This is the peer reviewed version of the article accepted for publication in Food Microbiology, Volume 98, September 2021, 103788, which has been published in final form at https://doi.org/10.1016/j.fm.2021.103788

Production of volatile compounds by wild-type yeasts in a natural olive-derived culture medium

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Abstract

The production of volatile compounds in naturally fermented green table olives from Manzanilla cultivar was investigated. A total of 62 volatile compounds were detected after 24 weeks of fermentation. To clarify the contribution of yeasts to the formation of these compounds, such microorganisms were isolated from the corresponding fermenting brines. Five major yeast strains were identified: *Nakazawaea molendinolei NC168.1, Zygotorulaspora mrakii NC168.2, Pichia manshurica NC168.3, Candida adriatica* NC168.4, and *Candida boidinii* NC168.5. When these yeasts were grown as pure cultures in an olive-derived culture medium, for 7 days at 25 ºC, the number of volatiles produced ranged from 22 (*P. manshurica NC168.3*) to 60 (*C. adriatica NC168.4*). Contribution of each yeast strain to the qualitative volatile profile of fermenting brines ranged from 19% (*P. manshurica* NC168.3) to 48% (*Z. mrakii* NC168.2 and *C. adriatica* NC168.4). It was concluded that *C. adriatica* NC168.4 presented the best aromatic profile, being a solid candidate to be part of a novel starter culture to enhance the organoleptic properties of naturally fermented green table olives.

Keywords: table olives, fermentation, yeast, aroma, volatile compounds, SPME, GC-MS

1. Introduction

The economic and social importance of table olives in the Mediterranean countries has been outstanding for centuries, being the most widespread fermented vegetable in this area (Campus et al., 2018). Table olives are elaborated in many different ways but all of them pursue in the first instance to eliminate, or at least reduce, the bitter taste conferred by phenolic compounds such as the ubiquitous glucoside oleuropein. Natural table olives are elaborated from freshly collected fruits that, after a rinse in water to clean up the fruit surfaces, are submerged without any further treatment into brine with a concentration ranging 4-10 % (w/v) NaCl (Romero et al., 2004; Rejano et al., 2010; Fadda et al., 2014). This type of olives is partially debittered by diffusion of the bitter polyphenolic compounds from the flesh to the brine over a period of time ranging from 5 to 8 months. Concomitantly, a spontaneous fermentation takes place in these brines which is mainly supported by different yeast species (Rejano et al., 2010). These yeast species have been reported belonging to a wide diversity of genera such as *Candida, Pichia, Saccharomyces, Debaryomyces, Issatchenkia, Zygotorulaspora* and *Wickerhamomyces* (Heperkan, 2013). The presence of lactic acid bacteria (LAB) along this fermentation is variable, being dependent on the initial NaCl concentration of the brines as well as the polyphenol content of the olive cultivar used (Rejano et al., 2010), which in turn varies according to the crop season (El Qarnifa et al., 2019). Finally, some authors have pointed to the fact that the nutritional value of table olives depends mostly on the balanced profile of polyunsaturated and monounsaturated fatty acids and the contents of health-promoting phenolic compounds, which are best retained in natural table olives (Conte et al., 2020).

The role of yeasts in table olive fermentations has been discussed many times in the past. Some authors have considered their beneficial effects as contributors to the flavor of the fermented product (Arroyo-López et al., 2008, 2012; De Angelis et al., 2015), B-group vitamins producers (Ruiz-Barba and Jiménez-Díaz, 1995), debittering by beta-glucosidase activity (Bonatsou et al., 2017), or even exhibiting probiotic properties (Arroyo-López et al., 2012). In contrast, other authors have pointed out some detrimental properties associated with some yeast species that are common in olive fermentations, including softening of the fermented olive fruits by pectinolytic yeast (Golomb et al., 2013) and production of off-flavors (Arroyo-López et al., 2008). In addition to ethanol production, yeast metabolism in naturally fermented black olives has been associated to the increase in several alcohols, mainly isoamyl alcohol, characterized by a fruity-winey aroma (Bleve et al., 2014, 2015). The use of starter cultures of *Saccharomyces cerevisiae* has been reported to improve quality and safety aspects of naturally fermented table olives in comparison with spontaneously fermented olives (Tufariello et al., 2019). However, until now, the selection of yeast or LAB starters in table olives has been based on their specific technological and safety traits (beta-glucosidase activity and absence of production of biogenic amines) rather than the production of desirable aroma compounds. The aim of the present work was to study the volatile profile produced by yeasts isolated from natural green-olive fermentations growing in a culture medium derived from the same green olives. We have further correlated such profiles with those obtained from the actual natural green-olive fermentations from which these yeasts were actually isolated. This knowledge will undoubtedly be very useful to design appropriate starter cultures for natural green-olive fermentations.

2. Materials and methods

2.1. Olive fermentation set up

Olives of Manzanilla cv. were kindly provided by a local company, located in Albaida del Aljarafe, Seville, Spain. In our laboratories, olives were subjected to quality control to remove damaged fruits, washed with tap water and directly immersed in brine, containing 5%

(w/v) of NaCl. Fermentation was carried out in triplicate using cylindrical vessels made of polyethylene, each containing 5.2 kg fruits plus 3.4 L of brine. Fermentation took place at room temperature (ca. 20–22 °C) for a period of 24 weeks. Along the fermentation, microbiological and physico-chemical characteristics of the olive brines were monitored at 1, 2, 4, 7, 15 and 24 weeks.

2.2. Microbiological analyses

For routine control of the fermentations, brines were serially diluted in sterile saline and plated onto different culture media using a Spiral Plater (Don Whitley Sci. Ltd., Shipley, UK). Culture media used were De Man, Rogosa, Sharpe (MRS) agar (Biokar, Beauvais, France) containing 0.02% (w/v) sodium azide (Sigma-Aldrich), oxytetracycline-glucose-yeast extract (OGYE) (Oxoid Ltd., Basingstoke, UK) agar, and Violet Red Bile Glucose Agar (VRBG) (Oxoid). These culture media were aimed to enumerate total lactic acid bacteria, yeasts and *Enterobacteriaceae*, respectively. Plates were incubated at 32 ºC for up to five days and the number of colony forming units counted with a Scan 500 (Interscience, St Nom la Bretèche, France) colony counter.

2.3. Isolation and molecular identification of yeast strains

After 24 weeks of fermentation, brine samples of the three 5-kg fermenters under study were serially diluted in sterile saline and plated onto OGYE agar plates. Isolated colonies of yeasts were observed under the binocular magnifier to select for all different morphologies. Cell morphology of each yeast isolate was observed under the microscope. For each distinct morphology, up to 10 representative single colonies were picked out, streaked onto a fresh GYE (glucose, 20 g; yeast extract, 5 g; per liter) agar plate and purified by successive subculturing. Total DNA from yeast isolates was extracted directly from colonies by the rapid chloroform method described by Ruiz-Barba et al. (2005). Yeast isolates were identified to the species level by PCR amplification and further sequencing of the D1/D2 domain of the 26S rDNA gene as previously described in Lucena-Padrós et al. (2014).

2.4. Production of a natural olive-derived culture medium (OCM)

Four kg of the same batch of olives of Manzanilla cv. described above in section 2.1 were heated by immersion in a hot water bath (60 ºC, 10 min) to deactivate enzymes (Ramírez et al., 2017) and pitted. Pitted olives (ca. 2 kg) were added with 1 L distilled water and homogenized using a hand mixer. The homogenate was filtered through cheesecloth and the filtrate was centrifuged at 20,000 g for 15 min to remove oil. The aqueous fraction was first filtered using a Whatman grade 40 filter paper and then filtered again using a M.E. cellulose membrane of 0.45 μm pore size (Teknokroma, Barcelona, Spain). Finally, the filtrate was subjected to ultrafiltration (UF) using a regenerated cellulose membrane with a molecular weight cut-off of 1 kDa (Sigma-Aldrich, St Louis, USA). The UF permeate obtained was named as natural olive-derived culture medium (OCM) and aliquots were stored frozen at -20 ºC until use. Before microbiological experiments, OCM aliquots were sterilized using 0.22 μm-pore-size Q-max syringe filters (Frisenette ApS, Denmark).

2.5. Production of aroma compounds in OCM

Five mL GYE were inoculated with a single colony of each yeast strain and incubated overnight at 25 ºC. One mL of this culture was centrifuged and the resulting pellet washed twice in sterile saline. The washed pellet was finally resuspended in 1 mL of sterile saline and used to inoculate filter-sterilized OCM at a rate of 1:100. The inoculated OCM was dispensed, in triplicates, in 3 mL-aliquots into 15-mL screw-capped glass vials and incubated at 25 ºC for 7 days. Uninoculated OCM tubes were prepared in a similar way and used as controls. The three vials of each tested yeast strain (triplicates) were used for chromatographic analyses as described below. Inoculum concentration as well as growth after 7 days of incubation was estimated by plating serially diluted samples onto GYE agar plates.

