic research efforts at the international level, it is not yet possible to determine the sensory quality of a food product using only instrumental analytical techniques. Human senses remain uniquely capable of successfully describing the sensory attributes of food. As a result, methodologies for the sensory evaluation of foods have significantly advanced in recent years, including the application of specialized statistical analysis techniques (sensometrics) for data processing. It is worth noting that, despite the extensive development and application of sensory analysis methods for the assessment of final food products and individual processing steps, limited information exists concerning the sensory evaluation of table olives, especially compared to their nutritional properties and biological value, which have been studied to a satisfactory extent.

Currently, several empirical methods exist for the sensory evaluation of table olives, often resulting in varying conclusions regarding the quality of the final product. It is therefore crucial to develop a universally accepted and reliable method that, along with microbiological and chemical characteristics, will serve as a criterion for determining the commercial quality of table olives. To this end, the IOC established a working group comprising experts from various member countries with the goal of developing a standardized method for the sensory evaluation of table olives. On November 21, 2008, the IOC adopted the proposal of the working group titled "Method for the Sensory Analysis of Table Olives" (COI/OT/MO/Doc No 1), which was formalized on November 25, 2011, through Decision No DEC-18/99-V/2011. The objective of the method is the sensory classification of table olives according to the intensity of perceived defects, as determined by a tasting panel composed of 8–10 appropriately selected and trained assessors.

Alterations of Olive Fruit Related to Sensory Quality.

The primary factor affecting the quality of table olives is spoilage due to microbial activity, predominantly by undesirable microorganisms, which can lead to fermentation failure and degradation of the final product's sensory properties. Undesirable conditions in processed olives may result from physical, chemical, or physiological factors and include: (a) fruit shrinkage or wrinkling, (b) blister formation on the skin, (c) textural degradation (softening), and (d) fruit discoloration (e.g., bluish or cyanotic appearance). Additionally, abnormal conditions caused by microbiological activity are frequently observed, including gas pocket formation, off-odor development, and butyric fermentation.

Gas Pocket Formation ("Fish-eye", "Alambrado").

This is a common defect across all commercial types of table olives. It typically occurs at the initial stage of fermentation, where the fruit's surface appears as if it has been scored with wire. The alteration is attributed to specific microorganisms, primarily Gram-negative Enterobacteriaceae belonging to the genera *Enterobacter*, *Citrobacter*, *Klebsiella*, *Escherichia*, and *Aeromonas*. These microbes metabolize the sugars present in the fruit and release gases (CO_2 and H_2) as by-products of fermentation. These gases accumulate within the fruit, forming blisters that result in the rupture of the pulp and its detachment from the skin. In advanced stages, these blisters resemble a "fish-eye," which is the origin of the term used in American literature. Eventually, the blisters grow large enough to reduce the fruit's specific

gravity, causing affected olives to float to the surface of the packaging. At this stage, the texture of the fruit is severely compromised. Although affected olives do not pose a health risk to consumers, the defect substantially devalues the product commercially. The severity of the defect depends on the cultivar, the load of enterobacteria in the brine, the temperature during early fermentation, and the salt concentration of the initial brine. Preventative measures include rigorous hygiene practices throughout the facility, fermentation tanks, equipment, and personal hygiene of staff handling the fruit. Effective brine composition includes 5–7% NaCl and a pH below 4.5. Prompt dominance of lactic acid bacteria in the fermentation environment—facilitated by starter cultures of Lactobacillus, acidification with lactic or acetic acid, or CO_2 injection—can limit the survival of undesirable microbes and significantly reduce the prevalence of affected fruit.

Butyric Fermentation.

Butyric fermentation is attributed to the growth of saccharolytic bacteria, notably *Clostridium butyricum*, which imparts a rancid butter-like odour to the fruit. This spoilage typically occurs at the initial stage of fermentation when the concentration of fermentable substrates is high in the brine. In the absence of sufficient populations of lactic acid bacteria, *Cl. butyricum* proliferates and dominates the microbial ecosystem, causing undesirable changes. This defect is characteristic of the Spanish-style processing of green olives, and it is rarely observed in naturally black olives due to unfavourable growth conditions for saccharolytic clostridia. These conditions include: (a) brine salt concentration exceeding 6.5%, (b) fermentation temperatures below 20°C, and (c) low pH values. The presence of sugars in high concentrations is a prerequisite for the growth of these organisms, as clostridia are obligate saccharolytic anaerobes. Thus, the occurrence of butyric fermentation requires the simultaneous presence of low salt levels, elevated temperatures, and abundant fermentable sugars. Olives exhibiting this defect are sensorially unacceptable and should not be marketed. Partial mitigation may be achieved by transferring the olives into fresh brine supplemented with fermentable substrates and a starter culture of lactic acid bacteria.

Zapateria.

The characteristic of this spoilage is a penetrating off-odour that intensifies over time and is consistently associated with low acidity in the brine. In the initial stage, the aroma resembles that of worn leather shoes, hence the Spanish term "zapateria" (from "zapatos"). This defect may not be immediately discernible to consumers, and some may even mistake it for the typical aroma of table olives. Zapateria can be observed in both green and naturally black olives, particularly when stored in brines with low salt content and elevated pH. It typically manifests after the primary lactic acid fermentation has subsided, especially as ambient temperatures rise. This spoilage is attributed to the action of proteolytic clostridia and propionic acid bacteria, which thrive at pH values above 4.2. In advanced cases, malodorous acids such as formic, butyric, caproic, and caprylic acids are produced, conferring a putrid aroma to the product. The development of zapateria can be prevented by maintaining brine salt levels above 8.0% and adhering strictly to hygienic practices throughout the processing environment.

Conclusion.

The sensory evaluation of table olives constitutes a critical component in the comprehensive assessment of their overall quality, encompassing not only visual and textural attributes but also olfactory and gustatory properties. The sensory profile of table olives is highly susceptible to various microbiological and physicochemical alterations that may arise throughout processing, fermentation, and storage. Defects such as gas-pocket formation (fish-eye), butyric and zapateria not only compromise the sensory acceptability of the final product but also significantly reduce its commercial value. Despite advances in analytical chemistry and microbiological monitoring, human sensory perception remains indispensable for the reliable classification of sensory defects and quality grading. The establishment of a harmonized, scientifically robust sensory evaluation methodology by the International Olive Council (IOC) represents a pivotal development toward the standardization of quality control practices across the olive industry. The systematic training and calibration of tasting panels, coupled with rigorous environmental and procedural controls, are essential for ensuring consistency, objectivity, and reproducibility in sensory assessments. In this context, integrating sensory analysis with microbiological, chemical, and technological data can offer a more holistic approach to quality assurance

and product development in the table olive sector. Furthermore, the implementation of such methodologies across industrial and regulatory settings may foster transparency, enhance consumer trust, and facilitate international trade by ensuring that products meet clearly defined sensory standards.

Table Olives: From Fermentation to Taste

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Table olives are among the most emblematic food products of the Mediterranean region, offering not only nutritional value but also a rich sensory experience. Their distinct flavour develops during fermentation, a process that is both scientifically driven and influenced by tradition. Greek manufacturers have decades of experience in olive processing, while they continuously explore how fermentation contributes to flavour development in order to improve product quality, consistency, and consumer satisfaction. This presentation explores the full production journey of table olives — from the initial stages of fermentation to the final expression of taste. Raw olives are naturally bitter due to the presence of oleuropein, a phenolic compound that must be reduced or eliminated to make the fruit tastily acceptable. Fermentation, whether physically or starter-culture-driven, promotes the debittering process of the olives, while also preparing a suitable environment for the formation of desirable taste and aroma compounds. Lactic acid bacteria (LAB) and yeasts play important roles in this transformation. Different fermentation styles, such as the Greek natural black olive method (i.e in Kalamata olives) and the Spanish-style green olive process (i.e. in Chalkidiki Olives), lead to significantly different flavour effect. Variables such as olive variety, salt concentration, temperature, oxygen presence, duration of fermentation, and processing type all influence microbial activity. For example, a slower, natural fermentation can result in more complex, earthy, and slightly fruity flavours, while controlled fermentations may offer more consistent acidity and milder flavour. Each olive variety has different nutritional profile, which contributes to the final olives' taste. Traditional practices adoption and fermentation under controlled conditions to optimize flavor, in combination with ensuring food safety and meeting regulatory standards, can lead to a standardized aroma, texture, and overall consumer acceptance. This approach strengthens consumer trust in the sensory quality of the Greek olives. The industrial expertise of Greek Olive Manufacturers and collaboration with academic research, can result in more advanced processing technologies and quality control standards that develop the sensory profile of the final product. We will discuss how consumer preferences and market demands are driving innovation in product development and sustainable practices. This integrated approach offers significant insights for both industry professionals and researchers aiming to link tradition with science for high-quality table olives with optimal taste and safety.

An integrated metagenomic and volatilomic approach to advance research in Spanish-style cv. Chalkidiki green table olives

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Introduction.

Table olives represent one of the most widespread plant-based fermented foods in the Mediterranean region, holding a prominent position within both the agricultural and food processing sectors of these countries. Global consumption has steadily increased in recent years (10.37349/eff.2024.00052). Spanish-style green table olives from cv. Chalkidiki, one of the most economically significant Greek cultivars, are appreciated in the local and international markets due to their superior quality characteristics.

The traditional processing method of cv. Chalkidiki green olives involves debittering with a lye treatment (NaOH, 2% w/v), followed by multiple washing steps. This is succeeded by spontaneous fermentation in high salinity brine (typically 8% w/v NaCl) over a period of four months. The spontaneous fermentation process is primarily driven by the indigenous microbiota associated with the raw olives, including lactic acid bacteria and yeasts, which play key roles in the development of the final product's organoleptic and safety attributes (10.1002/ejlt.201800171). Several factors that shape this microbial community are cultivar, geographical origin, raw fruit integrity, harvesting conditions, and postharvest treatment. Additionally, its activity is further influenced by competitive interactions with contaminating microorganisms introduced through fermentation equipment and associated processing infrastructure that come into contact with the olives and the brine (10.1002/jsfa.1336). This complex and dynamic ecosystem has a direct impact on the safety and key quality attributes of cv. Chalkidiki green table olives.

