Effect of photostimulation through red LED light radiation on natural fermentation of table olives: An innovative case study with Negrinha the Freixo variety

Fátima Martins [a,b], Elsa Ramalhosa [a,b,*], Nuno Rodrigues [a,b], José Alberto Pereira [a,b], Paula Baptista [a,b], Maria Filomena F. Barreiro [a,b], Pedro J.L. Crugeira [a,b,*]

[a] Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal
[b] Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal

ARTICLE INFO

ABSTRACT

In this study, for the first time, red LED light radiation was applied to the fermentation process of table olives using the Negrinha de Freixo variety. Photostimulation using LED light emission (630 ± 10 nm) is proposed to shorten and speed up this stage and reduce time to market. Several physical-chemical characteristics and microorganisms (total microbial count of mesophilic aerobic, molds, yeasts, and lactic acid bacteria) and their sequence during fermentation were monitored. The fermentation occurred for 122 days, with two irradiation periods for red LED light. The nutritional composition and sensory analysis were performed at the end of the process. Fermentation under red LED light increased the viable yeast and lactic acid bacteria (LAB) cell counts and decreased the total phenolics in olives. Even though significant differences were observed in some color parameters, the hue values were of the same order of magnitude and similar for both samples. Furthermore, the red LED light did not play a relevant change in the texture profile, preventing the softening of the fruit pulp. Similarly, LED light did not modify the existing type of microflora but increased species abundance, resulting in desirable properties and activities. The species identified were yeasts - *Candida boidinii*, *Pichia membranifaciens*, and *Saccharomyces cerevisiae*, and bacteria - *Lactobacillus plantarum* and *Leuconostoc mesenteroides*, being the fermentative process dominated by *S. cerevisiae* and *L. plantarum*. At the end of fermentation (122 days), the irradiated olives showed less bitterness and acidity, higher hardness, and lower negative sensory attributes than non-irradiated. Thus, the results of this study indicate that red LED light application can be an innovative technology for table olives production.

1. Introduction

Table olives can be produced by several methods. The Greek process is very much used in Portugal because it does not imply the addition of any compound other than salt. This process is also known as natural fermentation, where the fruit is only placed directly in brine with a salt concentration that can vary between 6 and 10% (m/v) [1]. The result is a unique product with different organoleptic, sensory, and textural characteristics [2]. Natural fermentation is an ancient, empirical, slow, and complex method that occurs spontaneously, driven by microorganism communities naturally present in the fruit pulp [3]. In this process, consortia of microorganisms (gram-negative bacteria, lactic acid bacteria, and yeasts) originating from the autochthonous flora olives co-interact and develop, resulting in synergistic and "quorum sensing" systems [4]. The olive sugars are metabolized and converted into lactic acid and other sub-products. Water-soluble compounds diffuse through osmosis from the olive skin, partially reducing the natural bitterness of the fruits and forming desirable aromatic compounds [5].

The natural fermentation process typically takes several months, depending on temperature, salt percentage, and olive's ripening stage [5,6]. Although technical improvement has been taking place over several generations by introducing some practices, e.g., the addition of acidifying substances, glucose, or sucrose supplements, that stimulate

---

* Corresponding authors at: Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal.
  E-mail addresses: elsa@ipb.pt (E. Ramalhosa), pedro.crugeira@ipb.pt (P.J.L. Crugeira).

microbial activity, the time needed to eliminate bitterness is still very long [7,8].