2.6. Analysis of volatile compounds

Volatile compounds were analyzed by headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) following the procedure described by Sánchez et al. (2018) with few modifications. For analysis of inoculated and uninoculated OCM vials, 50 µL of internal standard (5-nonanol, 2 mg/L) were added to each vial immediately before extraction of volatile compounds. Then, the vial was closed and placed in a water bath adjusted to 40 ºC. The vial was equilibrated for 15 min at 40 ºC and stirred at 600 rpm using a stirring bar. The headspace volatile compounds were extracted for 30 min on a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (2 cm, 50/30 µm; Supelco, Bellefonte, PA). The volatile compounds adsorbed on the SPME fiber were desorbed at 265 ºC for 15 min in the injector port of a GC interfaced with a mass detector (internal ionization source: 70 eV) with a scan range from m/z 30 to 400 (GC model 7890A and mass detector model 5975C, Agilent Technologies, Santa Clara, CA). Separation was achieved on a VF-WAX MS capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) from Agilent. The initial oven temperature was 40 ºC (5 min), then 40-195 ºC at 3 ºC min^{-1}, and then 195-240 ºC at 10 ºC min^{-1} and held there for 15 min. The carrier gas was helium at a constant flow of 1 mL min^{-1}. MassHunter software version B.09.00 (Agilent Technologies) was used to detect and quantify peaks based on areas as determined by the deconvolution algorithm. A library search of the NIST 17 MS library was utilized for tentative identification of deconvoluted peaks. Chemical names were assigned to peaks that had a minimum mass spectral similarity > 80 (100 is an exact match). Confirmation was conducted by comparison of the retention indices with literature data reported for equivalent columns and with authentic standards when available. The volatile compounds were semi-quantified by comparison of peak areas to that of internal standard (5-nonanol).

For the analysis of brine from naturally fermented olives, 3 mL of brine was placed into a 15 mL glass vial with 50 µL of internal standard (5-nonanol, 2 mg/L) and volatiles were extracted, identified and semi-quantified as mentioned above for OCM samples.

2.7. Analysis of major substrates and fermentation end-products

Both carbohydrates (sucrose, glucose, fructose, and mannitol) and fermentation end-products (lactic acid, acetic acid, and ethanol) were determined by HPLC with a refractive index detector following the methods described by Sánchez et al. (2000). Concentrations were calculated by comparison of peak areas with those of external standards for each compound.

2.8. Analysis of physico-chemical characteristics

The pH and titratable acidity were measured following the routine procedures used in our laboratories (Cortés-Delgado et al., 2016).

2.9. Statistical analyses

The Student´s t-test was used to determine the significance of concentration changes of individual volatiles in inoculated OCM compared with uninoculated OCM. One-way analysis of variance (ANOVA) and Duncan tests were used for volatiles comparison between the selected yeasts. These analyses was performed using SPSS version 26 (IBM, Armonk, NY, USA), where $p < 0.05$ was considered to be significant. Principal component analysis (PCA) based on the contents of volatile compounds in OCM after 7 days of incubation (112 variables) was performed with SIMCA 14.1 software (Umetrics, Umea, Sweden).

3. Results

3.1. Microbiological analyses, isolation and identification of yeast strains

Evolution of the microbial population along the fermentation of natural green olives is shown in Figure S1. No LAB was detected in the brines of any of the three fermenters under study, while Enterobacteriaceae could not be detected after 15 weeks of fermentation. In contrast, yeast population was always above 4 log CFU/mL after the second week and reached 4.6 log CFU/mL at the end of the fermentation. At this point, up to five distinct yeast colony and cell morphologies were found in the OGYE agar plates seeded with 24-week (168 days) brine samples. Molecular identification of the isolates (up to 50), representative of each of the five yeast morphologies found, resulted in the five yeast species described in Table 1, where their respective closest relative type strain and sequence accession numbers are also shown. Counts of individual yeast species in the brines of each of the three fermenters after 24 weeks of fermentation are shown in Table S1.

3.2. Physicochemical analyses, major substrates and fermentation end-products

Evolution of the physicochemical characteristics of olive brine during fermentation is shown in Figure S2. Final values of pH and titratable acidity (expressed as percentage of lactic acid) were 4.49 ± 0.02 and 0.45 ± 0.03, respectively (mean ± SD, n=3). Of the major free sugars present in fresh olives (i.e. glucose, fructose, sucrose, and mannitol; Guillen et al., 1992), only mannitol (at a concentration of 1.68 ± 0.04 g/L) was found after 24 weeks of fermentation, indicating that this sugar alcohol was not utilized by the microbial population present in the olive brines. The major end-products of fermentation were ethanol, acetic acid, and succinic acid, which reached final concentrations of 7.88 ± 0.13, 0.22 ± 0.02, and 0.20 ± 0.02 g/L, respectively (mean ± SD, n=3). Lactic acid was not detected in any fermenter, indicating that LAB did not grow during the fermentation, as supported by the microbiological analyses (see section 3.1).

3.3. Volatile profile of natural green-olive fermentations

The volatile profile of naturally fermented green olives after 24 weeks of fermentation was determined. Sixty-two volatile compounds were identified and grouped into different families, including acids (8), alcohols (15), carbonyl compounds (7), esters (20), and others (12) (Table 2). Alcohols and esters were the predominant families (76% and 17% of the total concentration of volatile compounds, respectively), whereas the amounts of the remaining families were quite lower (< 3% each). Ethanol (representing 54.5% of all volatile compounds), isopentanol (17.3%), ethyl acetate (13.6%), acetic acid (1.8%), phenylethyl alcohol (1.3%), and dimethyl sulfide (1.3%) were the major volatile compounds.

3.4. Sugar metabolism, major end-products and production of aroma compounds by selected yeast strains in OCM

All of the selected yeast strains were able to grow in OCM and reached concentrations ca. 8 log CFU/mL after 7 days of incubation (Table S2). Preliminary experiments involving yeast cultures in OCM incubated for 2, 4 and 7 days at 25 ºC indicated that maximum number and amount of volatile compounds were reached after 7 days (not shown). Values of pH and consumption of major sugars present in OCM after 7 days of incubation in the presence of each yeast strain are shown in Table S3. The concentration of major end-products, i.e. ethanol and acetic acid, are also shown in Table S3. Lactic acid was not detected in any case.

Changes in the content of volatile compounds in OCM as a result of yeast growth after 7 days of incubation are shown in Table S4, where concentrations obtained were compared with those from uninoculated OCM. A total of 112 volatiles were identified. The volatile compounds produced significantly by the selected yeast strains in OCM are shown in Table 3, where comparisons among the different yeast strains can be visualized. In this sense, *Candida adriatica* NC168.4 originated the highest number of volatiles (60), either formed *de novo* or producing a concentration significantly higher than that found in the control OCM, being ethyl acetate, ethanol, theaspirane, (Z)-3-hexen-1-ol, and acetic acid the most abundant compounds, in decreasing order of concentration. *Candida boidinii* NC168.5 produced up to 43 volatiles, being ethanol, isopentanol, acetic acid, isopentyl acetate, and isobutanol the predominant compounds. A similar number of volatiles (42) was produced by *Zygotorulaspora mrakii* NC168.2, with ethanol, isopentanol, dimethyl sulfide, ethyl acetate, and acetaldehyde as the major compounds. *Nakazawaea molendinolei* NC168.1 produced up to 38 volatiles, the majority ones being ethanol, ethyl acetate, phenylethyl alcohol, isobutanol, and (*Z*)-3-hexen-1-ol. Finally, *Pichia manshurica* NC168.3 originated the lowest number of volatiles (22), of which dimethyl sulfide, acetic acid, isopentanol, and 2-methylbutanoic acid were the most abundant.

In order to determine the proportion of volatile compounds found in the brines of naturally fermented green olives that could be specifically produced by the selected yeast strains, common volatiles produced in OCM were identified (Table 3). Recall that, as described above, all these yeast strains were isolated from those same brines. Thus, the main contributors to the formation of the volatilome of naturally fermented olives (Manzanilla cv.), at least regarding to the number of different volatiles, were *Z. mrakii* NC168.2 and *C. adriatica* NC168.4, both with a score of 48%, followed by *C. boidinii* NC168.5 and *N. molendinolei* NC168.1 with 44 and 40%, respectively (Table 3). *P. manshurica* NC168.3 (19%) was the lesser contributor to the formation of volatile profile of naturally fermented olives.