To better understand and control this intricate microbial system, the application of advanced high-throughput methodologies from the omics sciences is critical. Amplicon metagenomics, a widely utilized technique in table olive research, facilitates detailed characterization of the microbial dynamics throughout fermentation. Volatilomics, which focuses on the profiling of volatile metabolites, provides insights into the metabolic activities of microbial communities, thereby contributing to the understanding of flavor development, consumer acceptance, shelf life, and overall product quality (10.37349/eff.2024.00052; 10.1007/s11947-023-03211-0). Despite the knowledge offered by single-omics, a multi-omics strategy can unravel the changes and interactions taking place among the microbial community during fermentation and reveal the interplay between the microbial profile and volatilome, offering a more holistic view of this complex ecosystem (10.3389/fmicb.2022.1044820). Although multi-omics approaches, such as the integration of metagenomic and volatilomic datasets with the assistance of appropriate bioinformatics tools, have been widely employed in fermented foods, their application in table olives fermentation system remains limited.

Importance of multi-omics data integration.

Deciphering the microbiota and its functional properties involved in Spanish-style green cv. Chalkidiki green table olives could enhance the comprehension of the mechanisms of fermentation and enable the identification of potential biomarkers (e.g., specific volatile compounds linked to distinct microbial taxa). These biomarkers could become a valuable tool in monitoring the fermentation process under different novel treatments, such as low NaCl content in brine or inoculation with starter cultures. Moreover, they could open the possibility to design starter cultures for the tailored production of VOCs, hence providing the ability to model the flavour and quality aspects of table olives, extended shelf-life, and potential health-promoting properties, and contribute to quality evaluation and optimization of final product development with desirable features. When the fermentation takes place spontaneously, as in this case, there is a risk of microbial spoilage occurring with a direct impact on the volatilome of the final product. There have

been major volatile compounds identified that have been associated with each type of malodorous microbial spoilage**,** most commonly zapatera spoilage, butyric and putrid spoilage, that could be employed in monitoring the fermentation process. However, advancing the understanding of the microbial and volatilomic profile of table olives could further elucidate the mechanisms underlying each spoilage type (10.3390/metabo8040073).

In 2012, table olives from cv. Chalkidiki received the Protected Designation of Origin (PDO) "Prasines Elies Chalkidikis" indication according to the regulation (EU) No 426/2012 (http://data.europa.eu/eli/reg_impl/2012/426/oj). A global issue of significant attention affecting such products is food authenticity. One of the most popular types of fraud appears to be misleading geographic origin (10.1016/j.foodchem.2022.133856). The application of such a multi-omics approach for the discrimination of table olives based on the growing region or cultivar could become a tool in the assurance and protection of these economically significant products. Overall, this approach could provide novel insights into the development of different organoleptic properties among olives grown in different locations, thus enhancing their PDO or PGI character, significantly endorsing the local economy. While both technologies have been applied separately for this purpose, to the best of our knowledge, no paper employing this multi-omics approach has been published so far (10.37349/eff.2024.00052).

Research challenges.

Evident became the variations in various steps of the workflow, e.g., the sequencing of different regions of the 16S rRNA gene to unravel the microbial diversity of table olives. Further research comparing and detecting the most suitable region could lead to the establishment of a standardized workflow to uphold the quality and interoperability of the generated amplicon metagenomic data. Such a consistent pipeline could also be applied to volatilomics, up to the bioinformatics section of the analysis to achieve the same purpose. The integration of omics datasets, such as amplicon metagenomics and volatilomics, while providing a valuable opportunity to unravel the profile of the microbial ecosystem of table olives, poses several challenges. Both omics techniques generate an extensive volume of complex biological data, often presented in different formats (10.37349/eff.2024.00052). Advanced equipment and specialized computational resources are required to analyse, integrate, and interpret these datasets efficiently (10.34133/bdr.0059). These challenges highlight the need to establish standardized workflows and consistent bioinformatics pipelines to ensure the quality and interoperability of the generated data.

Conclusions and future perspectives.

The application of a multi-omics framework in table olive fermentation holds significant promise, though it remains in the early stages of development. A key goal in integrating amplicon metagenomics and volatilomics is the identification of microbial biomarkers—particularly VOCs—indicative of specific microbial activities or spoilage events. These markers could inform quality control, starter culture development, microbial safety assessments, and authenticity verification based on geographic origin.

Future efforts should focus on the integration of metagenomic and volatilomic data with other omics datasets, such as metatranscriptomics, metaproteomics, and shotgun metagenomics. This will allow for a comprehensive systems biology approach to unravel the functional interactions within the microbial consortium and their effects on product quality. In the present study, we aim to present the microbial and volatilomic profiles of Spanish-style *cv.* Chalkidiki green olives, exploring the dynamic relationships between these datasets and establishing metagenomic-phenotypic correlations. Ultimately, this knowledge will support the development of science-based strategies for controlled fermentation, consistent high-quality product generation, and robust quality assurance frameworks for PDO/PGI products.

Innovative Strategies for Enhancing the Shelf Life and Safety of Naturally Fermented Table Olives

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Background.
Table olives are a key component of the Mediterranean diet, but conventional preservation often relies on high salt concentrations and synthetic additives. With growing consumer demand for clean-label, low-sodium products with extended shelf life, alternative preservation strategies are needed. This talk will present two complementary research efforts: (1) the *OLIVEPACK* project, funded by PRIMA, which aims to develop natural antimicrobial packaging solutions for low-salt olives, and (2) a study investigating near-infrared (NIR) light treatment as a non-thermal microbial reduction technique.

Methods.
In the OLIVEPACK project, biocompatible cellulose-based foams are being developed as packaging inserts, loaded with natural antimicrobial agents such as hydroxytyrosol extracted from table olive wastewater and bay laurel essential oil. These agents are encapsulated in halloysite nanotubes and incorporated into cellulose foams. The effectiveness of the antimicrobial bionanocomposite foams will be evaluated by monitoring their ability to reduce the microbial load of packaged olives during storage under real-life conditions.
In the NIR study, fermented black olives were irradiated with NIR light under controlled conditions. Microbiological, physicochemical, and sensory analyses were conducted over a 6-month storage period to assess the treatment's effectiveness.

Results.
Antimicrobial packaging inserts based on cellulose foams loaded with hydroxytyrosol and bay laurel essential oil have been successfully developed. In vitro tests confirmed their antimicrobial activity against common spoilage microorganisms. Following final optimization of formulation and loading conditions, the inserts will be tested under real storage conditions to evaluate their effectiveness in different types of fermented table olives.
NIR treatment have been shown to effectively reduce initial microbial load, without altering the olives' physicochemical, or sensory acceptance. The treated olives maintained physicochemical stability and microbial safety over storage.

Conclusions.
Both strategies offer promising, complementary approaches for preserving fermented table olives naturally. The OLIVEPACK system supports clean-label, active packaging innovations, while NIR irradiation provides a chemical-free, non-thermal method to enhance microbial safety. Together, these approaches contribute to the development of sustainable, health-conscious olive products aligned with industry and consumer trends.

Microorganisms drive a virtuous way to ferment table olives with improved safety, nutritional and sensorial traits. A story born in lab, moved to producing context and reaching the consumer's table

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Background.

Table olives represent a fundamental food source in the Mediterranean diet owing to their nutritional and bioactive properties. Indeed, they contain high levels of bioactive compounds and are highly appreciated by the consumers for their aromatic properties. Natural black olives are traditionally produced by a spontaneous fermentation since they cannot be readily consumed, due to their characteristic bitter taste.

Microbial starters, temperature and pasteurization conditions heavily influence production, quality, and safety aspects of fermented table olives. Starter cultures represent a new technological approach to control and standardize the fermentation process. Selected strains of lactic acid bacteria and yeasts have been proposed, individually or in combination, to improve the organoleptic and nutritional traits of the final product, also controlling the development of undesirable microorganisms and production losses. However, the use of starter culture is scarcely diffused due to several reasons, including cost, complexity, limited availability of knowhow and validation at industrial scale.

Methods.

Lactic acid bacteria and yeasts from the CNR-ISPA microbial collection were selected. Lab-scale fermentation assays using several microorganisms as starter, in different conditions of temperature and considering the effects of the pasteurization treatment of the final product, were optimized. Pilot-scale fermentation tests were performed in an industrial facility to validate the best conditions identified at lab-scale. Sensorial analyses were performed on the final products by a panel of experts. The effects of a usual diet supplemented with fermented table olives were tested on gut microbiota and metabolome of healthy volunteers.

Results.

Tarantini and collaborators (10.1016/j.fm.2024.104537) investigated the impact of selected microbial starters from various sources on the physical, chemical, and microbiological parameters of black olive fermentation at "laboratory-scale". For each fermentation assay, the effects of controlled temperature (20 °C) vs not controlled environmental conditions (7-17 °C), as well as the consequences of the pasteurization were assayed on the final products. Among the tested microbial starters, the combination of two LAB strains, *Leuconostoc mesenteroides* KT 5-1, and *Lactiplantibacillus plantarum* BC T3-35, and two yeasts, *Debaryomyces hansenii* A 15-44 and *Saccharomyces cerevisiae* LI 180-7, was useful to obtain fermented products showing the most promising characteristics in terms of enzymatic activity, total phenolic content, and antioxidant activity. Fermentation under uncontrolled temperature, which is commonly used in industrial process, improved the technological and functional properties of table olives. Moreover, the fermented final products maintained their functional properties even after the pasteurization treatment.

Process reproducibility at the industrial scale often poses challenges, given to the numerous external factors that could influence the whole process "outside the lab". Validating fermentation conditions at a pilot industrial scale can confirm the feasibility and reliability of applying selected microbial starters to enhance both production efficiency and quality of table olives. Thus, both starter-driven, and spontaneous fermentations of the *Leccino* cultivar were studied in an industrial facility.

A single inoculum of lactic acid bacteria, LAB1 (*Leuconostoc mesenteroides*), LAB2 (*Lactiplantibacillus plantarum*), yeast, YST1 (*Debaryomyces hansenii*) and YST2 (*Saccharomyces cerevisiae*) strains, and a co-inoculum LAB1+YST2 (*Leuconostoc mesenteroides*+*Saccharomyces cerevisiae*) were used as starters to conduct the fermentation process and to counteract the development of pathogens and undesired microorganisms. Chemical and biochemical analyses confirmed the positive outcome of the process and the improvement of nutritional traits, especially in the case of the LAB1 and LAB1+YST2 treatments. These two strategies enhanced the total phenolic content and the corresponding antioxidant activity, compared to spontaneous fermentation and the raw material, respectively. Additionally, the same treatments resulted in the most appreciated sensory attributes with low levels of defects and off-flavours in the final products. The Volatile Organic Compound (VOCs) analysis highlighted the predominance of esters in the LAB1+YST2 sample.

After *in vitro* digestion, the total bio-accessibility of phenolic compounds was quite high in olives fermented by the mixed starter culture LAB1+YST2 and those fermented by the LAB1 strain.