In this regard, accelerating this process is extremely important due to the decrease in in the production time, cost reduction, and consequent quicker market readiness. So, finding methods to accelerate the fermentation process will add value to producers. Photostimulation using LED light is widely explored in several areas, including agriculture and food processing [9]. The ability of cellular photo acceptors to absorb photons at specific wavelengths can promote the development of changes in metabolic activity, interfering in bioprocesses [10]. Light is a promoter of primary reactions in the respiratory chain, cell membrane, or enzymes carrying out anabolic or catabolic processes, forming a metabolic cascade that triggers cellular responses [11]. The ability of low-power electromagnetic irradiation to stimulate terminal enzymes of the respiratory chain (cytochromes), flavoproteins, enzyme cofactors, and their protein conformations induces an increase in microbial proliferation, RNA and DNA synthesis, protein activation, and ATP synthesis [12–14]. Furthermore, it is a low-cost and environmentally friendly technique with high potential. One of the targets of red spectrum irradiation is the cytochrome C complex, which, when photoexcited, induces an increase in proton pumping capacity and a consequent increase in the amount of cellular ATP available for cell division and the enzymatic activity necessary for the performance of metabolic pathways. The aim is to photostimulate table olives' fermentation process to speed up this stage and reduce the time to market. To the authors' best knowledge, no irradiation studies have been performed on table olives fermentation; thus, the effect of LED light on the natural fermentation of Negrinha de Freixo table olives is a case study of high interest. Negrinha de Freixo is an important cultivar in Trás-os-Montes region because the climatic conditions of this area allow the production of quality table olives without pesticides [1]. Furthermore, it is the raw material for the Portuguese Protected Designation of Origin (PDO) entitled "Azeitona de Conserva Negrinha de Freixo" [15]. Natural fermentation is one of the most applied processes for this cultivar, originating a product rich in valuable bio compounds with different beneficial properties for health [16].

In this context, this study aimed to evaluate the effect of photostimulation by $\lambda 630 \pm 1$ nm LED light on the natural fermentation of Negrinha de Freixo green olives. The microbial dynamics, flavor profile, general quality of the olives, and physicochemical parameters were evaluated throughout the fermentation process, and sensory analyses were performed at the end of the process. The insights gathered from this study hold significant importance as they can assist table olive producers in achieving a superior product within reduced production times.

2. Materials and Methods

2.1. Sampling and Fermentation Conditions

Green olives of the Negrinha de Freixo cultivar were supplied by a producer from Mogadouro, Trás-os-Montes region (NE Portugal) in November 2022. The fruits were harvested manually and transported in plastic containers to the Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança. Afterward, the olives were washed in running water and selected by their caliber. Fruits free of wounds or pests were selected, and the fermentation was performed in cylindrical clear glass jars. The brine was prepared at 7% NaCl (m/v), and 300 g of olive was immersed in 300 mL of brine (olive/brine ratio of 1.0/1.0 (m/v). The fermentation occurred naturally and spontaneously over 122 days, without brine refuel and agitation at an average temperature of 22 °C. The samples of brine and olives were collected over the process days (6 days equilibration time; 32, 47, 77, 92, and 122 days), and the assessments were performed in triplicate (three individual jars for each sampling time).

2.2. Irradiation Process

The irradiations were carried out using a low-power LED device (Emilight, MMOptics, São Carlos, SP, Brazil) with a power of 100 mW at wavelengths of 630 ± 10 nm, depositing an energy density of 14 J/cm^2 continuously (Table 1). The irradiations were carried out on the bottom outside of the jars at an irradiation angle of 90° and a distance of 0.5 cm. Two irradiations occurred during fermentation: the first occurred at 32 days, and the second at 77 days. Each irradiation persisted for 15 days, alternating with 30 days without irradiation (Fig. 1). The experiment was performed in triplicate, and the irradiation process was done in a dark environment, thus avoiding interference. The jars without irritation (control jars) were in the same experimental conditions. The LED device was calibrated, and the energy absorption in the initial fermentation medium (7% (m/v) saline solution) was evaluated using a potentiometer (Thorlabs Power Meter Sensor PM 30, Newton, New Jersey, United States) to establish the energy density of 14 J/cm^2 to be delivered [13].