3.5. PCA analysis of volatile production in OCM

To investigate the potential that volatile compounds has to discriminate among samples, SPME-GC/MS data of yeast-inoculated and control OCM were subjected to PCA (Figure 1). *C. adriatica* NC168.4 showed high positive PC1 and low negative PC2 score, being located at the right side of the score plot (Figure 1a). This yeast was highly correlated with a great number of volatiles, which were located at the right side of the loading plot (Figure 1b), such as 1-hexanol (v47), methyl salicylate (v86), benzyl alcohol (v93), (Z)-3-hexen-1-ol (v50), theaspirane A (v60), theaspirane B (v66), 6-methyl-5-hepten-2-ol (v58), β-myrcene (v28), 3-octanone (v37), and β-linalool (v68), among others. Actually, these volatiles reached the highest concentrations or even were uniquely formed in OCM inoculated with *C.*

adriatica NC168.4, as supported by ANOVA (Table 3). The principal component PC2 was able to establish differences among the rest of the samples, including the control, except for *N. molendinolei* NC168.1and *Z. mrakii* NC168.2 which could not be separated along PC2 (Figure 1a). This principal component was positively linked with carbitol (v73), 2-bornene (v62), 1-butanol (v27), ethanol (v10), dimethyl sulfoxide (v69), and 2-phenylethyl acetate (v87), among others, and inversely linked with 1-dodecanol (v97), 1-tridecanol (v102), 2-ethyl-1-hexanol (v61) and a great deal of aldehydes such as 3-methylbutanal (v9), hexanal (v24), phenylacetaldehyde (v75), and 2-phenyl-2-butenal (v95).

4. Discussion

The study of volatile metabolite profile of naturally fermented green olives has attracted the attention of researchers in recent years. Such studies evidenced that the volatile composition of the fermented products was cultivar dependent. A total of 52 volatile compounds were identified by Aponte et al (2010) using five different olive tree cultivars (Brandofino, Castriciana, Nocellara del Belice, Passalunara, and Manzanilla), which showed clear differences in their volatile profiles and considerable changes during storage. Using olives from Nocellara del Belice cultivar, Martorana et al. (2015) identified 49 volatile compounds, whereas 82 volatiles were identified by De Angelis et al. (2015) using Bella di Cerignola cultivar. A comparative study between naturally green table olives from Giarraffa and Grossa di Spagna cultivars was conducted by Randazzo et al. (2014), who detected clear differences in their volatile composition (35 compounds in Giarraffa samples vs. 24 in Grossa di Spagna ones). In addition, Randazzo et al. (2017) demonstrated that the use of bacterial cultures as starters clearly influenced the fermentation process and hence the volatile profile of the final product. In the present work, 62 different volatile compounds were identified using olives of the Manzanilla cultivar. The fact that yeasts represented the vast majority (virtually the only) of microorganisms growing all throughout the fermentations studied here, whereas LAB were not detected and *Enterobacteriaceae* were only present in low numbers up to the 7th week, is not surprising in the case of Manzanilla cultivar. Medina et al. (2010) also observed the absence of LAB in natural olive fermentations involving this cultivar when using 5% NaCl in the brines, while Aponte et al. (2010) obtained the same result using this cultivar with a NaCl concentration of 8%. It is known that the main factors that can limit the growth of LAB in naturally fermented olives are the ambient temperature, the initial salt concentration, the nutrient availability and the presence of natural inhibitory compounds (Ruiz-Barba et al., 1993; Medina et al., 2010). The conditions of salt concentration and temperature (average value > 18 ºC) of the present work should not be inhibitory for LAB (Tassou et al., 2002). Therefore, shortage of nutrients and/or the presence of relatively high concentrations of natural inhibitory compounds (polyphenols) could be the reasons for the absence of LAB growth throughout the fermentation. Actually, Manzanilla cv. is known to present relatively high levels of phenolic compounds (bitterness) compared to other "sweeter" Spanish varieties such as Gordal and Hojiblanca (Ramírez et al., 2017). Although the average pH (4.49) reached after 24 weeks of fermentation is slightly over that required by the current normative (IOC, 2004), it is a common practice to adjust that parameter at 4.3 or below by adding the necessary amount of acids (e.g. lactic acid) to the packaging brine.

It is acknowledged that the components of the culture medium (carbohydrates, proteins and lipids) supply the precursors for aromatic compounds (Smid and Kleerebezem, 2014), and also that each microbial strain shows a specific way to metabolize those substrates and therefore different volatile producing abilities (Ricci et al., 2018). In this line, the ability of each microbial isolate to produce relevant volatile compounds was examined in a natural culture medium obtained from olive fruits (OCM). It is noteworthy to point out that, for the production of OCM, it was found to be necessary a final step of ultrafiltration (see Materials and Methods) to reach the medium stabilization, as in its absence this medium turned cloudy in a short time. It appears to be that the initial heat treatment (60 ºC, 10 min) applied to the raw olives was not severe enough to totally deactivate the enzymatic activity present in olives. However, this hypothesis needs to be confirmed.

The five yeast strains isolated in this study, and used to analyze their metabolite profiles in OCM, belong to species that have been reported previously from different types of olive fermentations or olive-related products. Thus, *C. adriatica* was firstly isolated from extra virgin olive oil (Čadež et al., 2012), while *C. molendinolei* was later found to be one of the dominant yeast species in Kalamata natural black-olive fermentations (Bonatsou et al., 2018). Also, *Z. mrakii, P. manshurica* and *C. boidinii* were isolated from different green and black directly-brined table olive preparations (Bonatsou et al., 2017). All yeast strains isolated in this work were found to grow at good rates in OCM, reaching concentrations around 8 log CFU/mL, in average, after 7 days of incubation (Table S2). This fact indicates that this natural culture medium is appropriate to investigate the metabolite profiles of these and, in the future, other yeast strains. Furthermore, preliminary experiments indicated that strains of *Lactobacillus* sp., a common inhabitant of table olive fermentations with an important role as lactic acid producer, were able also to grow in this natural culture medium (not shown). The fact that we could observe growth of a LAB such as a strain of *Lactobacillus* sp. in OCM, when no LAB growth was observed during the fermentation of the same fruits, could indicate that some inhibitory compounds present in the processed fruits were removed during the protocol to obtain OCM. To get this effect, we observed that it was crucial the final ultrafiltration step through a cellulose membrane with a molecular weight cut-off of 1 kDa.

Our study showed that specific yeasts, in our case mainly strains of the species *C. adriatica* and *Z. mrakii*, could play a key role in the production of aroma compounds of naturally fermented olives. Both *C. adriatica* NC168.4 and *Z. mrakii* NC168.2 showed the same contribution (48%) to the aroma of naturally fermented olives based on number of different volatile compounds produced (Table 3). However, *C. adriatica* NC168.4 exhibited the highest number of volatiles produced in OCM after 7 days of incubation, i.e. up to 60 different compounds (Table 3). Besides, the concentrations of most esters, alcohols and terpenes with positive aroma descriptions (fruity, floral, sweet, apple, banana, green, etc; see Table 2), were significantly higher or were only formed in OCM fermented by *C. adriatica* NC168.4 (Table 3). Therefore, it can be concluded that, of the five yeasts studied here, *C. adriatica* NC168.4 presented the best aromatic profile. Further studies will be carried out in our laboratories in order to evaluate other biochemical features such as β-glucosidase, esterase and lipase activities, which could also contribute to desirable organoleptic characteristics of the fermented product when this strain is used as a starter for natural green olive fermentations.

5. Conclusion

The work presented here is an attempt to clarify the role of yeasts in the formation of the aroma characteristics of naturally fermented olives. Production of volatile compounds both in the brines of naturally fermented green olives as well as in a natural olive-derived culture medium was investigated. Up to 62 volatiles were detected in the brines of naturally fermented green olives. In parallel, five major yeast strains were isolated from these brines and production of volatile compounds by these strains was further examined in a natural olive-derived culture medium (OCM). It was found that one of these strains, i.e. *Candida adriatica* NC168.4, produced the maximum number of volatiles in OCM, representing a contribution of 48% to the volatilome of the original olive fermenting brines. In addition, the concentrations of most esters, alcohols and terpenes with positive aroma descriptions were significantly higher, or were actually only formed, by this strain. In conclusion, *C. adriatica*

NC168.4 presented the best aromatic profile and is a solid candidate to be used as a novel starter culture to enhance the organoleptic properties of naturally fermented green table olives.

Acknowledgements

This research was partially funded by the Spanish Ministry of Economy and Competitiveness from the Spanish Government through Projects AGL2014-54048-R and AGL2012-33400, as well as by the Junta de Andalucía Excellence Project AGR-07345, and by CSIC Project 201670E028. These projects included European Regional Development Funds (ERDF).

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Figure captions

Figure 1. Principal component analysis (PCA) of volatile compounds in uninoculated OCM (control) and OCM inoculated with selected yeast strains (7 days of fermentation): (a) distinction between the samples (score scatter plot); (b) relationships between the variables (loading scatter plot). C, control ; Y1, *Nakazawaea molendinolei* NC168.1; Y2, *Zygotorulaspora mrakii* NC168.2; Y3, *Pichia manshurica* NC168.3; Y4, *Candida adriática* NC168.4; Y5, *Candida boidinii* NC168.5. Volatile compounds (variables) are represented by the codes shown in Table S4.