Finally, a study was conducted on healthy volunteers, both women and men, who introduced fermented table olives (LAB1 treated sample), together with black kale, into their usual diet associated with physical activity habits, over a period of 4 consecutive weeks. Serum and faecal samples for biomarker screening were collected at the run-in (T0) and after the 4 weeks dietary intervention (T1). At both sampling times, volunteers were also screened for anthropometric measurements. As preliminary results, a shift of the gut metabolome accounting to an increase on short chain fatty acids, was recorded. In addition, the improvement of serum biomarkers of well-being and an increase in health promoting taxa of the gut microbiota, were observed. In particular, haemato-chemical profiling revealed that the dietary intervention was able to induce, a significant decrease in total cholesterol and also contributed to the control of HDL/LDL levels.

Conclusions.

A multiparametric approach was firstly applied at laboratory scale to determine the most favourable conditions for table olives fermentation, processing, and stabilization. The best strategy selected foreseen the use specific starter strains, to carry out the fermentation at not controlled environmental conditions and to stabilize the final product by pasteurization.

These conditions were successfully validated on table olive starter-driven fermentation strategies in an industrial environment at pilot-scale. A deep characterization of sensory attributes, nutritional traits and the bio-accessibility of bioactive compounds, mainly polyphenols, was performed on pasteurized selected samples. Finally, preliminary effects on health-related parameters, on gut microbiota and metabolome, resulting from the consumption of fermented vegetables (table olives in combination with black kale), were observed.

How machine learning and microbial metataxonomic data can help to classify table olives.

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Background.

In recent years, the use of metataxonomic analysis to characterize microbial communities in fermented foods has increased significantly. Specifically, in the field of table olive microbiology, the number of studies focusing on the microbial communities associated with different types of olive processing methods, cultivars, and countries of origin has grown steadily. Moreover, advances in bioinformatics and machine learning (ML) have expanded the analytical tools available for studying these complex microbial datasets.
The aim of this study is to identify patterns in the microbial profiles of table olives that can support their classification based on processing type, olive cultivar, and country of origin. To achieve this, several supervised ML algorithms based on decision tree models were compared. Furthermore, the most accurate model could potentially be used to predict metadata for new olive samples based on their microbial profiles.

Methods.

A comprehensive database was constructed using microbial profiles from sequence reads available in published table olive bioprojects studies from the NCBI and ENA repositories, along with their associated metadata (processing type, cultivar, and country of origin). A new taxonomic reassignment was conducted to standardize the dataset. Taxonomic assignment was carried out using DADA2 and phyloseq, followed by data preprocessing to filter out low- abundance microbial genera (<0.1%) and underrepresented metadata categories (<1%).
Three tree-based classification algorithms—Classification and Regression Trees (CART), Random Forest (RF), and eXtreme Gradient Boosting (XGB)—were evaluated and compared independently for each metadata category.

Results.

The results demonstrated that ML-based approaches can effectively classify microbial profiles from table olives. Among the models tested, RF yielded the highest accuracy, exceeding 95% when classifying samples by country of origin, and reaching around 80% accuracy for classification by processing type and cultivar. The kappa coefficient was above 0.7 in all cases, with the highest value of 0.94 observed in the country-based classification.
Moreover, the use of tree-based models allowed for the identification of key variables (microbial genera) in the classification process. For processing type classification, the most relevant genera were *Sporolactobacillus*, *Pichia*, *Candida*, and *Alkalibacterium*. For cultivar classification, the most influential genera were *Candida*, *Ogataea*, and *Lactiplantibacillus*.

Finally, for classification by country of origin, the most important genera included *Candida*, *Halomonas*, *Sporolactobacillus*, *Alkalibacterium*, and *Citeromyces*. Interestingly, unassigned taxa also played a significant role in all three classification tasks.

Conclusions.

In conclusion, metataxonomic databases of food products can be highly valuable when analysed using ML models. The findings from this study show that ensemble learning methods such as RF and XGB outperform single decision tree models in classifying the microbial metataxonomic profiles of table olive samples. Additionally, these models offer meaningful insights into microbial genera importance enabling rapid identification of microbial markers linked to

specific metadata categories. These results underscore the practical applications of ML models in the table olive sector and broader food industry for tasks such as authenticity verification, traceability, and quality and safety control.

Functional Table Olives: Fortification with probiotic *Lactiplantibacillus pentosus*

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Background.

Table olives are rich in essential micronutrients, fatty acids, and biologically active phytochemicals, including polyphenols, which have been linked to various health benefits. The growing awareness of the role of gut microbiology in human health has fuelled the development of probiotic and prebiotic-based products. Research suggests fruits and vegetables, including table olives, are suitable carriers for probiotics, offering benefits to vegetarian consumers (10.1016/j.tifs.2012.01.006). Table olives possess unique properties for probiotic impregnation, facilitated by their porous structure, which can be filled with beneficial microorganisms. Techniques like vacuum impregnation and microencapsulation are used to enhance viability and sensory properties without damaging the food (10.3390/ijms150916577). This study focuses on evaluating the probiotic properties of *Lactiplantibacillus pentosus* NRRL B-227, including its antioxidative ability, antibiotic resistance, and survivability after digestion, followed by microencapsulation in a whey protein concentrate/xylan complex and impregnation onto table olives.

Methods.

This study was carried out using the Turkish olive variety Gemlik, Sele type black olives. *Lactiplantibacillus pentosus* NRRL B-227 was microencapsulated using xylan and whey protein concentrate (WPC) using the water-in-oil emulsion technique to maintain cell viability (10.1016/j.jbiosc.2015.04.014). A vacuum impregnation process was performed to coat olive surfaces with *L. pentosus.* Antioxidative ability, antibiotic resistance, and survivability after simulated digestion tests (INFOGEST) were carried out. The antioxidant activity of *L. pentosus* at 9 log CFU/mL was evaluated using DPPH and ABTS free radical scavenging assays. (10.1016/j.foodchem.2012.06.048). Freeze-dried Gemlik olives were analysed using scanning electron microscopy (SEM) and phase-contrast microscopy. EDX analysis was done with a gold-coated olive surface, while the ESEM detector showed no coating.

Results.

The antioxidant DPPH activity was 71.6% for *Lact. pentosus* cells and 17.7% for the intracellular cell-free extract. ABTS activity was 9.7% for cells and 7.7% for the extract. *Lact. pentosus* showed resistance to all antibiotics tested after 24 hours. After 48 hours, it exhibited resistance to seven antibiotics (amoxicillin, gentamicin, streptomycin, penicillin, vancomycin, cephalothin, and kanamycin) and sensitivity to five others.
The microencapsulation efficiency of *Lact. pentosus* was assessed using water-in-oil emulsion with varying xylan concentrations. The highest efficiency was seen at a 1:1 ratio of whey protein concentrate (WPC) and xylan, with over 90% efficiency and cell viability between 10.13–10.58 log CFU/g. After one month, viability decreased by 0.41–0.86 log cycles. Storage at 4°C for 72 weeks led to a 2.27 log CFU/g reduction, but counts remained above 6 log CFU/g, meeting the recommended probiotic dose for functional foods. Both free and microencapsulated *Lact. pentosus* showed similar results in simulated digestion, with a 1 log reduction after the oral phase and a 3 log reduction after the gastric phase. However, microencapsulated cells exhibited higher viability in the intestinal phase, indicating enhanced protection.
Lact. pentosus and *Lact. plantarum* were vacuum impregnated onto Gemlik table olives. Non- microencapsulated LAB lost viability quickly, but freeze-drying improved cell viability. Microencapsulated cells maintained stable counts (6.02–6.29 log CFU/g) during storage, while non-microencapsulated cells decreased by 0.62 log cycles. No significant bacterial viability change occurred in the impregnation fluid during 4 weeks, though a 1.5–2 log reduction was seen between the

fluid and olive surfaces.
Gemlik olives showed no microbial growth before or after freeze-drying, and no mould was observed on the table olives. Phase-contrast and scanning electron microscope (SEM) images of microencapsulated *Lact. pentosus* cells, prepared with whey protein concentrate and xylan, were obtained. The microcapsules were lyophilized, gold-plated, and examined under SEM.
Microencapsulated and non-microencapsulated LAB-impregnated Gemlik olives retained similar appearance and colour after freeze-drying, though the oily and juicy look was lost for some extent.
Consumers are increasingly seeking functional foods, with table olives providing a potential vehicle for probiotics due to their ready-to-eat nature. *Lact. pentosus* demonstrated antioxidant activity (71.6% DPPH and 14.96 µg Trolox/mL ABTS) but showed resistance to common antibiotics, which helps maintain their health benefits during antibiotic use.
Microencapsulation with whey protein concentrate (WPC) and xylan increased *Lact. pentosus* survival during storage, with over 90% efficiency and a 78% survival rate after 72 weeks. Microencapsulated probiotics showed better survival in simulated gastrointestinal conditions compared to non-microencapsulated cells. Microencapsulation also enhanced the stability of probiotics on Gemlik olive surfaces, maintaining viable cell counts for up to 30 days.
Freeze-drying further preserved the probiotics on olives, helping retain their viability. The water-in-oil emulsion technique proved effective for microencapsulation, making Gemlik table olives a suitable carrier for probiotics, with *Lact. pentosus* showing better stability than *Lact. plantarum* during storage and impregnation.

Conclusions.

This study showed that *Lact. pentosus* has antioxidant properties and is resistant to antibiotics and simulated digestive system. Xylan and whey protein concentrate-based microencapsulation improved the viability of *Lact. pentosus,* which successfully impregnated onto the surface of the Gemlik table olives. After being stored at 4°C for four weeks, microencapsulated *Lact. pentosus* showed cell levels above the recommended threshold of probiotics necessary for functional foods. Findings in this study showed the ability of this strain to survive under packing conditions for a 1-month-storage period and to colonize the table olive surface. Table olives can be a suitable model foodstuff to produce a new and deliciously functional herbal product; the level of live *Lactobacillus* strains on the olive surface ranged from 106 to 107 CFU/g. This means that given the intake of about 10–15 olives per serving (corresponding to 40–60 g of olives), the strains can be taken into the body in an amount ranging from 10^7-10^8 CFU. In the light of these findings, *Lact. pentosus* with antibiotic and digestion fluids resistant and antioxidant properties were successfully microencapsulated. Table olives can be considered as a suitable carrier for beneficial microorganisms that satisfies with the expectations of regulations for functional foods.

Phenolic compounds content in cv. Chalkidiki olives as a critical factor for Spanish style processing conditions.