2.3. Physicochemical Characterization

2.3.1. Color and Texture

The olives' color was measured during the fermentation process by a Konica Minolta CR-400 colorimeter and the computer software Spectra Magic Nx (version CM-S100W 2.03.0006, Konica Minolta Company, Osaka, Japan), using the CIELab scale. The instrument was previously calibrated for the standard white color. The assessment was done randomly on the surface of 10 olives taken from each jar. The L^* (lightness), a^* ($^-$green to red$^+$), b^* ($^-$blue to yellow$^+$), C^* (purity or intensity of the color), and h (hue) were determined. Texture was evaluated in fresh olives through a compression test using a TA. XT Plus Texture Analyzer equipped with a 30 kg load cell. Ten fruits were collected from each jar, and ten readings were taken. All analyses were carried out at room temperature, with data acquisition and integration obtained using the Texture Exponent TPA32 software and applying the Texture Profile Analysis (TPA) test that includes two compression cycles. The compression of the olives was carried out using a flat cylindrical probe (P/36R, diameter 36 mm) at a speed of 5 mm/s and a penetration length of 7 mm. Multiple textural parameters were quantified, such as hardness (maximum force of the first compression), adhesiveness (negative work between the two cycles), springiness (ratio of the distance of the detected height during the second compression and the original compression distance) (Distance 2/Distance 1), cohesiveness (ratio of the areas of the second compression and first compression (Area 2/Area 1) and chewiness (Hardness × Cohesiveness × Springiness).

2.3.2. NaCl Content

NaCl content was determined by refractometry using the KERN-Digital Refractometer. The brine samples were previously filtered and measured directly on the refractometer. The olive pulp was crushed, and 5 g were taken to prepare an aqueous solution in 5 mL of previously boiled water. After homogenization, the solution was squeezed through cheesecloth, and the NaCl concentration was determined in the solution. The samples were at room temperature, and three replicates were always evaluated. The results were expressed as the percentage of NaCl per 100 g of olive flesh (g NaCl/100 g pulp) and per 100 mL of brine (g

Table 1
Light emission parameters used in the irradiation process.

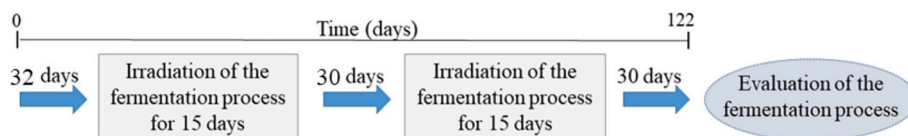| Parameter	LED
Wavelength (nm)	630 ± 10
Energy density (J/cm^2)	14
Emission	CW
Spot size (cm^2)	9
Power density (mW)	100

Fig. 1. Description of irradiation times during the natural fermentation process of table olives.

NaCl/100 mL brine).

2.3.3. pH and Titratable Acidity

pH determination was carried out in triplicate by potentiometry with a Hanna (HI 99163) pH electrode. For the brine, 20 mL of solution was used, and for the measurement, the pH electrode and the temperature probe were directly introduced into the sample. Regarding olives, it was prepared as a solution with 5 g of olive pulp in 20 mL of boiled water, being the pH the one measured in this solution. The titratable acidity (TA) was evaluated by titration with NaOH 0.1 M up to pH 8.1, following the Portuguese Standard NP 1421 (1997) [17]. In more detail, around 5 g of crushed olives were weighed and mixed with 50 mL of distilled water in a round bottom balloon. A reflux condenser was adapted to the balloon, and the mixture heated for 30 min, then allowed to cool to room temperature, and the solution transferred to a 100 mL test tube, with the volume completed with water. After homogenization and filtration, 25 mL were taken and titrated with the NaOH. The TA of the olives was expressed in grams of lactic acid per 100 g of olives. Regarding brine, TA determination was carried out using the same methodology as described above, directly titrating 20 mL of brine solution. Acidity was expressed as grams of lactic acid per 100 mL of brine. All measurements were performed in triplicate.