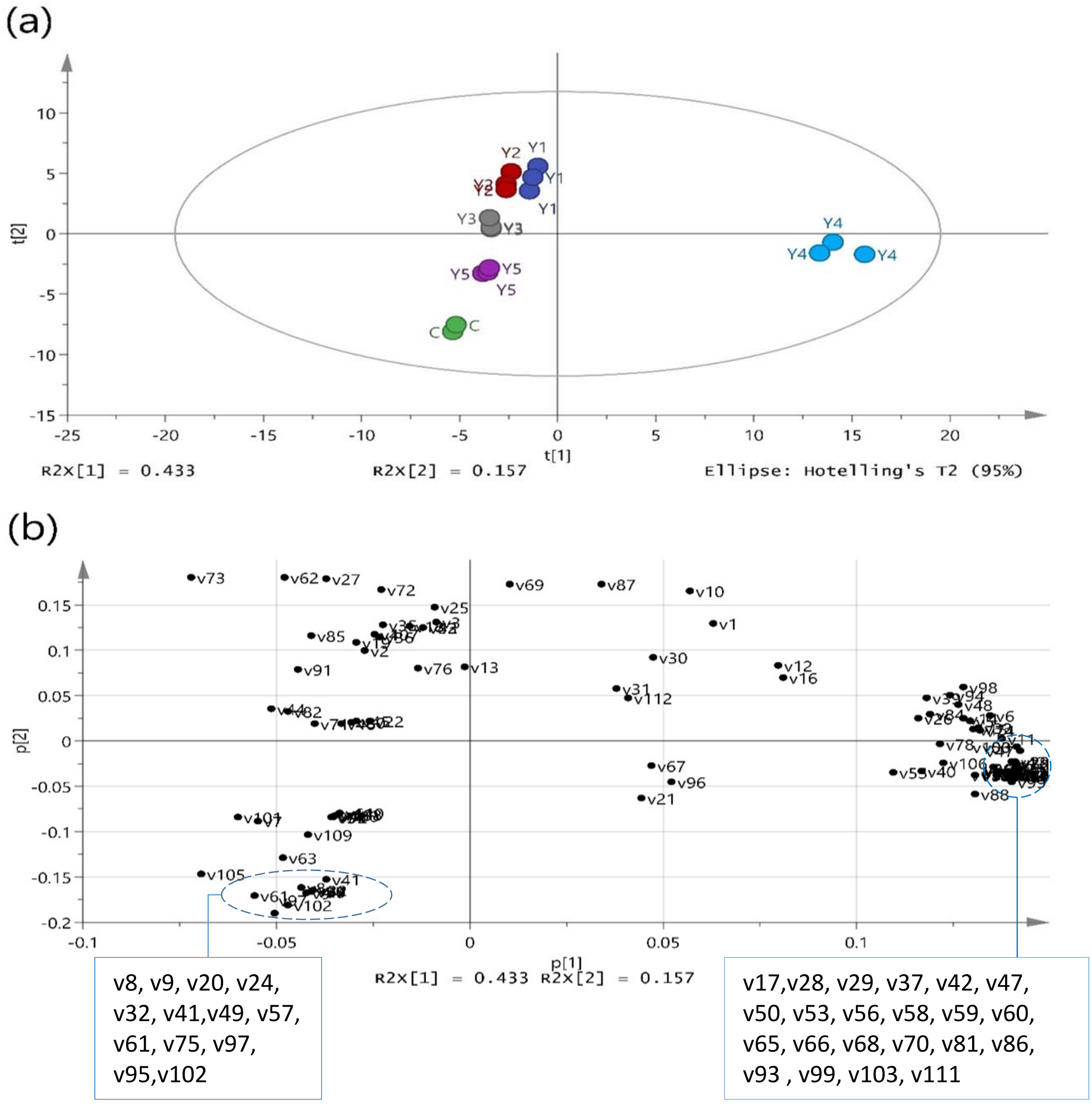

Figure 1. Alfredo MONTAÑO, Amparo CORTÉS-DELGADO, Antonio Higinio SÁNCHEZ and José Luis RUIZ-BARBA

Table 1. Molecular identification of yeast strains, isolated from natural green table-olive fermentations, through D1/D2 domain of the 26S rDNA gene sequence homology.

Strain	Length (bp)	Accession number	Closest relative type strain (accession number)	Similarity (%)
Nakazawaea molendinolei NC168.1	547	MT154798	*Nakazawaea molendinolei* CBS 12508^T (NG058353)[1]	100
Zygotorulaspora mrakii NC168.2	553	MT154799	*Zygotorulaspora mrakii* CBS 4218^T (KY110301)[2]	100
Pichia manshurica NC168.3	526	MT154800	*Pichia manshurica* CBS 209^T (MK394164)	100
[Candida]adriatica NC168.4[3]	533	MT154801	*[Candida]adriatica* ZIM 2334^T (NG060386)	100
[Candida] boidinii NC168.5	555	MT154802	*[Candida] boidinii* NRRL Y-2332^T (JQ689009)[4]	99.82

[1]Synonym (Syn.): *Candida molendinolei.*
[2]Syn.: *Zygosaccharomyces mrakii*, *Saccharomyces mrakii*, *Torulaspora mrakii*.
[3] Square brackets ([ ]) around a genus indicates that the name awaits appropriate action by the research community to be transferred to another genus.
[4]Syn.: *Candida koshuensis*, *Candida olivaria*, *Candida methanolica*, *Candida methylica*, *Candida alcomigas*, *Kloeckera boidinii*, *Torulopsis enokii*, *Candida queretana*, *Candida silvicola* var. *melibiosica*, *Candida ooitensis.*

Table 2. Volatile profile of naturally fermented green olives (Manzanilla cv.)

Compound	ID[a]	Odor description[b]	Concentration[c]
Acids			
Acetic acid	A	Sharp, pungent, vinegar	10.0 ± 1.6
Isobutanoic acid	A	Sour, cheesy, buttery	0.22 ± 0.12
Butanoic acid	A	Cheesy, sharp, dairy-like	0.19 ± 0.05
2-Methylbutanoic acid	A	Pungent, acidic, cheesy	0.94 ± 0.31
Hexanoic acid	A	Sour, sweaty, cheesy	0.16 ± 0.04
Octanoic acid	A	Fatty, waxy, cheesy	0.14 ± 0.04
Nonanoic acid	A	Waxy, cheesy, dairy	0.82 ± 0.64
Benzoic acid	A	Balsamic, urine	1.85 ± 1.4
Alcohols			
Ethanol	A	Alcoholic, ethereal, medical	306.8 ± 24.7
1-Propanol	A	Alcoholic, fermented, musty	1.17 ± 0.19
Isobutanol	A	Ethereal, winey	5.91 ± 0.55
1-Butanol	A	Fermented, balsamic, whiskey	0.84 ± 0.14
Isopentanol	A	Fermented, fruity, alcoholic	97.7 ± 8.4
Isoprenol	A	Sweet, fruity	0.32 ± 0.06
1-Pentanol	A	Fermented, pungent	0.45 ± 0.06
4-Penten-1-ol	B	N/A	0.25 ± 0.08
Prenol	A	Fruity, sweet, alcoholic	0.45 ± 0.06
1-Hexanol	A	Herbal, pungent, alcoholic	2.69 ± 0.38
(E)-3-Hexen-1-ol	A	Green, leafy, floral	5.09 ± 1.15
1-Nonanol	A	Floral, fresh, rose	0.22 ± 0.04
1-Decanol	A	Fatty, waxy, floral	0.14 ± 0.04
Benzyl alcohol	A	Floral, sweet, phenolic	0.48 ± 0.16
Phenylethyl alcohol	A	Floral, sweet, bready	7.47 ± 1.59
Carbonyl compounds			
Isobutanal	B	Fresh, aldehydic, herbal	0.20 ± 0.03
2-Methylbutanal	A	Musty, cocoa, nutty	1.36 ± 0.26
3-Methylbutanal	B	Aldehydic, chocolate	1.21 ± 0.3
3-Pentanone	B	Ethereal, acetone	0.12 ± 0.05
Nonanal	B	Aldehydic, waxy, citrus	0.99 ± 0.6
Benzaldehyde	A	Fruity, almond, nutty	1.67 ± 1.02
3-Ethylbenzaldehyde	C	Bitter almond[d]	0.11 ± 0.02
Esters			
Methyl acetate	B	Ether, sweet, fruity	5.18 ± 0.22
Ethyl acetate	A	Ether, sweet, fruity	76.4 ± 4.2
Ethyl propanoate	B	Fruity, sweet, grape	0.39 ± 0.29
Ethyl isobutanoate	B	Fruity, sweet, ethereal	0.58 ± 0.2
Methyl 2-methylbutanoate	A	Fruity, tutti-fruti, green	0.15 ± 0.04
Isobutyl acetate	B	Fruity, sweet, banana	0.41 ± 0.02
Methyl isopentanoate	A	Fruity, apple, pineapple	0.32 ± 0.09
Ethyl butanoate	A	Fruity, sweet, tutti-frutti	1.19 ± 0.02
Ethyl 2-methylbutanoate	A	Fruity, sweet, berry	1.08 ± 0.17