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Table olives is a product that demands special attention by the relevant sector in Greece and elsewhere in terms of latest scientific and technological advances in every step of the manufacturing process. Green table olives, cv. Chalkidiki, is a brand product of great interest in internal and international market. We present our views and experimental findings as well as recommendations on why phenolic compounds content should be considered as a critical factor in the course of Spanish style processing steps. Our know how for the cv Chalkidiki table olives can be adapted for other cultivars, too, and is strongly related with the quality and nutritional value of the final product.

A new fermentation system for the production of Spanish-style green table olives

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Background.
The traditional system of fermenting Spanish-style green table olives in underground fibreglass containers has remained unchanged for decades (10.3989/gya.1997.v48.i5.806). Despite its longstanding use, this system presents challenges in terms of hygiene, homogeneity, and operational efficiency. In response, a novel under-vacuum and connected olive fermentation system using stainless steel fermenters has been developed.

Methods.
A comparative study in the fermentation of Spanish-style table olive inoculated with *Lactiplantibacillus* strains was conducted between the traditional fiberglass fermenters and the new stainless-steel system. This new system integrates two pumps (recirculation and gas purge) and aims to modernize the process while improving control and sanitary conditions. Both physicochemical and microbiological parameters were analysed. Particular attention was given to the variability of measurements, microbial growth dynamics, and metataxonomic profiling of bacterial and fungal populations. The efficiency of post-fermentation cleaning and disinfection was also evaluated.

Results.
For most physicochemical and microbiological parameters analysed, the new stainless-steel system exhibited lower variability, indicating greater homogeneity. Lactic acid bacteria growth was favoured in the conventional system, which also showed higher titratable acidity, although both systems achieved similar final pH levels (~4.2), typical values for spanish-style table olives (Garrido-Fernández et al., 1997). The traditional system produced olives with better colour and sensory acceptability. In contrast, the new system favoured yeast growth, enhanced CO_2 release, improved fruit texture, and promoted inoculum imposition, though it resulted in lower combined acidity. Metataxonomic analysis revealed greater microbial diversity in the traditional system, both bacterial and fungal, indicating a lower effect of the inoculum, while the new system showed a higher presence of *Lactiplantibacillus* sequences and no foodborne pathogens. Finally, at the end of the fermentation process, cleaning and disinfection were more effective in the stainless-steel system compared to fiberglass fermenters.

Conclusions.
This study supports the potential for modernizing table olive fermentation through stainless steel systems, with the aim of reducing spoilage risks and enhancing product standardization.

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Microbial innovation in table olive fermentation: a decade of research on *Lactiplantibacillus pentosus* OM13

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Background.

Table olive fermentation is a longstanding biotechnological tradition practice integral to the Mediterranean diet and agri-food sector. Despite its cultural and economic relevance, spontaneous fermentation often suffers from unpredictable microbial dynamics, inconsistent sensory outcomes, and safety concerns due to the presence of spoilage and pathogenic microorganisms. These limitations have generated interest in controlled fermentation strategies based on microbial ecology and starter culture development and application.

At the University of Palermo, the food microbiology research group has undertaken a comprehensive programme aimed at improving the quality, safety, and reproducibility of table olive fermentation. Focusing on Sicilian cultivars, particularly Nocellara del Belice, the research group has explored both Spanish-style and Greek-style fermentation methods. This work contributes to a broader scientific effort to understand and manage microbial communities in fermented vegetables, with a special emphasis on the role of lactic acid bacteria (LAB) and yeasts in shaping product quality and safety (10.1016/j.ijfoodmicro.2022.109670).

Methods.

Central to this research was the application and characterisation of the autochthonous strain *Lactiplantibacillus pentosus* OM13, was consistently applied across all experimental trials. Originally isolated from Sicilian table olives, OM13 was selected for its robust fermentative capabilities, tolerance to high salinity and phenolic compounds, and ability to dominate the microbial ecosystem during fermentation. Its performance was assessed under various conditions, including different harvesting methods (manual *vs.* mechanical), irrigation regimes, and fermentation strategies. One of the most innovative approaches was the adaptation of the "pied de cuve" technique, commonly used in winemaking, to table olive fermentation. This involves preparing a small volume of partially fermented brine to inoculate larger batches, promoting early establishment of beneficial microbial populations while reducing the need for large quantities of starter cultures. Additional strategies included OM13 acclimatisation in brine before inoculation, supplementation with nutrients to support microbial growth, and co-inoculation with selected yeast strains (*Candida boidinii* and *Candida norvegica*) known for their enzymatic activity and contribution to flavour development. Trials were conducted at both laboratory and industrial scales in collaboration with local producers. Microbial populations were monitored using culture-based methods and molecular profiling such as randomly amplified polymorphic DNA (RAPD)-PCR and 16S rRNA gene sequencing. Physicochemical parameters (pH and salt concentration), volatile organic compounds (VOCs), and sensory attributes were systematically evaluated throughout the fermentation process. Multivariate statistical analyses were used to interpret microbial dynamics and compare fermentation treatments.

Results.

Across all experiments, *Lact. pentosus* OM13 consistently dominated the fermentation microbiota, rapidly acidified the brine, and suppressed spoilage organisms including *Enterobacteriaceae* and *Pseudomonads*. Molecular analyses confirmed that LAB isolates from inoculated trials closely matched the OM13 genetic profile based on RAPD-PCR and 16S rRNA sequencing. The strain demonstrated strong adaptability to diverse environmental conditions, including high salinity, low pH, and phenolic presence. The "pied de cuve" approach proved particularly effective in promoting native LAB growth while reducing reliance on freeze-dried cultures. This approach preserved microbial diversity and enhanced the expression of cultivar-specific sensory traits. The acclimatisation of OM13 prior to inoculation further improved fermentation performance, accelerating acidification and stabilising microbial communities. Nutrient supplementation supported OM13 persistence and improved fermentation kinetics. Sensory evaluations consistently showed that olives fermented with OM13 scored higher in aroma, flavour complexity, and overall acceptability compared to spontaneous fermentations. OM13 use was associated with a favourable VOC profile, including elevated levels of phenylethyl alcohol, ethyl acetate, and hexanal, compounds linked to freshness and green fruit notes. Co-inoculation with selected yeasts contributed to improved colour retention, pulp firmness, and reduced off-flavours.
Inoculated trials consistently demonstrated superior microbiological stability, reduced variability, and enhanced sensory quality across different processing styles and environmental conditions, underscoring the robustness and versatility of OM13 as a starter culture.

Conclusions.

The use of *Lact. pentosus* OM13 has proven to be a reliable and effective strategy for guiding table olive fermentation. Its consistent dominance, environmental resilience, and positive impact on product quality making it a key element in the advancement of table olive fermentation methods. By integrating microbial selection, process optimisation, and sensory science, producers can achieve greater consistency, safety, and authenticity in their products. The development of tailored starter cultures and fermentation aids has advanced microbial biotechnology, offering scalable solutions for both artisanal and industrial production. Moreover, the implementation of OM13 has contributed to the valorisation of local cultivars and traditional methods, enhancing the competitiveness of Sicilian table olives in domestic and international markets. Techniques such as "pied de cuve", starter acclimatisation, and yeast co-inoculation are replicable and adaptable across the Mediterranean, providing a model for sustainable innovation in fermented vegetable production. This decade-long research effort has significantly deepened scientific understanding of microbial ecology in table olive fermentation while delivering tangible benefits to the agri-food sector. In conclusion, this work serves as a paradigm of how microbiological research can drive innovation, safeguard traditional practices, and contribute to the enhancement of the quality and identity of emblematic Mediterranean fermented foods.

A new generation of biological debittered olive paté enriched with beneficial *Lactiplantibacillus plantarum*

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Background.

The quality of food and the binomial diet-health have now attracted extreme interest with scientific and technological implications. Fermented foods have regained popularity in Western diets, for their health-promoting potential, mainly related to the role of lactic acid bacteria (LAB) during the fermentation process. Moreover, due to health, sustainability and ethical concerns, there is an increasing attention in the development of non-dairy fermented foods by using vegetable food matrices (10.1038/s41575-020-00390-5). Plant- based matrices are in line with that, and among them, olives and their co-products represent suitable matrices to produce healthy innovative and environmentally sustainable fermented foods. Olive oil production is one of the main agro-industrial activity of the Mediterranean countries that generates a great amount of olive pomace as one of the major by-products, in which end up a high amount of the olive total phenols, giving it disposal problems but also potential benefits. The destoned olive pomace can represent an important source of fibre and bioactive molecules such as phenolic compounds, whose exploitation until present is limited to animal feeding (10.3389/fnut.2024.1467724). The palatability of the destoned olive is achieved after a fermentation process hydrolyzing bitter secoiridoids and enhancing final aromatic profile. The biological biotransformation with selected LAB is an alternative to chemical hydrolysis (10.3390/foods9020178). Among LAB, *Lactiplantibacillus* (*Lact.*) *plantarum* strains, widely used as starter cultures in the production of fermented foods, showed the potential to enhance fermented foods functionality and affect host health by modulating the immune and inflammatory response (10.3390/microorganisms9020349). Moreover, fermented foods and their associated microbes can positively modulate gut microbiota structure and composition, that is of paramount importance to guarantee gut homeostasis and health. Based on that, the study aimed at investigating selected food-associated *Lact. plantarum* strains, characterized for their ability to restore inflammation *in vitro*, as a tool in a lab scale fermentation process, to enrich the functionality of an innovative biologically debittered olive patè (FDP), recently patented.

Methods.

Two selected *Lact. plantarum* strains isolated from fermented foods, including table olive, and previously characterized for their probiotic traits (10.3389/fmicb.2018.02392; 10.1093/femsle/fnaa076; 10.1038/s41598-020-58069-5), have been investigated for their anti-inflammatory activity in an *in vitro* human inflamed intestinal model (10.1038/s41598-020-73201-1; 10.1016/j.isci.2023.108481). Then, a lab scale biological debittering process has been carried out by using a mixture of *Lactiplantibacillus* (*Lact.) pentosus* starter strains, already isolated from olives brines and characterized for their oleuropeinolytic activity (10.1021/jf053206y) mixed with the selected *Lact. plantarum* strains (FDP samples). Subsequently, olive patè samples have been subjected to a simulated gastro-duodenal digestion (INFOGEST protocol, 10.1038/s41596-018-0119-1) followed by an ex-vivo colonic fermentation by the means of a faecal gut fermentation model inoculated with human faeces collected from healthy donors. Data were analysed by omics approaches (microbiomics by qPCR and metabolomics by SPME GC-MS and statistical multivariate analysis), in order to investigate the impact of samples in modulating human gut microbiota, as different composition of principal microbial taxa and their metabolites, prior and after food sample fermentation. Several ecological indicators were

then analyzed, as the prebiotic index, the bifidogenic effect, the eubiosis/dysbiosis state expressed as *Firmicutes*/*Bacteroidetes* ratio. In addition, a mouse model of dextran sodium sulphate (DSS)-induced chronic colitis has been used to confirm *in vivo* the anti-inflammatory effect of the administration of *Lact. plantarum* combined with the biologically debittered olive pomace. Clinical features and large bowel macroscopic, histologic, and immunohistochemical findings were evaluated.