2.3.4. Total Phenols

A total of 2 mL of each brine was filtrated, and 100 μL of the filtrate was diluted in 25 mL of distilled water. Then, 0.5 mL of the diluted brine was added to a test tube containing 0.5 mL of saturated sodium bicarbonate solution, 0.5 mL of Folin-Ciocalteu phenol reagent (Sigma-Aldrich), and 3.5 mL of distilled water. The colorimetric reaction occurred in the dark at room temperature for 90 min. After briefly shaking, sample absorbances were measured in a spectrophotometer using a wavelength of 725 nm. The total phenols were extracted and determined following the protocol developed by [18] with some adaptations. To 1.5 g of olive pulp, 7.5 mL of acetone were added to remove the oil fraction. The samples were homogenized for 1 min and then centrifuged at 9000 $\times g$ for 10 min. The supernatant was removed, and 3 mL of methanol added to the pellet, followed by homogenization and centrifugation. This procedure was repeated at least four times. The extracts were combined, the methanol was evaporated under vacuum, and the residue dissolved in methanol to form extracts at a concentration of 50 mg/mL, then and analyzed using a spectrophotometer at a wavelength of 725 nm. The analyses were performed in triplicate, and the results were expressed in mg gallic acid/ 100 g of brine or mg gallic acid/100 mL of olive pulp.

2.4. Nutritional Analysis

The nutritional composition of table olives was determined, namely, ash, total fat, protein content, and dietary fiber, and the values were expressed as g/100 g dry weight (d. w.). The ash content was determined after calcination at 550 °C for 5 h to obtain white ashes. Total fat was determined on 5 g of freeze-dried sample extracted with petroleum ether with butylated hydroxytoluene (BHT) (0.01%, m/v), using a Soxhlet extractor (40–60 °C). After 8 h of extraction, the petroleum ether was evaporated in a rotary evaporator. Then, the flasks with the oil were taken to an oven at 30 °C until they reached a constant weight. The protein content of the olive samples was calculated by analyzing total nitrogen according to the Kjeldahl method, with a conversion factor of 6.25, and following the procedure defined by [19]. Dietary fiber was estimated using the enzymatic-gravimetric technique based on the AOAC official method No. 985.29 [20].

After determining the ash, protein, and fat content, the carbohydrate content was calculated by difference, and the energy value was calculated and expressed in kcal/100 g of dry weight. All determinations were made in triplicate.

2.5. Microbiological Analyses

2.5.1. Microbial Counts

To understand the evolution of different microbial communities during fermentation, viable counts of mesophilic aerobics, yeasts, molds, and LAB present in olives and brine samples were measured. Thus, the total microorganisms at 30 °C (mesophilic aerobic), molds, yeasts, and lactic acid bacteria were counted. A volume of 25 mL of brine solution was removed, and 25 g of fruits weighed. The fruits were placed in stomacher bags containing 225 mL of 0.015% peptone water (m/v), followed by homogenization and incubation at 25 °C for 10 min with slight agitation. Subsequently, successive decimal dilutions were made in 9 mL of the same solution. The samples were inoculated into specific culture media for the respective microorganisms to determine the mesophilic aerobic on Plate Count Agar (PCA, 30 °C, 48 h); molds and yeasts on Sabouraud Dextrose Agar (SDA) and Malt Extract Agar (MEA) (25 °C, 72 h) with the addition of 0.1% (m/v) chloramphenicol; lactic acid bacteria (LAB) on Man, Rogosa, and Sharpe (MRS) agar supplemented with 0.01% (m/v) cycloheximide, at pH 5.7 (30 °C, 72 h). Results were expressed as log CFU (colony forming units) per mL or g, depending on the sample type (brine or olive pulp), and the mean values of 3 replications with the standard deviation are presented. At the end of fermentation, the quality and microbiological safety conditions of table olives were evaluated in agreement with EU Regulation n° 2073/2005 [21] through the detection/research of pathogenic microorganisms, namely Enterobacteriaceae, *Clostridium perfringens*, *Listeria monocytogenes*, and *Salmonella* spp. Total Enterobacteriaceae counts were performed by ISO 21528-2:2004 [22], using the standard Compact Dry ETB plates (R-Biopharm) at 37 °C for 24 h.

The detection of *Clostridium perfringens* was done following the recommendations of the ISO 7937:2004 [23], standard on the Tryptone-Sulfite Cycloserine Agar (TSC, BioKar) culture medium, supplemented with egg *gem* emulsion and D-cycloserine 0.02% (m/v) at 37 °C for 48 h. The absence of L. *monocytogenes* and *Salmonella* spp. pathogens was verified in 25 g of olives following the ISO 11290-1:2017 [24], and ISO 6579:2002 [25], respectively.