Ethyl isopentanoate	B	Fruity, sweet, pineapple	1.77 ± 0.38
Isopentyl acetate	B	Fruity, sweet, banana	4.63 ± 0.1
Ethyl hexanoate	B	Fruity, sweet, pineapple	0.51 ± 0.09
(Z)-3-Hexenyl acetate	A	Green, floral, banana[d]	0.16 ± 0.05
Ethyl octanoate	B	Waxy, sweet, musty	0.63 ± 0.4
Methyl 2,5-dimethyl-3-furoate	C	N/A	3.2 ± 1.2
Ethyl 2,4-dimethyl-3-furoate	C	N/A	0.5 ± 0.17
Ethyl benzoate	B	Fruity, musty, wintergreen	0.08± 0.03
Methyl salicylate	A	Minty, wintergreen	0.07 ± 0.02
2-Phenylethyl acetate	B	Floral, sweet, honey	0.22 ± 0.01
Phenethyl 2-methylbutanoate	B	Floral, green, sweet	0.7 1± 0.48
Other compounds			
Phenol	A	Phenolic, plastic, rubbery	0.28 ± 0.05
p-Vinylguaiacol	B	Woody, roasted peanut	0.08 ± 0.02
D-Limonene	A	Citrus, sweet, peely	0.75 ± 0.39
β-Linalool	A	Floral, citrus, terpenic	0.53 ± 0.52
β-Damascenone	A	Floral, woody, herbal	0.49 ± 0.05
Dimethyl sulfide	A	Sulfurous	7.1 ± 2.5
Styrene	A	Balsamic, sweet, plastic	1.9 ± 0.54
Theaspirane A	A	Camphor[e]	0.26 ± 0.11
Theaspirane B	A	Fruity, naphtalene[e]	0.44 ± 0.12
Butyrolactone	B	Creamy, oily, fatty	0.41 ± 0.03
Carbitol	B	Ethereal	2.3 ± 0.44
2,3-Dihydrobenzofuran	B	N/A	0.52 ± 0.04

[a] Identification: A, identified, mass spectrum and RI were in accordance with standards; B, tentatively identified, mass spectrum matched in the standard NIST 2017 library and RI matched with literature; C, tentatively identified, mass spectrum agreed with the standard NIST 2017.

[b] Odor descriptions from the Perflavory web (www.perflavory.com) with the exception of those marked by superscript letters. N/A, not available.

[c] Values are mean ± standard deviation (n=3) in brine, expressed as μg L^{-1} of 5-nonanol, after 24 weeks of fermentation.

[d] Burdock (1997).

[e] Schmidt et al. (1992).

Table 3. Volatile compounds produced by yeast strains grown in OCM after 7 days of incubation at 25 ºC

Compound[a]	Yeast strain				
	Nakazawaea molendinolei NC168.1	*Zygotorulaspora mrakii* NC168.2	*Pichia manshurica* NC168.3	*Candida adriatica* NC168.4	*Candida boidinii* NC168.5
Acids					
Acetic acid	19 (7)ab[b]	5 (1)a	83 (13)c	136 (40)d	53 (5)bc
Propanoic acid	-[c]	-	2.0 (0.5)b	-	0.7 (0.2)a
Isobutanoic acid	-	0.7 (0.1)a	19 (2)c	-	2.0 (0.4)b
Butanoic acid	0.28 (0.04)b	-	-	0.57 (0.02)c	0.09 (0.01)a
2-Methylbutanoic acid	0.84 (0.09)a	-	21 (2)b	0.90 (0.07)a	1.0 (0.2)a
Hexanoic acid	-	0.10 (0.03)a	0.20 (0.01)b	-	-
2-Ethylhexanoic acid	0.09 (0.02)a	0.09 (0.02)a	0.16 (0.01)ab	0.28 (0.05)bc	0.4 (0.1)c
Octanoic acid	-	-	-	-	0.9 (0.1)
Nonanoic acid	-	-	-	-	0.19 (0.07)
Decanoic acid	-	-	-	-	0.7 (0.2)
Geranic acid	-	-	-	2.1 (0.2)	-
Alcohols					
Ethanol	256 (49)c	350 (45)d	4 (1)a	248 (29)c	84 (9)b
1-Propanol	-	5 (1)b	-	-	0.7 (0.1)a
Isobutanol	37 (6)c	9 (1)b	2.6 (0.4)a	5 (1)ab	9 (3)ab
1-Butanol	0.6 (0.1)a	0.6 (0.1)a	-	-	-
Isopentanol	-	130 (13)b	78 (7)a	95 (17)a	77 (11)a
Isoprenol	1.8 (0.4)b	-	-	-	0.15 (0.05)a
(*E*)-2-Penten-1-ol	1.7 (0.3)	-	-	-	-
1-Hexanol	13 (4)b	0.38 (0.02)a	-	62 (7)c	0.16 (0.04)a
(*E*)-3-Hexen-1-ol	-	-	-	1.0 (0.1)	-
(*Z*)-3-Hexen-1-ol	37 (9)a	-	-	152 (5)b	-
3-Octanol	-	-	-	2.1 (0.6)	-
(*E*)-4-Hexen-1-ol	0.64 (0.09)	-	-	-	-
2-Octanol	-	-	-	1.4 (0.2)	-
1-Heptanol	-	-	-	5.3 (0.3)	-
6-Methyl-5-hepten-2-ol	-	-	-	2.4 (0.2)	-
2-Ethyl-1-hexanol	-	-	-	-	0.11 (0.02)
2-Nonanol	-	-	-	2.1 (0.2)	-
1-Octanol	3.0 (0.3)b	0.14 (0.05)a	-	32 (4)c	0.36 (0.07)a
1-Nonanol	1.1 (0.2)a	0.8 (0.1)a	-	4 (1)b	-
1-Decanol	-	0.12 (0.03)	-	-	-
Benzyl alcohol	0.34 (0.02)b	-	-	19 (1)c	0.04 (0.00)a
Phenylethyl alcohol	41 (1)b	4.2 (0.3)a	7.6 (0.4)a	59 (4)c	5.4 (0.3)a
Carbonyl compounds					
Acetaldehyde	15 (4)a	15 (5)a	-	-	-
Acetone	0.41 (0.05)ab	0.6 (0.2)b	1.1 (0.3)c	0.37 (0.03)ab	0.08 (0.01)a
3-Pentanone	-	2.07 (0.05)a	-	5.5 (0.7)b	-
2-Heptanone	-	0.9 (0.1)a	-	7.1 (0.9)b	-
3-Octanone	-	-	-	4.0 (0.2)	-
Acetoin	-	1.02 (0.09)a	0.7 (0.2)a	1.9 (0.5)b	-
4-Methyl-4-hydroxy-2-pentanone	-	-	0.14 (0.02)	-	-
Acetophenone	-	-	-	-	0.10 (0.01)
Esters					
Ethyl formate	-	0.75 (0.02)	-	-	-
Methyl acetate	0.8 (0.1)b	-	-	1.6 (0.5)c	0.09 (0.02)a
Ethyl acetate	214 (35)b	40 (5)a	-	491 (131)c	7 (1)a
Ethyl propanoate	3.1 (0.4)a	4 (1)a	-	23 (7)b	-