Results.

Results from colonic fermentations showed the ability of FDP to finely modulate gut microbiota with a potential prebiotic, eubiotic and bifidogenic activity. In particular, FDP sample, which serves as a carrier for probiotic, was also able to bring live probiotics, that stand active also after colonic fermentation. FDP was also able to positively modulate the colon microbiota, acting as a prebiotic for beneficial commensal bacteria (e.g. *Bifidobacteriaceae, Facelibactrium prausnitzii*) and limiting the growth of opportunistic bacteria (e. g. *Enterobacteriaceae*). Metabolomic analysis revealed that FD and FDP samples enriched with beneficial *Lact. plantarum* strains stimulates the production of healthy bioactive molecules (short- and medium-chain fatty acids) and attenuates detrimental compounds, related to proteolytic fermentations, such as indole, skatole and p-cresol. Moreover, the simultaneous oral administration of a diet enriched with fermented olive patè and *Lact. plantarum* significantly improve the macroscopic and microscopic colitis scores with a significant reduction of inflammatory and pro-fibrotic cytokines (IL-1b, IL-6, and TNF-a) in the mouse model of DSS-induced chronic colitis.

Conclusions.

Lab-scale fermentations with a multifunctional starter mix containing beneficial *Lact. plantarum* strains clearly suggested destoned olive pomace as a good matrix for deliver probiotics as well as a good source of high value molecules. Results from colonic fermentations showed the ability of FDP to finely modulate gut microbiota with a potential prebiotic, eubiotic and bifidogenic activity. The concomitant oral administration of a diet enriched with biologically debittered olive cream and a specific *Lact. plantarum* strain can exert synergistic anti- inflammatory and antifibrotic action in DSS-induced chronic colitis in mice. Overall, our results highlight the beneficial contribution of a new generation of fermented olive patè and the application of *Lact. plantarum* species as a promising tool to enhance the functionality of olive- based fermented foods in ameliorating intestinal inflammation and ultimately impact the human gut microbiota, that need to be further investigated for the development of new olive- based functional foods.

Characterization of Lactic Acid Bacteria Isolated from Spontaneous Fermentation Brines of Green Olives for Their Potential Use as Starter Cultures

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Background.

Table olive fermentation is a traditional practice in various regions of Argentina, especially in northwestern provinces (NOA region) of Argentina. This process usually occurs spontaneously, driven by the native microbiota present on the fruits and in the medium.
Lactic acid bacteria (LAB) play a key role in fermentation, producing lactic acid and other antimicrobial compounds that preserve the product, reduce pH, and improve its safety and stability. The use of selected starter cultures with appropriate technological properties can improve the quality of the final product, control the fermentation process, and reduce the presence of undesirable microorganisms. For this purpose, it is necessary to isolate and study native strains capable of tolerating the extreme conditions of the fermentation environment: high salinity, low pH, and the presence of phenolic compounds. The aim of this study was to isolate LAB from spontaneous fermentation brines of green olives from the NOA region, to characterize them phenotypically and to evaluate their relevant technological properties, in order to select promising strains for potential use as starter cultures.

Methods.

Brine samples from different stages of spontaneous fermentations of green Arauco and also Manzanilla and Aloreña olives were collected. Serial dilutions were plated on modified MRS agar supplemented with 4% NaCl and cycloheximide (0.1 mg/L). Plates were incubated at 30 °C for 48–72 h under anaerobic conditions. Colonies with typical LAB morphology were selected. Each isolate was subjected to Gram staining, the catalase test and determination of its ability to ferment glucose and gluconate with CO2 production. A total of 256 isolates were recovered (Gram-positive and catalase-negative, compatible with LAB). First pre - selection of LAB was performed applying a 2^{3-1} fractional factorial statistical design with repetition of a central point, considering the effect of NaCl concentration (6, 7, 8 %), temperature (15, 25, 35 ºC) and initial culture pH (6, 7, 8) on the growth of each strain. These treatments were six experimental runs for each strain (6 x 256), four factorial points and two central points.
Growth of each isolate was monitored in modified MRS broth (1% glucose), supplemented with 0,004% (w/v) bromocresol green for 7 days, every 24 h, and colour changes of the medium were compared with a previously standardized pH scale. The experimental results were analysed using appropriate statistical programs. Relevant technological properties were evaluated in pre-selected LAB: tolerance to pH (3.5, 4.5, 6.5, 7.5, 8.5) and osmotic stress (NaCl: 0, 4, 6, 8, 10, 12%), growth under lower glucose condition (0.5 % and 1 % w/v glucose), enzymatic activities, compatibility study, antimicrobial activity. The results were analysed and the selected strains were identified by biochemical and molecular characterization. The results were analysed using multifactorial ANOVA and Tukey's test ($p < 0.05$), to identify statistically significant differences in growth.

Results.

A total of 256 strains (86 % bacilli and 14% cocci) were isolated from different brines olives. These isolated strains were taxonomically identified as LAB by phenotypic tests (positive Gram stain, morphology, non-motility, absence of catalase activity, ability or not to ferment glucose and gluconate with CO2 production). 98% of the isolates were homofermentatives, of which 25% were strictly homofermentatives. The application of statistical design allowed us to evaluate the behavior of 256 strains. It was observed that their growth differed depending on the treatment considered: the limiting parameters were mainly pHi and, to a lesser extent, temperature and NaCl concentration. Data analysis allowed us to: i) determine the optimal and critical conditions for the development of the 256 isolates; ii) discriminate the degree of importance of the limiting growth parameters; iii) preselect 13 strains (all lactobacilli) belonging to different fermentation stages. Growth under different stress conditions showed a wide variability among pre-selected isolates. Most strains grew well under moderate stress conditions (pH 4.5–6.5, NaCl 4–8%). However, as salinity increased and pH decreased, growth was significantly reduced in many strains. The 13 isolates were compatible with each other; none of them produced inhibitory activity against *Listeria* and sensitive LAB. The pre-selected isolates grew more slowly in media with lower glucose concentration. This selection narrowed the group to 6 strains, which maintained acceptable growth under various extreme conditions, especially at low pH and high salt levels. Enzyme profiling using API Zym allowed quantitative differentiation of the six strains. All strains exhibited low esterase and lipase activity (<2), except for one lactobacillus; they had good peptidase activity and low proteinase activity. A large variation was observed among glycosylated substrates depending on the strain tested. Three strains presented maximum beta-glucosidase values, confirmed by arbutin test. The six strains of lactobacilli were identified as four *Lactiplantibacillus (Lact.) pentosus*, one strain as *Lact. plantarum* and one strain as *Lacticaseibacillus paracasei* subsp *paracasei*. These strains showed the best adaptation to the fermentation environment.

Conclusions.

Lactic acid bacteria isolated from spontaneous fermentation brines of green olives from NOA region, Argentina, exhibited significant phenotypic diversity and variable tolerance to environmental stress. Through a stepwise selection process —from 256 initial isolates to 13 compatible strains, homofermentative candidates, and finally 6 highly tolerant strains— this study identified native LAB with strong potential for use as starter cultures.
These selected strains demonstrated robust growth under combinations of low pH, and high salinity, conditions typical of olive fermentation. Their application could help standardize the process, improve product safety and sensory quality, and preserve the regional identity of Argentine fermented olives.

Development and validation of Fast Blue BB Assay for the time-/cost-effective determination of phenolic compounds in extra virgin olive oil

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Background.
Phenolic compounds contribute critically to define the nutritional value, sensory characteristics, and oxidative stability of extra virgin olive oil (EVOO). According to Commission Regulation (EU) No. 432/2012, EVOO may carry the EFSA-approved health claim: "Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress," provided it contains at least 5 mg of hydroxytyrosol and its derivatives (such as the oleuropein complex and tyrosol) per 20 g of oil (equivalent to ≥250 mg/kg of polyphenols). However, the recommended analytical methods both time-consuming and costly, requiring liquid-liquid extraction and HPLC-UV analysis of the polar fraction. Therefore, this health claim remains significantly underexploited for differentiating EVOO within the market. The aim of this research is to develop a rapid and cheap method to determine phenolic compounds in EVOO, for categorizing oil samples according to the parameters of the EFSA claim.

Methods.
The Fast Blue BB (FBBB) spectrophotometric method, recently described by us to determine total phenolic compounds in unfractionated EVOO samples (10.1016/j.foodchem.2021.130990; 10.3390/molecules28073108), was optimized and inter-lab validated. The method involves the spectrophotometric (absorbance 420 nm) determination of azo derivatives resulting from coupling of EVOO phenolic compounds with FBBB diazonium salt in alkali pH. For the coupling reaction, unfractionated EVOO samples (1.0 g) is mixed with 1.5 mL of 0.1% w/v FBBB in ethanol and 1.5 mL of 1.25 N NaOH for 15 min, skipping the extraction of polar compounds.

Results.

Linearity was validated with a series of samples resulting from blending sunflower seed oil with an EVOO sample having a content of phenolic compounds previously determined by HPLC-UV with the method recommended by International Olive Council (IOC). Inter- laboratories analyses of phenolic compounds in dozens EVOO samples demonstrated reproducibility (CV<5%), striking correlation of FBBB assay with HPLC-determinations ($R^2 > 0.95$), and limit of detection < 50 mg/kgoil. FBBB is highly specific for phenolic compounds, and it is marginally affected by interfering compounds such as tocopherols. The FBBB assay is simple, rapid, cheap, repeatable, and robust. The use of on-purpose designed cuvettes suitable for centrifugation further speeds up, simplifies the protocol and is suited to develop commercial analytical kits. Results can be assessed using a printed colorimetric scale, reducing the need for spectrophotometric analysis to only those EVOO samples with phenolic content near the threshold of 250 mg/kg.

Conclusions.

The FBBB assay is suited for the routine classification of EVOO according to the EFSA health claim related to polyphenols. It might be extended to the determination of phenolic compounds in other olive-based matrices. The FBBB assay does not require specific skills, and it can be executed on-site (e.g. at the olive mill) to categorize the EVOO at the time of bottling.

Posters

Bioprotection of Nocellara del Belice table olives: effects of *Candida boidinii* LC1 and *Candida norvegica* OC10 under different thermal storage conditions

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Background.