2.5.2. Molecular Identification of the Microbial Population

After microbial quantification, the colonies (yeasts and LAB) were sub-cultured in a fresh culture medium (PCA and MRS) to obtain pure cultures. The isolates from each biological replicate (glass container) and each dilution were grouped into morphotypes, and two representatives were selected for molecular identification. Genomic DNA was extracted using the REDExtract-N-Amp Plant PCR kit (Sigma, Poole, UK), following the manufacturer's instructions. The DNA obtained was amplified by polymerase chain reaction (PCR) using a MyCycler thermocycler (BioRad Hercules). Two primers were used: 27F and 534R for bacteria [26,27]; TS1 and ITS4 for yeast [28] for amplifying a portion of the internal transcribed spacer (ITS) region and the 16S rRNA gene, respectively. The PCR reaction contained the primers (0.4 μL of each at

10 mM), 2 μL PCR buffer (10 mM), 0.4 μL dNTPs (10 mM), 2 μL DNA, 0.1 μL Taq polymerase (5 U/μL) and 14.7 μL of ultrapure H_2O for a final volume of 20 μL. The PCR program consisted of an initial denaturation at 94 °C for 3 min (1 cycle), followed by denaturation at 94 °C for 30 s, primers annealing at 52 °C–56 °C for 50 s, extension at 72 °C for 2 min (35 cycles); and a final extension at 72 °C for 10 min (1 cycle). The amplified products were sequenced by the services of Macrogen Inc. (Madrid, Spain) and subsequently analyzed with the DNASTAR v.2.58 software. Taxonomic identification was achieved by using the National Center for Biotechnology Information (NCBI), UNI, and UNITE databases, performing the BLAST (Basic Local Alignment Search Tool) algorithm. Operational taxonomic units (OTUs) presenting the lowest *E*-value and the highest identity score were identified as bacterial and yeast species when identity presented a value >98% or genus when presenting 95% to 97% identity. All identified isolates were cryopreserved and maintained in the microbial culture collection at the Mountain Research Center (CIMO), Instituto Politécnico de Bragança.

2.6. Sensory Analysis at the End of the Fermentation Process

The table olives were evaluated at the end of fermentation (122 days) by a trained panel of the Instituto Politécnico de Bragança, who were invited to assess the acceptability of the table olives and express their preference for the samples submitted to LED light or not. The organoleptic characteristics were evaluated according to the COI/OT/MO No 1/Rev.2 document [29]. The sensory analysis of table olives considered the negative attributes or defects (abnormal fermentation; putrid, butyric, zapateria), the gustatory attributes (bitter, acid, salty), and the kinesthetic sensations (hardness, fibrousness, crunchiness). The table olive profile sheet consisted of an intensity scale (unstructured line 10 cm long) that ranged from 1.0 (no perception) to 11.0 (extreme). The obtained data are presented after applying the method for calculating the mean and the confidence intervals, according to Annex 1 of COI/OT/MO No 1/Rev.2 [29].

2.7. Statistical Analysis

The physicochemical parameters were statistically analyzed using the Minitab software version 4 (Minitab, LLC, State College, Pennsylvania, USA). The normality of the data was verified by the Shapiro-Wilk test. To determine if there were significant differences ($p < 0.05$) between samples, an analysis of variance (ANOVA) or ANOVA Welch was carried out, depending on the existence or not of homogeneity of variances, respectively. If significant differences were detected between samples, a post hoc analysis was performed. When variances in the samples were identical, Tukey's honestly significant difference test was performed. On the contrary, the Games-Howell test was done when sample variances differed. The homogeneity of the variances was tested using Levene's test.