Ethyl isobutanoate	-	0.50 (0.08)ab	-	0.36 (0.06)a	0.8 (0.2)b
Propyl acetate	1.5 (0.2)a	-	-	2.8 (0.8)a	-
Isobutyl acetate	3.4 (0.3)c	0.20 (0.04)a	-	2.7 (0.6)c	1.2 (0.3)b
Ethyl butanoate	-	-	-	14 (2)	-
Ethyl 2-methylbutanoate	-	0.21 (0.03)a	-	0.37 (0.04)b	0.6 (0.1)c
Butyl acetate	-	-	-	0.25 (0.09)	-
Isopentyl acetate	14 (2)b	5.3 (0.6)a	-	22 (4)c	12 (2)b
Ethyl hexanoate	0.43 (0.09)a	0.23 (0.09)a	-	1.3 (0.5)b	0.22 (0.00)a
Hexyl acetate	0.22 (0.05)a	-	-	3 (1)b	-
(*Z*)-3-Hexenyl acetate	0.80 (0.04)a	-	-	7 (1)b	-
Ethyl octanoate	-	-	-	-	1.41 (0.05)
Octyl acetate	-	-	-	0.92 (0.05)	-
Ethyl decanoate	-	-	-	-	2.3 (0.1)
Benzyl acetate	-	-	-	0.7 (0.1)	-
Methyl salicylate	3.6 (0.7)b	-	-	57 (5)c	0.16 (0.01)a
2-Phenylethyl acetate	2.3 (0.1)a	3.7 (0.2)b	-	1.72 (0.4)a	-
Ethyl dodecanoate	-	-	-	-	2.0 (0.2)
Ethyl tetradecanoate	-	-	-	-	2.1 (0.5)
Ethyl hexadecanoate	-	-	-	-	0.4 (0.1)
Ethyl 9-hexadecenoate	-	-	-	-	0.23 (0.09)
Hydrocarbons					
Toluene	3.8 (0.3)b	-	0.33 (0.09)a	-	-
Styrene	-	9 (3)	-	-	-
2-Bornene	1.5 (0.6)b	0.7 (0.2)a	0.58 (0.05)a	-	0.42 (0.01)a
Phenols					
Phenol	0.27 (0.05)a	0.20 (0.07)a	-	0.7 (0.1)b	-
p-Cresol	0.13 (0.02)a	-	-	0.51 (0.04)b	-
4-Ethylphenol	-	-	0.06 (0.00)a	0.15 (0.04)b	-
***p*-Vinylguaiacol**	-	0.17 (0.02)	-	-	-
Sulfur compounds					
Dimethyl sulfide	-	28 (4)b	70 (7)c	8 (3)a	-
2-Methyltetrahydrothiophen-3-one	-	-	-	-	0.11 (0.04)
Dimethyl sulfoxide	0.58 (0.08)a	1.0 (0.2)b	1.0 (0.3)b	0.5 (0.1)a	-
Methionol	-	0.14 (0.01)a	-	-	0.13 (0.04)a
Terpenes					
β-Myrcene	-	-	-	2.5 (0.2)	-
(*Z*)-β-Ocimene	-	-	-	0.8 (0.2)	-
6-Methyl-5-hepten-2-one	-	0.06 (0.01)a	0.31 (0.06)b	-	-
β-Linalool	0.18 (0.01)a	-	-	49 (3)b	0.19 (0.03)a
(*E*)-β-Farnesene	-	-	-	0.8 (0.2)	-
α-Terpineol	-	-	-	4.6 (0.5)	-
Geranyl acetate	-	0.34 (0.04)a	-	0.56 (0.09)b	-
β-Damascenone	-	-	0.8 (0.1)b	2.5 (0.2)c	0.36 (0.03)a
Isogeraniol	-	-	-	3.4 (0.5)	-
Geraniol	-	-	-	10 (1)	-
α-Nerolidol	-	-	-	2.4 (0.1)b	0.20 (0.01)a
Others					
2,5-Dimethylfuran	-	-	-	0.21 (0.02)	-
Theaspirane A	7.4 (0.1)c	1.4 (0.2)b	1.7 (0.1)b	178 (35)d	0.68 (0.09)a
Theaspirane B	6.5 (0.5)c	1.0 (0.1)a	1.6 (0.1)b	162 (24)d	0.64 (0.03)a
Butyrolactone	-	0.35 (0.05)	-	-	-
Carbitol	1.3 (0.3)a	1.1 (0.2)a	-	-	-
2,3-Dihydrobenzofuran	-	0.59 (0.07)	-	-	-
Total[d]	38	42	22	60	43

In common (fermented olives)[e]	25	30	12	30	27
Yeast strain contribution (%)[f]	40	48	19	48	44

[a] Compounds in common with naturally fermented green olives are written in bold. Compounds identified in naturally fermented green olives are described in Table 2.
[b] Values, expressed as µg L^{-1} of 5-nonanol, are means of triplicate fermentations (standard deviation in parenthesis). Values in the same row with different letters indicate that they are significantly different at $p < 0.05$.
[c] -, compound not detected or not produced in significant amounts as compared to uninoculated OCM.
[d] Total number of volatile compounds produced by each yeast strain.
[e] Total number of compounds produced by each yeast strain which are in common with those found in naturally fermented green olives and described in Table 2.
[f] Percentage of the number of volatile compounds that are in common with naturally fermented green olives, considering that the total number of volatile compounds found in naturally fermented olives was 62 (Table 2).

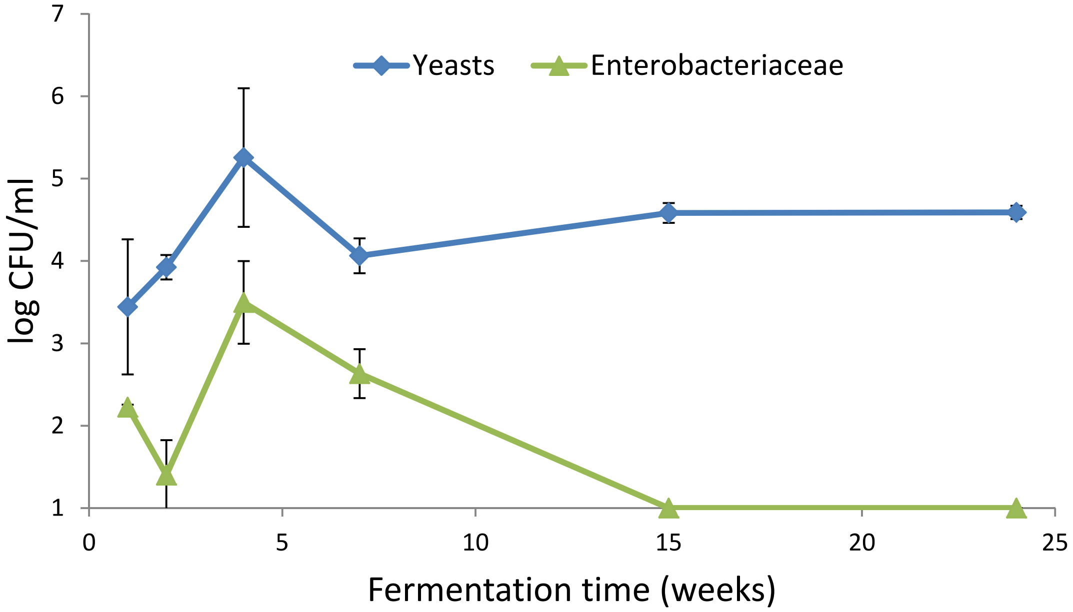

Figure S1. Evolution of microbial counts along natural green olive fermentations. Data are averages of three independent fermentations. Standard deviations are shown by the error bars (n=3)

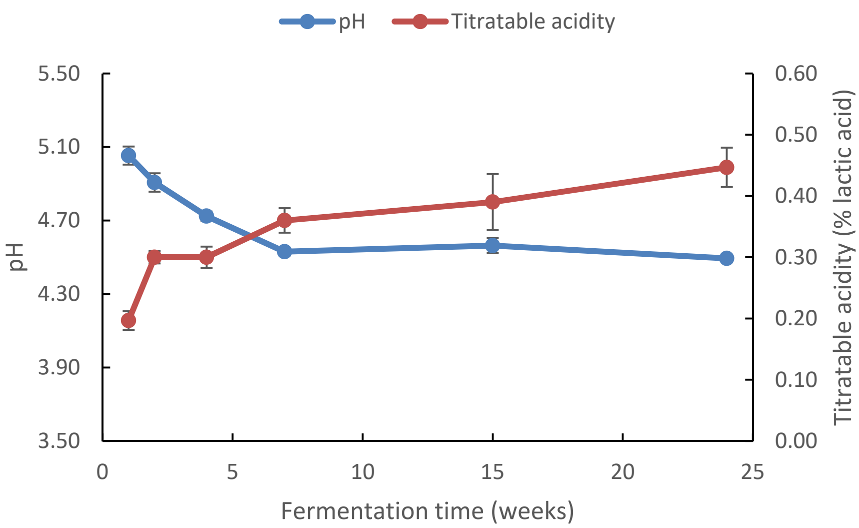

Figure S2. Evolution of pH and titratable acidity (expressed as percentage of lactic acid) during natural green olive fermentations. Data are averages of three independent fermenters. Standard deviations are shown by the error bars (n=3).

Table S1. Counts of individual yeast species identified in the brines of three fermenters of natural green olives after 24 weeks of fermentation.

Yeast species	Fermenter	Log CFU/ml	Mean log CFU/ml	SD
Nakazawaea molendinolei	NC1	4.30	4.29	0.28
	NC2	4.56		
	NC3	4.00		
Zygotorulaspora mrakii	NC1	4.68	4.43	0.31
	NC2	4.08		
	NC3	4.53		
Pichia manshurica	NC1	3.60	3.76	0.28
	NC2	3.60		
	NC3	4.08		
Candida adriatica	NC1	4.00	3.94	0.31
	NC2	4.20		
	NC3	3.60		
Candida boidinii	NC1	3.60	3.45	0.21
	NC2	ND		
	NC3	3.30		

ND, not detected (detection limit=$2x10^3$ CFU/ml)

Table S2. Averaged counts of yeast strains grown in a natural olive-derived culture medium (OCM) at 25 ºC.

Yeast strain	**t = 0 days**	**t = 7 days**
Nakazawaea molendinolei NC168.1	5.51 (0.05)[a]	8.04 (0.06)
Zygotorulaspora mrakii NC168.2	6.97 (0.01)	7.86 (0.01)
Pichia manshurica NC168.3	4.35 (0.49)	8.00 (0.06)
Candida adriatica NC168.4	5.13 (0.18)	7.98 (0.03)
Candida boidinii NC168.5	4.81 (0.05)	7.92 (0.02)

[a]Mean log CFU/ml (standard deviation), n=2.