The Castelvetrano method for processing Nocellara del Belice table olives is a well- established Sicilian agrifood tradition, celebrated for producing olives with a distinctive sweet taste and bright green colour. This method has achieved notable commercial success at both national and international level. However, it faces challenges, particularly during warmer months, when the shelf life of the product is compromised due to the growth of spoilage and pathogenic microorganisms. To mitigate microbial spoilage and extend shelf life, producers often rely on refrigerated storage. Although partially effective, refrigeration significantly increases energy consumption and operational costs. To address these limitations and reduce the energy costs associated with cold storage, this study investigated the bioprotective potential of two yeast strains, *Candida boidinii* LC1 and *Candida norvegica* OC10, previously selected for their technological and antimicrobial properties (10.1016/j.fm.2024.104477).

Methods.

Experimental batches of olives were processed using the traditional Castelvetrano method and inoculated with the selected yeast strains. These batches were then stored under three thermal regimes: constant refrigeration (8±1 °C); ambient temperature; and a combined regime: 90 days at ambient temperature followed by 90 days at 8±1 °C. Over a 180-day period, various aspects were monitored, including: dynamics of microbial groups such as lactic acid bacteria, yeasts, *Enterobacteriaceae*, *Pseudomonadaceae*, and *Staphylococcaceae*; kinetics of physicochemical parameters such as pH, salinity, temperature, pulp firmness, fruit colour; and finally sensory attributes evaluated by a trained panel using a structured profile method in accordance with ISO standards.
Additionally, the presence of mycotoxigenic fungi was assessed to ensure food safety.

Results.

Yeast inoculation had a significant impact on controlling spoilage and pathogenic microorganisms, particularly under refrigerated and combined storage conditions. In these treatments, microbial populations such as *Enterobacteriaceae* and *Staphylococcaceae* were considerably reduced compared to uninoculated controls, and no Clostridia were detected.
Conversely, olives stored solely at ambient temperature exhibited increased levels of pathogens, including *Salmonella spp.* and *Escherichia coli*, especially toward the end of the storage period. The inoculated batches also exhibited more stable pH values, better retention of green colour, and improved pulp firmness. Sensory evaluation revealed enhanced organoleptic qualities in inoculated batches, with *C. norvegica* OC10 outperforming in terms of colour intensity, texture, and overall appreciation. Importantly, no significant presence of mycotoxigenic fungi was detected, confirming the microbiological safety of the batches inoculated with the selected strains.

Conclusions.

This study confirms the effectiveness of bioprotective yeasts, particularly *C. norvegica* OC10, in enhancing both the microbiological safety and sensory quality of Castelvetrano-style olives. Notably, combining yeast inoculation with a reduced refrigeration period (from 180 to 90 days) resulted in a 50% energy cost reduction without compromising product quality. This approach offers a sustainable and cost-effective alternative for the olive processing industry, while also supporting the preservation and enhancement of traditional food products.

Further research is recommended to explore the application of these yeasts in other processing styles and under extended ambient storage, with the aim of developing safe and functional table olives with a reduced environmental footprint.

Bioprotective co-inoculation strategies for optimising the fermentation of Nocellara del Belice table olive

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Background.

Green split table olives from the Nocellara del Belice cultivar are a traditional Sicilian product, typically undergo spontaneous fermentation driven by indigenous microbiota. However, this natural process is microbiologically uncontrolled and often leads to inconsistent product quality, including pulp softening, browning, and the development of off-flavours. These factors compromise both safety and marketability. These issues are particularly pronounced in small-scale artisanal productions, where standardisation is often lacking. Recent studies have shown that using selected microbial starters, particularly lactic acid bacteria (LAB) and yeasts, can improve fermentation by enhancing microbial control, sensory attributes, and overall product stability (10.1016/j.fm.2020.103497). Co-inoculation strategies involving LAB and yeasts have demonstrated potential in modulating fermentation dynamics and inhibiting spoilage organisms. Employing defined starter cultures also promotes reproducibility and adherence to food safety standards.

Methods.

To optimise the fermentation process for Nocellara del Belice green split olives, three experimental treatments were tested under controlled conditions. The first treatment (SO1) used the commercial LAB strain *Lactiplantibacillus pentosus* OM13 as a reference control.
The second (SO2) and third (SO3) treatments involved co-inoculation of *Lact. pentosus* OM13 with *Candida boidinii* LC1 and *Candida norvegica* OC10, respectively. Fermentation was carried out over 90 days in 8% NaCl brine. Several parameters such as pH, salinity, and microbial population dynamics were monitored throughout the transformation process.
Randomly amplified polymorphic DNA (RAPD-PCR) analysis was used to assess the dominance of inoculated strains. At the end of fermentation, olives were evaluated for colour (CIELab), pulp hardness, and sensory attributes, with a trained sensory panel of 16 tasters, following International Olive Council protocols.

Results.

All inoculated strains successfully dominated the fermentation, with over 80% prevalence. In the co-inoculated treatments (SO2 and SO3), acidification occurred more gradually, reaching pH < 4.5 after 75 days, compared to 21 days in the control. Despite the slower acidification, co-inoculation effectively suppressed spoilage microorganisms, including *Enterobacteriaceae*, coliforms and pseudomonads. Pulp hardness was significantly higher in SO2 and SO3, likely due to the absence of polysaccharolytic activity of the yeast strains. Colour differences were minimal, though SO3 exhibited a more desirable yellow hue. Sensory evaluation confirmed these findings, with SO3 receiving the highest scores for flavour, texture, and overall appreciation. No off-flavours or defects were detected in any treatment, confirming the effectiveness of the selected strains in guided fermentation.

Conclusions.

The co-inoculation of *Lact. pentosus* OM13 with selected yeast strains (*C. boidinii* LC1 and *C. norvegica* OC10) presents a promising strategy to enhance the quality and safety of Nocellara del Belice green split table olives. This approach

improves microbial control, preserves texture, and enhances sensory quality. Although acidification was slower in co-inoculated trials, the bioprotective effects of the yeasts effectively limited the growth of undesirable microorganisms. These findings support the use of LAB–yeast consortia in standardised fermentation protocols, offering small-scale producers a practical and scalable solution for achieving consistent, high-quality, naturally fermented table olives.

Integrative Microbiome Engineering and Nanobiotechnology Approaches for Precision-Controlled Fermentation of Table Olives

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Background.

Worldwide, table olive has economical importance, rich in nutrients and provide health benefits. According to International Olive Oil Council (IOC), production rate of table olive in the 2020-2021 year was 2,661,000 tons whichwas 10.1% less yield than previous year (https://www.internationaloliveoil.org/what-we-do/chemistry-standardisation-unit/standards-and- methods/). Olive fruit is produced from olive tree *Olea europaea* L., its bitter taste causes it unsuitable for consumption. Bitterness is due to the presence of oleuropein, a bitter compound, high oil content of range12-30% and less sugar content of range 2.6-6% depending on varieties (https://www.internationaloliveoil.org/olive-world/table-olives/). Oldest biotechnological application such as fermentation in food processing is carried out to remove this bitterness. However, lack of control over processes cause spoilage microorganism and organoleptic problems during these old processes (10.3390/applmicrobiol5020052). Recent advances in microbiome related research enables to identify difference between beneficial and spoilage causing microorganisms. This approach helps us to use nanotechnology as biosensor and also help to deliver natural antimicrobials (10.1016/j.copbio.2016.11.012). Despites these advances, nanotechnology in olive industry is still unexplored. This study aimed to incorporate nano encapsulated microbial modulator (which promote slow release of probiotics, also act as enzyme inhibitor) and nano biosensor for real time monitoring of metabolites production and microbial activity.

Methods.

Microbiome Characterization

Fresh green olives were subjected to spontaneous and inoculated fermentations. Microbial communities were identified by using 16S rRNA and ITS (Internal Transcribed Spacer) metagenomic sequencing to identify dominant bacterial and yeast species during fermentation stages.

Development of Nano-enabled Delivery System

Chitosan nanoparticles were used to encapsulate nisin at optimized concentrations such as 250- 500 IU/mL using ionic gelation. Sodium Tripolyphosphate were added in drops to initiate the nanoparticle formation by placing it on ultrasonication for better particle size. It was gradually release in 10 to 20 days of fermentation. Its target microorganisms are *Clostridium spp*., *Listeria spp*., and spoilage LAB. Encapsulation efficiency, particle size and release kinetics were evaluated by using UV-visible spectroscopy.

Application and Monitoring

Nano-formulations were added in brine solution at the start and mid-point of fermentation. Monitoring of fermentation process was carried out using pH, titratable acidity, microbial plate count and volatile compound using GC-MS. Meanwhile a nano-biosensor prototype was also developed using gold nanoparticle functionalized using oligonucleotide probes was tested for real time detection of spoilage biomarkers such as biogenic amines.

Results.

Metagenomic analysis indicates the presence of *Lactiplantibacillus plantarum, Leuconostoc mesenteroides* and *Candida boidinii* as well as *Clostridium spp*. and *Pichia membranifaciens.* Encapsulation efficiency of chitosan nanoparticles recorded was >80% and its sustained release in 72 hours under fermentation conditions. Inhibitory activity of nano-encapsulated nisin was observed that inhibit the spoilage microorganisms without affecting the viability of lactic acid bacteria, significantly reduce pH variability and improve acidification ($p < 0.05$). The biosensor prototype detected cadaverine and putrescine levels successfully at non-molar concentration, enable the early identification of faults in fermentation. Sensory evaluation indicates reduced bitterness and enhances texture and aroma in treated groups compared to control.

Conclusion.

This research study showed promising strategy of controlled olive fermentation and food grade nanotechnology. This approach enhances product quality and safety by using selectively controlling microbial activity as well as real time monitoring of fermentation. Results highlighted that biosensor and nanocarriers can be used in traditional olive fermentation. Promising results are not only limited to revolutionize traditional fermentation practices but it will also set innovative standards in olive as well as in fermented food industry that contribute to global efforts of sustainable health-oriented product development.

Controlled fermentation of Kalamata olives using multifunctional yeast starters: the role of yeasts in enhancing process control and product stability

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Background.

Table olives are among the oldest fermented fruits in the Mediterranean region; however, their fermentation remains an empirical process that can yield inconsistent results. As consumer demand for functional foods grows, controlled fermentation using selected microbial cultures with both technological and probiotic potential is gaining importance. This study evaluated the controlled fermentation of natural black olives (cv. Kalamata) using selected yeast strains—four *Candida boidinii* and one *Saccharomyces cerevisiae*—previously isolated from spontaneous olive fermentations and selected for their multifunctional properties.

Methods.