3. Results

3.1. Color Characterization and Texture Profile Analysis

Color and texture are critical parameters influencing the rejection of olives by the consumer. Table 2 shows the values of the color parameters (*L**, *a**, *b**, *C**, and h) assessed for the olive surface, for which some significant changes during the fermentation process were detected. About the *L** parameter, the values obtained for non-irradiated olives ranged from 48.19 (32 days) to 55.12 (77 days). For irradiated olives, the values obtained were 47.12 (92 days) and 52.76 (47 days). Regarding *a** values, a considerable increase occurred in irradiated olives compared to non-irradiated olives. In more detail, the irradiated olives showed the highest *a** value (1.93) after 122 days of fermentation. A decline in *b** and *C** coordinates values was observed after 92 days for the non-irradiated olives, while for the irradiated, the decrease was observed earlier after 47 days.

Nevertheless, no significant differences were observed between 92 and 122 days for both samples. Regarding the hue (h parameter), significant differences were always observed between treatments until 122 days of fermentation. Concerning the texture profile, the parameters evaluated by TPA are described in Table 3. According to the results, there was a decrease in the hardness of the olives throughout the fermentation process. However, this decrease was only significant between treatments at the end of fermentation (122 days). Furthermore, the hardness value of the irradiated olives was higher (73.02 N) than the non-irradiated olives (65.18 N). Comparing the values of the adhesiveness and springiness after 122 days with those at the beginning, there were no significant differences along time and between treatments. On the contrary, after 47 days of fermentation, irradiated and non-irradiated olives' cohesiveness and chewiness values significantly differed.

3.2. Evolution of Chemical Parameters through the Fermentation Process

The results of the chemical parameters (pH, titratable acidity, salt concentration, and total phenols) throughout the fermentation with and without LED light irradiation are represented in Fig. 2. The pH values of the two brines changed throughout the fermentation. They varied between 5.1 (both brines) in the initial phase to 4.6 (non-irradiated brine) and 4.0 (irradiated brine) in the final phase (Fig. 2A).

At 92 days, the irradiated brine had the lowest pH value (3.3) compared to the non-irradiated brine (4.3). The evolution of the pH profile was similar in the olives and the brine, showing the non-irradiated fruit the highest pH value at the end of fermentation (122 days). For titratable acidity (Fig. 2B), the results showed a progressive increase in the acidity of both brines until the 92nd day of fermentation. This increase was more pronounced for the irradiated brine (1.62% lactic acid) than non-irradiated brine (1.00% lactic acid). However, after
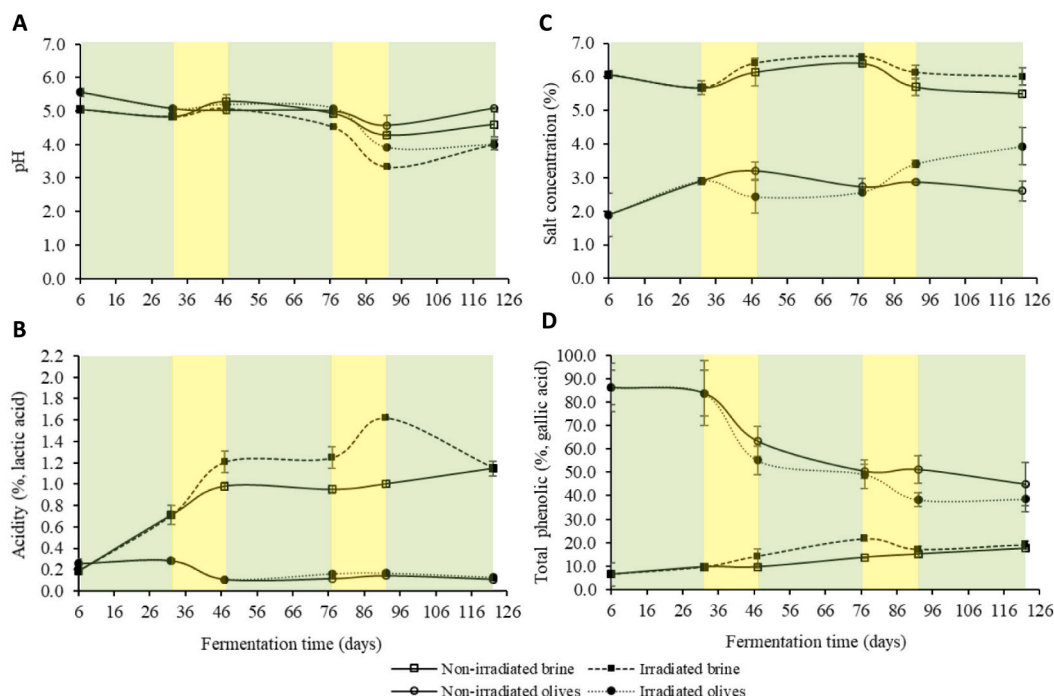
Table 2
Effects of red LED light on the color parameters in table olives fermented after 6, 32, 47, 77, 92, and 122 days.