Table S3. Values of pH, concentration of sugars and of major end-products in a natural olive-derived culture medium (OCM) inoculated with selected yeast strains after 7 days of incubation at 25 ºC

pH / sugar / end-product	Uninoculated OCM		*Nakazawaea molendinolei* NC168.1		*Zygotorulaspora mrakii* NC168.2		*Pichia manshurica* NC168.3		*Candida adriatica* NC168.4		*Candida boidinii* NC168.5	
	Mean[a]	SD	Mean[a]	SD	Mean[a]	SD	Mean[a]	SD	Mean[a]	SD	Mean[a]	SD
pH	5.24	0.05	5.02	0.08	5.04	0.01	4.77	0.03	4.63	0.04	4.70	0.06
Glucose	0.656	0.061	nd	-	nd	-	0.489	0.046	nd	-	nd	-
Fructose	0.256	0.061	nd	-	nd	-	0.288	0.050	nd	-	0.250	0.009
Sucrose	0.094	0.008	0.092	0.003	0.019	0.001	0.059	0.012	0.008	0.001	0.091	0.005
Mannitol	0.134	0.008	0.140	0.004	0.129	0.005	0.135	0.002	0.142	0.005	0.123	0.011
Ethanol	nd	-	0.372	0.008	0.356	0.013	nd	-	0.296	0.026	0.103	0.010
Acetic acid	nd	-	0.042	0.011	0.014	0.004	0.069	0.008	0.131	0.025	0.057	0.006

[a]Concentrations for sugars, ethanol and acetic acid are expressed in g/100 mL; n = 3.
SD = standard deviation
nd = not detected

Table S4. Changes in volatile compounds identified in a natural olive-derived culture medium (OCM) inoculated with selected yeast strains after 7 days of incubation at 25 ºC, as compared with uninoculated OCM (control). Concentrations are expressed as µg/L of 5-nonanol.

	Code	**ID**[a]	**OCM**[b]		***Nakazawaea molendinolei* NC168.1**			***Zygotorulaspora mrakii* NC168.2**			***Pichia manshurica* NC168.3**			***Candida adriatica* NC168.4**			***Candida boidinii* NC168.5**		
Volatile compound			Mean	SD[c]	Mean	SD	Change[d]	Mean	SD	Change	Mean	SD	Change	Mean	SD	Change	Mean	SD	Change
Acetaldehyde	v1	A	nd		14.59	4.74	F	15.12	4.87	F	nd			15.61	15.60	ns	nd		
Dimethyl sulfide	v2	A	14.76	0.59	27.69	6.10	ns	42.63	4.04	2.89	84.57	6.76	5.73	22.77	2.69	1.54	8.23	1.37	0.56
Acetone	v3	A	nd		0.41	0.06	F	0.64	0.20	F	1.10	0.34	F	0.37	0.03	F	0.08	0.01	F
Ethyl formate	v4	A	nd		nd			0.75	0.02	F	nd			nd			nd		
Methyl acetate	v5	A	nd		0.80	0.14	F	nd			nd			1.58	0.51	F	0.09	0.02	F
Ethyl acetate	v6	A	1.92	0.10	215.62	35.28	112.30	42.08	5.49	21.91	0.07	0.03	0.04	493.33	131.61	256.95	8.52	0.98	4.44
2-Butanone	v7	B	0.10		nd		E	nd		E	0.11	0.04	ns	nd		E	nd		E
2-Methylbutanal	v8	A	12.84	0.32	nd		E	0.69	0.14	0.05	0.14	0.04	0.01	nd		E	nd		E
3-Methylbutanal	v9	B	16.86	0.30	nd		E	nd		E	0.48	0.14	0.03	nd		E	nd		E
Ethanol	v10	A	1.15	0.08	256.78	49.78	222.47	350.96	44.68	304.07	5.50	1.34	4.76	248.92	28.51	215.67	84.75	9.44	73.43
Ethyl propanoate	v11	B	nd		3.06	0.43	F	4.49	1.59	F	nd			22.98	7.60	F	nd		
2,5-Dimethylfuran	v12	B	nd		0.23	0.17	ns	nd			0.06	0.05	ns	0.20	0.02	F	nd		
Ethyl isobutanoate	v13	B	nd		1.32	1.92	ns	0.50	0.08	F	nd			0.35	0.07	F	0.78	0.30	F
Propyl acetate	v14	B	nd		1.54	0.22	F	nd			nd			2.79	0.77	F	0.18	0.14	ns
3-Pentanone	v15	B	nd		nd			2.07	0.06	F	0.99	0.61	ns	5.48	0.74	F	nd		
Isobutyl acetate	v16	B	nd		3.42	0.35	F	0.20	0.04	F	nd			2.69	0.64	F	1.17	0.37	F
Ethyl butanoate	v17	A	nd		nd			nd			nd			14.18	2.24	F	nd		
Toluene	v18	A	nd		3.81	0.30	F	nd			0.33	0.10	F	nd			nd		
1-Propanol	v19	A	nd		nd			5.42	1.03	F	nd			nd			0.70	0.15	F
2-Butenal	v20	A	1.31	0.05	nd		E	nd		E	nd		E	nd		E	nd		E
Ethyl 2-methylbutanoate	v21	A	nd		nd			0.21	0.03	F	nd			0.37	0.05	F	0.58	0.12	F

2,3-Pentanedione	v22	B	nd		nd			nd			0.12	0.12	ns	nd			nd		
Butyl acetate	v23	B	nd		nd			nd			nd			0.25	0.09	F	nd		
Hexanal	v24	A	0.72	0.02	nd		E	nd		E	nd		E	nd		E	nd		E
Isobutanol	v25	A	nd		37.49	6.01	F	8.87	1.20	F	2.58	0.43	F	5.45	1.19	F	8.63	3.47	F
Isopentyl acetate	v26	B	nd		13.55	2.01	F	5.27	0.59	F	nd			22.28	4.36	F	11.63	1.70	F
1-Butanol	v27	A	nd		0.58	0.16	F	0.64	0.13	F	nd			nd			0.15	0.13	ns
β-Myrcene	v28	B	nd		nd			nd			nd			2.54	0.19	F	nd		
2-Heptanone	v29	B	nd		nd			0.93	0.12	F	nd			7.09	0.94	F	nd		
D-Limonene	v30	A	0.91	0.64	0.95	0.34	ns	3.37	1.93	ns	1.21	0.79	ns	2.38	0.80	ns	0.43	0.02	ns
Isopentanol	v31	A	0.14	0.02	nd		E	130.45	13.12	906.79	78.32	7.64	544.44	95.35	17.46	662.79	77.15	11.50	536.30
(E)-2-Hexenal	v32	A	0.23	0.04	nd		E	nd		E	nd		E	nd		E	nd		E
Ethyl hexanoate	v33	B	nd		0.43	0.09	F	0.23	0.09	F	nd			1.31	0.46	F	0.22	0.00	F
(Z)-β-Ocimene	v34	A	nd		nd			nd			nd			0.84	0.22	F	nd		
Isoprenol	v35	A	nd		1.77	0.40	F	nd			0.37	0.16	ns	nd			0.15	0.05	F
Styrene	v36	A	0.02		nd		E	9.43	3.74	392.23	nd		E	nd		E	nd		E
3-Octanone	v37	B	nd		nd			nd			nd			4.08	0.22	F	nd		
Hexyl acetate	v38	A	nd		0.22	0.05	F	nd			nd			3.40	1.49	F	nd		
Acetoin	v39	A	0.04	0.04	0.24	0.12	ns	1.06	0.09	26.71	0.70	0.24	17.69	1.90	0.58	47.66	0.10	0.03	ns
2-Octanone	v40	B	nd		nd			nd			nd			1.84	1.57	ns	nd		
Octanal	v41	A	0.09	0.05	nd		ns	nd		ns	nd			nd			nd		
(Z)-3-Hexenyl acetate	v42	A	nd		0.80	0.04	F	nd			nd			6.53	1.87	F	nd		
(E)-2-Penten-1-ol	v43	A	nd		1.75	0.29	F	nd			nd			nd			nd		
6-Methyl-5-hepten-2-one	v45	A	0.08		nd		E	0.14	0.01	1.71	0.40	0.06	4.80	nd		E	nd		E
2-Hydroxy-3-pentanone	v46	B	nd		nd			nd			0.20	0.13	ns	nd			nd		
4-Methyl-4-hydroxy-2-pentanone	v47	B	nd		nd			nd			0.14	0.02	F	nd			nd		
1-Hexanol	v48	A	0.11	0.02	12.65	3.61	112.61	0.49	0.02	4.40	nd		E	61.90	7.37	551.21	0.28	0.05	2.46
(E)-3-Hexen-1-ol	v49	A	0.21	0.01	0.63	0.19	ns	0.42	0.28	ns	nd		E	1.23	0.14	5.98	0.20	0.01	ns