Kalamata olives were processed via the traditional Greek-style anaerobic method in 8 L vessels containing 7.0% NaCl brine acidified with 0.5% vinegar. On the first day, olives were inoculated individually with four *Candida boidinii* strains (Y27, Y28, Y30, Y31) and one *Saccharomyces cerevisiae* strain (Y34) to reach a final concentration of 3×10^6 CFU/mL. All fermentations were conducted in duplicate at room temperature for 150 days.

Microbiological analyses were performed to determine the population of LAB, yeasts, and Enterobacteriaceae in the brines throughout fermentation and in the olives (at the end of fermentation). Yeast survival was evaluated in the brines at three different time points (days 0, 75, and 150), and in the olives at the end of fermentation using rep-PCR fingerprinting. Physicochemical analyses included pH, titratable acidity, and salt concentration. Multivariate statistical methods (HCA and PCA) were applied to identify relationships and correlations among variables during fermentation.

Results.

Inoculation with selected yeasts improved fermentation performance. *Enterobacteriaceae* were eliminated more rapidly (within 14–16 days) in inoculated fermentations compared to 20 days in the control. Yeasts coexisted with LAB, though LAB remained dominant except in fermentations with *C. boidinii* Y28 and Y30. Rapid acidification occurred in all inoculated treatments, with *C. boidinii* Y27 and Y31 achieving the lowest final pH (3.77), while the control and *S. cerevisiae* Y34 exceeded the safety threshold (pH > 4.3). Titratable acidity mirrored this trend, with Y27 showing the highest acid levels (0.815 g/100 mL), enhancing product safety. Yeast survival varied considerably. While all strains established well initially, only *C. boidinii* Y27 maintained high recovery rates by day 150 (50% in brine, 45% in olives). Other strains showed minimal survival, especially *S. cerevisiae* Y34, which was nearly undetectable at the end. Multivariate analyses grouped fermentations into two clusters: one (Y27, Y28, Y31) associated with superior acidification and microbial profiles, and another (control, Y30, Y34) with less favourable outcomes.

Conclusions.

Controlled fermentation using multifunctional yeast strains can enhance the safety and consistency of Kalamata olive fermentation. *C. boidinii* Y27 stood out for promoting rapid Enterobacteriaceae decline, stable acidification, and long-term survival, suggesting its suitability as a starter culture. While other strains also improved fermentation dynamics, they require process optimization to ensure microbiological stability. Overall, this work highlights the potential of targeted yeast starters to modernize traditional olive fermentations and support functional food development.

The impact of different starter cultures on the microbiome and volatilome profile of cv. Kalamata natural black olives during fermentation

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Background.

Table olives are among the most important fermented foods. Microbial ecology and subsequent production of volatiles are important for obtaining high-quality products. The purpose of this study was to investigate the fermentation of Kalamata black olives using different starter cultures to elucidate their impact on the microbiota and mycobiota and on the volatile profile during fermentation.

Methods.

Three fermentations were performed in 7% (w/v) brine namely, (a) spontaneous fermentation (control), and inoculated fermentations with (b) *Lactiplantibacillus pentosus* B281, a probiotic strain isolated from table olives, and (c) a commercial starter culture containing *Lactiplantibacillus plantarum*. At the beginning (day 1), middle (day 76), and end (day 145) of fermentation, olives were analysed using metataxonomics to identify the microbiota and mycobiota.

Results.

In total, 107 volatile compounds were identified, including acids, alcohols, esters, carbonyls, hydrocarbons, phenols, terpenoids and miscellaneous compounds. Differences in the volatile profiles were observed due to the different starter cultures used. Olives fermented with *Lact. pentosus* B281 presented a richer volatile profile at day 146 than the other two fermentations. Regarding the microbiota analysed, the number of genera identified in the spontaneous fermentation was higher, compared to inoculated fermentations. This was also observed in the mycobiota at the beginning and middle of fermentation. However, on Day 146, olives fermented with *Lact. pentosus* B281 were characterized by a richer mycobiota profile, similar to the volatile analysis, indicating the impact of starter cultures in shaping both the microbial ecology and e volatile profile.

Conclusions.
This work constitutes one of the first comprehensive evaluations of microbial and volatilome interactions in Kalamata natural black olives using advanced multi-omics tools. The findings underscore the potential of targeted microbial interventions in improving the quality and consistency of natural black table olives, contributing valuable knowledge to the field of fermented vegetable microbiology.

Acknowledgements.
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Co-Inoculation of *Lachancea thermotolerans* and *Lactiplantibacillus plantarum* enhances the Fermentation and Quality of Spanish-Style Table Olives

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Background.

Traditionally, the fermentation of Spanish-style table olives has been primarily driven by lactic acid bacteria (LAB), which contribute to the development of acidity, preservation, and characteristic sensory profiles. In recent years, there has been increasing interest in the use of other microorganisms, particularly yeasts, to enhance fermentation outcomes. *Lachancea thermotolerans*, a yeast species frequently isolated from wine fermentations, exhibits metabolic and functional properties that may be beneficial for table olive fermentation. The objective of this study was to evaluate the impact of co-inoculating *L. thermotolerans* and *Lactiplantibacillus plantarum* on the fermentation dynamics and quality parameters of Spanish- style table olives.

Methods.

Fermentations were conducted using Spanish-style green olives, inoculated at the start of fermentation with pure cultures of *L. thermotolerans* and *Lact. plantarum*. In addition to the co- inoculated treatment, single inoculations of each microorganism were also performed. A spontaneous fermentation (uninoculated Control) was included for comparison. All fermentations were carried out at 20 ± 2 °C for 120 days.

Results.

The presence of *L. thermotolerans* appeared to stimulate the growth of lactic acid bacteria during the initial 20 days of fermentation. Co-inoculation with *L. thermotolerans* and *Lact. plantarum* resulted in the highest concentrations of organic acids, which consequently led to a more pronounced decrease in pH compared to the control. Furthermore, the combined inoculation improved several key quality attributes of the final product, particularly enhancing olive texture and colour, as compared to the olives from the uninoculated fermentation.

Conclusions.

Mixed starter cultures of *L. thermotolerans* and *Lact. plantarum* can positively influence the fermentation dynamics and final quality of Spanish-style table olives. The use of such microbial consortia represents a viable strategy for producing table olives with distinctive sensory and technological characteristics.

Microbial and Physico-chemical Dynamics During the Fermentation of Conservolea and Kalamàta table olives

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Background.

Table olives, a traditional plant-based fermented product, are a key component of the Mediterranean diet and have been widely consumed for centuries (10.1002/jsfa.8443). Renowned varieties such as Conservolea, and Kalamàta are particularly valued for their culinary and nutritional properties (10.3390/microorganisms8050672; 10.3390/foods12071527). However, in their raw form, olives are bitter due to the presence of oleuropein, a phenolic compound (10.1002/jsfa.8443; 10.3390/foods12071527). Fermentation plays a crucial role in mitigating this bitterness. Greek-style fermentation involves immersing olives in a 6-10% NaCl brine for 8-12 months, promoting natural fermentation by Yeasts and Lactic Acid Bacteria (LAB) (10.3390/microorganisms8081241). The microbiota plays a crucial role in shaping the quality of table olives, prompting significant research efforts to identify microbial species and understand their dynamics. This research specifically focuses on the fermentation of Greek table olives, a process that remains largely craft-based and empirical, by monitoring the physicochemical properties and the microbial growth throughout the fermentation (10.3389/fmicb.2012.00248).

Methods.

Microbiological analysis was conducted alongside physicochemical parameter monitoring, ensuring a thorough assessment of fermentation conditions. Conservolea and Kalamàta varieties of black olives were used, and the fermentation was carried out with their autochthonous microbiota. Throughout the fermentation, brine salinity, pH, and titratable acidity were measured in olives and brine samples to monitor fermentation progress. The brine salinity was maintained at 6% (v/v), and the fermentation process was completed (based on pH levels) in 4 months for Conservolea olives and 5 months for Kalamàta olives at ambient temperature. Biological triplicates were used to ensure experimental reliability.
Additionally, microbial populations were tracked using enumeration via the plate count method, targeting the Total Viable Counts (TVC), LAB, yeasts and moulds, *Enterobacteriaceae*, *Staphylococcus aureus* and *Pseudomonas* spp.

Results.

In terms of physicochemical measurements, brine temperatures followed seasonal fluctuations, ranging between 12°C and 21°C. A significant decline in pH was observed in the brine of both Conservolea and Kalamàta olive varieties within the first 5 to 15 days of fermentation. In contrast, the pH decrease in the olives was more gradual and less pronounced during the initial 10 days. By the end of fermentation, all samples had pH values below 4.3. Conservolea showed greater acidification, reaching pH 3.5—about one unit lower than Kalamàta. Titratable acidity in the olives of both varieties remained lower and more stable than in the brine, which exceeded 1.0% lactic acid in Conservolea and reached about 0.6% in Kalamàta.
Regarding the microbiological analysis, the initial microbiota primarily consisted of *Enterobacteriaceae*, LAB, yeasts and moulds. *Enterobacteriaceae* were detected in the samples during the first 15 days of fermentation but became undetectable shortly thereafter. Additionally, *S. aureus* was present in Conservolea olives between days 4 and 10 of fermentation and also detected in olive samples on day 4. *Pseudomonas* spp. were identified in both the olives and

brines of Conservolea samples on day 13 of fermentation.
LAB became the dominant microbial group in Conservolea olives and brine from day 5 until the end of fermentation (day 115). In Kalamàta olives, LAB dominated from the beginning until day 30. Hereafter, LAB remained active alongside yeasts and moulds in Kalamàta brine, contributing to fermentation up to day 150 (end of fermentation).

Conclusions.

Early microbial communities included *Enterobacteriaceae*, *S. aureus*, and *Pseudomonas* which disappeared after the initial days, indicating a safe fermentation process. LAB dominated throughout Conservolea fermentation, while in Kalamàta, LAB prevailed early but were later accompanied by yeasts and moulds. Overall, Conservolea supported a more stable and efficient LAB-driven fermentation.
This study will be extended by analyzing phenolic compounds and triterpenoids throughout fermentation, and by performing both targeted and untargeted metabolomics to identify key metabolites associated with the sensory qualities and safety of table olives.

OliveFMBN: an extensively annotated database of the microbiota of table olives.

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Background.

Table olives are among the most ancient and important fermented foods of the Mediterranean basin. Their production is still strongly related to traditional practices, and the lack of thermal treatments, the reliance on natural contamination and selective factors determine the dynamics of the microbial community. The microbiota of table olives has been extensively reviewed (10.3390/foods12203783) but there is a need for a well annotated repository of existing data which might be useful for both meta-studies and for the design of microbiome-based starters.