Parameters	Treatment	Fermentation time (days)					
		6	32	47	77	92	122
L*	Non-irradiated olives	48.36 ± 3.58 [A,B]	48.19 ± 2.46 [A]	51.50 ± 3.96[a,C,D]	55.12 ± 2.79[a,D]	50.41 ± 5.4[a,A,B,C]	50.72 ± 3.08[a,B,C]
	Irradiated olives			52.76 ± 3.97[a,B]	52.41 ± 4.62[b,B]	47.12 ± 6.32[b,A]	49.46 ± 2.21[a,A]
a*	Non-irradiated olives	−3.74 ± 2.21 [A]	−1.19 ± 1.31[B]	0.32 ± 1.46[a,B,C]	−0.05 ± 0.98[a,C,D]	−0.48 ± 1.17[a,B,C,D]	0.38 ± 1.04[a,D]
	Irradiated olives			−0.62 ± 1.87[a,A]	3.06 ± 2.24[b,C]	0.27 ± 1.20[b,A]	1.93 ± 0.98[b,B]
b*	Non-irradiated olives	23.65 ± 4.17 [A]	25.86 ± 3.57 [A,B]	28.03 ± 5.44[a,C]	32.95 ± 3.62[a,D]	26.53 ± 6.07[a,B]	25.69 ± 4.00[a,A,B]
	Irradiated olives			30.43 ± 4.44[b,B]	28.51 ± 5.43[b,B]	22.39 ± 7.31[b,A]	23.57 ± 3.78[b,A]
C*	Non-irradiated olives	24.28 ± 4.18 [A]	25.92 ± 3.56 [A]	28.07 ± 5.44[a,B]	32.97 ± 3.61[a,B]	26.55 ± 6.08[a,A]	25.71 ± 3.10[a,A]
	Irradiated olives			30.49 ± 4.43[b,B]	28.79 ± 5.26[b,B]	22.43 ± 7.26[b,A]	23.67 ± 3.74[b,A]
h	Non-irradiated olives	99.08 ± 4.80[C]	92.54 ± 2.99[B]	99.08 ± 2.88[a,A,B]	90.07 ± 1.79[a,A]	90.85 ± 2.40[a,A,B]	89.00 ± 2.44[a,A]
	Irradiated olives			90.41 ± 5.19[b,A]	83.26 ± 5.43[b,A]	88.23 ± 5.40[b,B]	85.17 ± 2.78[b,A]

Values are expressed as mean ± standard deviation ($n = 10$). Uppercase letters -Values with different letters in the same line are statistically different ($p < 0.05$). Lowercase letters -Values with different letters in the same column are statistically different ($p < 0.05$).

Table 3

Textural parameters by TPA in table olives fermented after 6, 32, 47, 77, 92, and 122 days, subjected or not to LED light.