Nonanal	v50	B	0.79	0.03	nd		E	nd		E	nd		E	nd		E	nd		E
(Z)-3-Hexen-1-ol	v51	A	nd		36.72	8.67	F	nd			nd			151.75	5.32	F	nd		
3-Octanol	v52	A	nd		nd			nd			nd			2.14	0.60	F	nd		
(E)-4-Hexen-1-ol	v53	A	nd		0.64	0.09	F	nd			nd			nd			nd		
2-Octanol	v54	B	nd		nd			nd			nd			1.42	0.26	F	nd		
Ethyl octanoate	v55	B	nd		nd			nd			nd			nd			1.41	0.05	F
Acetic acid	v56	A	0.65	0.23	19.60	7.45	30.24	6.03	1.77	9.30	84.49	13.38	130.32	137.06	40.92	211.40	53.22	4.59	82.09
1-Heptanol	v57	A	nd		nd			nd			nd			5.29	0.33	F	nd		
Furfural	v58	A	0.11		nd		E	nd		E	nd		E	nd		E	nd		E
6-Methyl-5-hepten-2-ol	v59	A	nd		nd			nd			nd			2.37	0.18	F	nd		
Octyl acetate	v60	B	nd		nd			nd			nd			0.92	0.05	F	nd		
Theaspirane A	v61	A	0.64	0.05	8.04	0.13	12.65	2.01	0.18	3.16	2.33	0.12	3.67	178.34	35.67	280.57	1.31	0.09	2.07
2-Ethyl-1-hexanol	v62	A	0.27	0.04	nd		E	nd		E	nd		E	nd		E	0.37	0.02	1.41
2-Bornene	v63	C	nd		1.52	0.64	F	0.75	0.17	F	0.58	0.05	F	nd			0.42	0.01	F
Benzaldehyde	v64	A	0.99	0.11	nd		E	0.27	0.11	0.27	nd		E	nd		E	nd		E
2-Methyltetrahydrothiophen-3-one	v65	B	nd		nd			nd			nd			nd			0.11	0.04	F
2-Nonanol	v66	B	nd		nd			nd			nd			2.14	0.17	F	nd		
Theaspirane B	v67	A	0.71	0.04	7.24	0.49	10.22	1.72	0.15	2.43	2.26	0.11	3.20	162.88	24.93	229.90	1.35	0.04	1.91
Propanoic acid	v68	A	nd		nd			nd			2.04	0.49	F	1.44	1.01	ns	0.68	0.23	F
β-Linalool	v69	A	nd		0.18	0.02	F	nd			nd			49.33	3.24	F	0.18	0.03	F
Dimethyl sulfoxide	v70	A	0.42	0.08	1.00	0.08	2.36	1.41	0.25	3.33	1.43	0.28	3.38	0.94	0.14	2.23	0.31	0.02	ns
1-Octanol	v71	A	0.21	0.03	3.25	0.27	15.45	0.35	0.05	1.65	nd		E	31.95	3.81	151.96	0.57	0.08	2.72
Isobutanoic acid	v72	A	nd		0.38	0.33	ns	0.74	0.14	F	18.64	1.62	F	nd			2.04	0.43	F
Butyrolactone	v73	B	nd		0.37	0.36	ns	0.35	0.05	F	nd			nd			nd		
Carbitol	v74	B	0.56	0.06	1.85	0.35	3.29	1.66	0.23	2.96	0.93	0.48	ns	nd		E	0.50	0.06	ns
Butanoic acid	v75	A	nd		0.28	0.04	F	nd			nd			0.57	0.02	F	0.09	0.01	F
Phenylacetaldehyde	v76	A	2.32	0.04	nd		E	nd		E	nd		E	nd		E	nd		E

Acetophenone	v77	B	nd		1.07	0.93	ns	nd			nd			nd			0.10	0.02	F
Ethyl decanoate	v78	A	nd		nd			nd			nd			nd			2.34	0.12	F
1-Nonanol	v79	A	nd		1.14	0.19	F	0.79	0.15	F	nd			3.68	1.43	F	1.25	0.59	ns
(E)-β-Farnesene	v80	B	nd		nd			nd			nd			0.78	0.25	F	nd		
2-Methylbutanoic acid	v81	A	0.36		1.20	0.09	3.32	0.34	0.11	ns	21.46	2.39	59.23	1.26	0.07	3.48	1.41	0.19	3.88
α-Terpineol	v82	A	0.23	0.03	0.23	0.07	ns	0.16	0.03	ns	0.19	0.05	ns	4.80	0.46	21.20	0.21	0.04	ns
Methionol	v83	B	nd		nd			0.14	0.01	F	nd			nd			0.13	0.04	F
Benzyl acetate	v84	A	nd		nd			nd			nd			0.70	0.12	F	nd		
Geranyl acetate	v85	B	nd		nd			0.34	0.04	F	nd			0.56	0.10	F	nd		
1-Decanol	v86	A	nd		0.09	0.08	ns	0.12	0.04	F	nd			nd			0.07	0.06	ns
Methyl salicylate	v87	A	nd		3.64	0.71	F	nd			nd			56.77	4.94	F	0.16	0.01	F
2-Phenylethyl acetate	v88	B	nd		2.28	0.16	F	3.69	0.16	F	nd			1.72	0.43	F	nd		
β-Damascenone	v89	A	0.41	0.02	0.55	0.11	ns	nd		E	1.02	0.04	2.49	2.88	0.25	7.04	0.76	0.03	1.87
Isogeraniol	v90	B	nd		nd			nd			nd			3.44	0.54	F	nd		
Geraniol	v91	A	nd		nd			nd			nd			10.47	1.53	F	nd		
Hexanoic acid	v92	A	nd		nd			0.10	0.03	F	0.20	0.01	F	nd			nd		
Ethyl dodecanoate	v93	B	nd		nd			nd			nd			nd			1.96	0.24	F
Benzyl alcohol	v94	A	nd		0.34	0.03	F	0.07	0.03	ns	nd			18.59	1.39	F	0.04	0.01	F
Phenylethyl alcohol	v95	A	0.10	0.07	40.75	1.46	427.47	4.27	0.26	44.80	7.69	0.45	80.65	59.07	4.53	619.64	5.51	0.38	57.83
2-Phenyl-2-butenal	v96	B	0.14	0.01	nd		E	nd		E	nd		E	nd		E	nd		E
2-Ethylhexanoic acid	v97	B	nd		0.09	0.02	F	0.09	0.02	F	0.16	0.01	F	0.28	0.05	F	0.37	0.14	F
1-Dodecanol	v98	B	1.12	0.23	nd		E	nd		E	nd		E	nd		E	0.26	0.02	0.23
Phenol	v99	A	0.07	0.02	0.35	0.06	4.69	0.27	0.08	3.69	0.30	0.18	ns	0.76	0.16	10.38	0.11	0.02	ns
α-Nerolidol	v100	B	nd		nd			nd			nd			2.36	0.12	F	0.20	0.02	F
Ethyl tetradecanoate	v101	B	nd		nd			nd			nd			nd			2.12	0.55	F
Octanoic acid	v102	A	0.17	0.02	0.09	0.04	ns	0.10	0.02	ns	0.37	0.13	ns	nd		E	1.09	0.14	6.58
1-Tridecanol	v103	B	1.29	0.29	nd		E	nd		E	nd		E	nd		E	0.20	0.17	0.15

p-Cresol	v104	A	nd		0.13	0.02	F	nd			nd			0.51	0.04	F	nd		
Eugenol	v105	A	nd		nd			nd			nd			0.50	0.23	ns	nd		
Nonanoic acid	v106	A	0.23	0.03	0.04		0.19	nd		E	0.14	0.01	0.60	nd		E	0.42	0.07	1.84
4-Ethylphenol	v107	A	nd		nd			nd			0.06		F	0.15	0.05	F	nd		
p-Vinylguaiacol	v108	B	nd		nd			0.17	0.02	F	nd			nd			nd		
Ethyl hexadecanoate	v109	B	nd		nd			nd			nd			nd			0.40	0.14	F
Decanoic acid	v110	A	0.11	0.02	nd		E	nd		E	0.20	0.07	ns	0.05	0.01	0.42	0.84	0.22	7.39
Ethyl 9-hexadecenoate	v111	B	nd		nd			nd			nd			nd			0.23	0.09	F
Geranic acid	v112	B	nd		nd			nd			nd			2.13	0.24	F	nd		
2,3-Dihydrobenzofuran	v113	B	0.21	0.02	nd		E	0.59	0.07	2.81	nd		E	0.33	0.09	ns	nd		E
Total number of volatiles produced by each yeast strain					38			42			22			60			43		

[a] Identification: A, identified, i.e. mass spectrum and RI were in accordance with standards; B, tentatively identified, mass spectrum matched in the standard NIST 2017 library and RI matched with literature; C, tentatively identified, mass spectrum agreed with the standard NIST 2017.
[b] Uninoculated culture medium (OCM) after 7 days of incubation at 30 ºC.
[c] SD = standard deviation (n=3)
[d] Change in concentration as compared with control OCM. Values indicate the fold change (mean concentration in inoculated medium/mean concentration in control) in case of a significant change according to the Student test. E = elimination; F = formation; ns = not significant change.
nd = not detected