Methods.

We have used metataxonomic data extracted from the FoodMicrobionet database, together with those obtained from the METAOlive project to provide quantitative insights on the structure of bacterial and fungal microbial communities of table olives and to identify core genera in different trade preparations.

Results.

Metataxonomic data and metadata are publicly available on GitHub (https://github.com/ep142/Metaolive). Version 0.3.2 of the repository includes data for 18 studies, with 533 samples for bacteria and 453 samples for fungi. Version 0.3.3, in preparation, will include 350 more samples from the METAOlive project. Given the variety of the data in terms of type of samples, trade preparations, olive varieties, this is by far the largest and best annotated public resource on the microbiology of table olives.

Conclusions.

OliveFMBN can be easily used by researchers with minimum proficiency in R (it is available as phyloseq objects which can be processed with interactive apps available on the web, like MicrobiomeAnalyst or ShinyPhyloseq. Its potential uses include support for the writing of metastudies and for the design of new studies, inference of microbial association networks (see other posters in this workshop), inferences on core and accessory microbiota of different types of olives. Given the size of the dataset, it can also be used in training machine learning algorithms for evaluating typicality or specificity of the microbiota.

Is WL (Wallerstein Laboratory) Nutrient Agar of any use in yeast colony differentiation from table olives?

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Background.

Given the importance of yeasts in table olives quality and spoilage, their isolation and characterization are of interest for both academia and industries. Media used for counting yeasts are not typically differential and colony morphologies are quite similar. On the other hand, WL (Wallerstein Laboratory) nutrient agar (WLna), has been specifically developed for the differentiation of yeasts in winemaking and brewing (10.1002/j.2050- 0416.1971.tb03413.x) but, to our knowledge, its value in differentiating colony morphologies of yeasts typically isolated from olives and olive environments has never been tested systematically.

Methods.

A collection of 261 isolates from olives, olive mill wastewater, olive oil and wine or must was used. For 199 of these, identification by sequencing of the ITS1-5.8S-ITS2 region and by MALDI-ToF spectrometry was available. They included a variety of species (17) which included members of the genera *Pichia, Candida, Lachancea, Nakazawaea, Saccharomyces, Rhodotorula,* and *Wickerhamomyces* which are commonly isolated from olives and their environments. The isolates were streaked on WLna and colony and cell morphology was evaluated after 5 d of incubation at 25°C. A standardised set of binary characters regarding colony shape, elevation, surface and colour and cell morphology was used.

Results.

Exploratory data analysis (hierarchical cluster analysis and MDS of the Jaccard distance matrix, Kohonen Self Organizing Maps) were used to obtain a preliminary grouping. For some species (*S. cerevisiae*, *N. molendiolei, L. fermentati)* relatively homogeneous clusters were found with all methods. *P. membranifaciens* and *P. manshurica* had a remarkably similar colony morphology.

Conclusions.

Colony morphology on WLna is only partially useful for the isolation of yeasts from table olives. This is not surprising since this medium was especially developed for other ecosystems. Further work with supervised machine learning approaches is in progress.

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This work was funded by the project METAOLIVE: meta-omic approaches for traditional table olive fermentation, funded by the European Union - NextGenerationEU, NRRP Missione 4 "Istruzione e Ricerca" - Componente C2, Investimento 1.1, "Fondo per il Programma Nazionale di Ricerca e Progetti di Rilevante Interesse Nazionale (PRIN)" (2022NN28ZZ).

Culturable yeasts in table olives: the METAolive collection

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Background.
Yeasts, together with lactic acid bacteria and halophilic microorganisms, are key components of the microbiota of table olives (https://doi.org/10.3389/fmicb.2021.797295). They are able to stimulate LAB growth and contribute positively to the sensory properties of the product, but are also involved in spoilage, during the fermentation and storage. Within the framework of the METAolive project we isolated yeasts from 250 table olive samples belonging to 33 varieties, obtained from 23 firms in Italy, Cyprus, Spain and Greece and carried out preliminary identification and partial phenotypic characterization of selected isolates.

Methods.
Colonies were isolated and purified by streaking on GYEA and then on WL differential medium. Strains were maintained on slants and frozen. Identification was carried out using MALDI-ToF Biotyper Sirius (Bruker) "Extended Direct Transfer" SOP. For representative strains it was confirmed by partial sequencing of the ITS1- 5.8S – ITS2 region of the rRNA operon and evaluation of enzymatic activities using API Zym galleries was carried out.

Results.
MALDI-ToF Biotyper allowed the identification, confirmed by ITS sequencing, of 80% of isolates. The most frequent species matched those found by metataxonomic analysis (*Pichia membranifaciens, P. manshurica, P. kudriazevii, Candida boidinii, Saccharomyces cerevisiae, Wickerhamomyces anomalus*). Identification of *Nakazawea*, *Metschnikowia* and some members of the genus *Candida* systematically failed, possibly because of limitations of the database. The most frequent enzymatic activities were phosphatases and esterases and some arylamidases. No correlation between species assignment and zymogram was found.

Conclusions.
We confirmed that a group of yeast species are the most frequent members of culturable table olive microbiota. MALDI-Tof biotyping was effective for most of the important species, but not all, and is a promising tool for culturomics approaches. API ZYM was of little usefulness in the characterization of isolates.

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Modelling the effect of NaCl on the growth of selected *Lactiplantibacillus pentosus* strains.

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Background.

Lactiplantibacillus pentosus is one of the most frequently isolated lactic acid bacterium from table olives fermentations (https://doi.org/10.3390/foods9070948) and is frequently used as a starter for this and other vegetable fermentations because of its tolerance of salt and phenols and its ability to ferment a large variety of sugars. We selected 29 strains from a large collection of isolates obtained from >370 samples of table olives from several European countries analyzed during the METAOlive project and sequenced their genome. In this study we are reporting on the modelling of the effect of NaCl concentration on their growth in MRS broth.

Methods.

Stationary phase cultures of the strains were used to inoculate MRS with 0-9% NaCl in microtiter plates. Growth curve data were collected in anaerobiosis at 25°C using a Varioskan multiplate reader with SkanIt software. A R script was optimized for batch graphical and statistical analysis of the results using variations of the Baranyi and Roberts D-model. A simple secondary model was then used to model the effect of NaCl on maximum specific growth rate (μ_{max}).

Results.

The best fitting primary models (selected automatically on the basis of lowest AIC) were the complete D-model or variations of the biphasic model (without lag phase or without stationary phase). The maximum specific growth rate under optimal conditions (usually 0 % NaCl) varied significantly among strains (0.6-1.2 h^{-1}). Most strains were still able to show significant growth at 6% NaCl and some did grow at 9% NaCl, while a few strains were unable to grow at this concentration.

Conclusions.

We developed a rapid screening method for modelling the growth of *Lpl. pentosus* in microtiter plates. Further work to model the growth in synthetic brines and model green olive brines. As genomic data become available we plan to correlate the results with genes related to osmotic/salt stress response.

Acknowledgements
This work was funded by the project METAOLIVE: meta-omic approaches for traditional table olive fermentation, funded by the European Union - NextGenerationEU, NRRP Missione 4 “Istruzione e Ricerca” - Componente C2, Investimento 1.1, “Fondo per il Programma Nazionale di Ricerca e Progetti di Rilevante Interesse Nazionale (PRIN)” (2022NN28ZZ).

Impact of partial substitution of NaCl with KCl on the microbial dynamics and physicochemical attributes of Kalamata natural black olives fermented at semi-industrial scale

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Background.

Salt plays a critical role in the fermentation of table olives by diffusing into the olive tissue reducing water activity and increasing osmotic pressure. This environment enhances the microbiological stability by suppressing the growth of pathogenic and spoilage microorganisms while contributing to the development of the characteristic sensory profile of the final product. However, the high salt content commonly used in traditional fermentation practices has raised health concerns, particularly due to associations with hypertension and strokes. Consequently, research efforts have focused on the partial substitution of sodium chloride with other chloride salts as an alternative strategy to reduce the overall sodium content, without compromising the safety and quality of the final product. To this end, the purpose of this study was to investigate the impact of 50% substitution of NaCl with KCl on the microbiological and physicochemical profile of Kalamata natural black olives fermented at semi-industral scale.

Methods.

Fermentations were carried out at the Cooperative of Sterna (Messinia, Greece), using 220 L plastic vessels, containing 130 kg of Kalamata olives and 90 L of brine. Two fermentations were assessed in duplicate: (a) 7% (w/v) NaCl (control), and (b) 3.5% NaCl and 3.5 KCl (50% substitution). The changes in the microbiological (lactic acid bacteria-LAB, yeasts, and enterobacteria), and physicochemical (pH, titrable acidity) parameters were monitored for
135 days. Sensory analysis was performed at the end of fermentation by 10 judges, according to the official method of sensory analysis of table olives of the International Olive Council (COI/OT/MO No 1/Rev.2, November 2011).

Results.

In both fermentations, LAB and yeasts became the dominant microbial groups presenting similar growth profiles. Specifically, in control fermentation, the population of LAB increased rapidly during the first 9 days, reaching a maximum at 7.3 log CFU/mL, and gradually declined to 5.3-5.8 log CFU/mL by the end of fermentation. Yeast population presented also
a rapid increase and reached the same population with LAB after 60 days of fermentation. A different profile was obtained in NaCl/KCl fermentation, where the growth profile of LAB and yeasts was similar, presenting an increase until day 22 and then remained almost unchanged until the end of fermentation (5.6-6.4 log CFU/mL). The presence of KCl appeared to affect the population of LAB that was maintained in lower populations compared to control treatment. In contrast, yeasts were not affected by the salt substitution. This deviation was also reflected in the pH values of fermentation that were higher in the NaCl/KCl fermentation (4.8 at 135 days) compared to control treatment (4.2), as well as in the final acidity values (0.51% and 0.89% in NaCl/KCl and control fermentation, respectively). Sensory analysis indicated no signs of abnormal fermentation (butyric, zapateria, or putrid) and thus the olives were classified as “extra” category. Bitterness scores were similarly high for both fermentations, whereas some tasters detected a slight metallic taste in the NaCl/KCl-treated olives.

Conclusions.

The results of this work demonstrated that Kalamata natural black olives can be successfully fermented in brines with 50% substitution of NaCl with KCl at semi-industrial scale. However, special attention must to given to safety aspects of the final product, as the use of KCl in the brines favours the growth of yeasts against LAB, rendering a final product with higher pH values that do not meet the specifications of the trade standard of the IOC. For this reason, further experiments must be undertaken at industrial scale to ensure both product safety and regulatory compliance.

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