Parameters	Treatment	Fermentation time (days)					
		6	32	47	77	92	122
Hardness (N)	Non-irradiated olives	88.45 ± 29.68 [A]	85.98 ± 21.50 [A]	77.27 ± 13.82[a,A]	70.93 ± 14.56[a,A]	71.51 ± 1.38[a,A]	65.18 ± 7.88[a,A]
	Irradiated olives			79.30 ± 9.78[a,A]	74.01 ± 9.19[a,A]	73.42 ± 11.77[a,A]	73.02 ± 7.97[b,A]
Adhesiveness	Non-irradiated olives	−7.00 ± 7.18 [A]	−5.71 ± 5.80 [A]	−12.55 ± 6.10[a,A]	−10.11 ± 5.09[a,A]	−6.75 ± 6.55[a,A]	−10.91 ± 6.67[a,A]
	Irradiated olives			−11.28 ± 7.00[a,A]	−6.39 ± 6.46[a,A]	−4.17 ± 6.69[a,A]	−9.87 ± 5.24[a,A]
Springiness (mm)	Non-irradiated olives	0.65 ± 0.03 [A]	0.63 ± 0.06 [A]	0.63 ± 0.04[a,A]	0.66 ± 0.06[a,A]	0.65 ± 0.07[a,A]	0.68 ± 0.03[a,A]
	Irradiated olives			0.63 ± 0.05[a,A]	0.68 ± 0.05[a,A]	0.68 ± 0.03[a,A]	0.65 ± 0.05[a,A]
Cohesiveness	Non-irradiated olives	0.38 ± 0.04 [A,B]	0.37 ± 0.05 [A,B]	0.39 ± 0.03[a,A,B]	0.39 ± 0.06[a,A,B]	0.35 ± 0.02[a,A]	0.41 ± 0.04[a,B]
	Irradiated olives			0.36 ± 0.04[b,A]	0.35 ± 0.04[a,A]	0.39 ± 0.05[a,A]	0.39 ± 0.08[a,A]
Chewiness (N mm^{-1})	Non-irradiated olives	22.30 ± 8.40 [A]	20.16 ± 3.02 [A]	20.09 ± 2.16[a,A]	18.11 ± 3.04[a,A]	16.76 ± 4.19[a,A]	18.50 ± 3.92[a,A]
	Irradiated olives			18.26 ± 1.74[b,A]	18.31 ± 3.71[a,A]	19.36 ± 2.33[a,A]	18.48 ± 4.11[a,A]

Values are expressed as mean ± standard deviation ($n = 10$). Uppercase letters - Values with different letters in the same line are statistically different ($p < 0.05$).
Lowercase letters - Values with different letters in the same column are statistically different ($p < 0.05$).

Fig. 2. Chemical parameters evolution during the fermentation process with and without LED light. (A) pH, (B) titratable acidity (%, lactic acid), (C) salt concentration (%, NaCl), and (D) total phenolics (%, gallic acid). Each irradiation persisted for 15 days (yellow color), alternating with 30 days without irradiation (grey color). Data points are mean values of triplicate and standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

122 days of fermentation, the acidity of the irradiated brine showed a concentration of 1.16% lactic acid, while the non-irradiated brine had 1.14% lactic acid. The titratable acidity in the fruits varied slightly between 0.11 and 0.28% of lactic acid, varying around 0.17%, while in the brine, the maximum variation observed was 1.4%. Regarding salt concentration, Fig. 2C shows the variations observed during processing. A decrease in the salt percentage of the brines and an increase in the olives was observed due to salt and water diffusion. At the end of the process, the irradiated olives showed higher NaCl values (3.9%) than non-irradiated olives (2.6%). On the other hand, the brines presented very similar values, namely, irradiated brine (6.0%) and non-irradiated brine (5.5%). The effect of the LED light was evaluated on the content of total phenolic compounds (Fig. 2D). Significant differences were observed between treatments in two fermentation periods. The first period stands out between 32 and 47 days, and the second between 77 and 92 days, corresponding to the irradiation periods. Irradiated olives showed a greater decrease in the content of phenolic compounds. The values obtained in the irradiated olives varied between 86.3 and 38.6% gallic

acid.

3.3. Nutritional Composition

The ash, total fat, protein, and dietary fiber contents were determined to carry out the nutritional characterization of the olives. The results are presented in Table 4. After 122 days of fermentation, nutritional analyses indicated that fat was the most abundant component in the dry matter, as expected, followed by carbohydrates. Protein was the minority component. Olives fermented in the presence of LED light had a higher ash content ($p < 0.05$) but similar amounts of total fat, protein, dietary fiber, and carbohydrates as olives fermented in the absence of LED light.

3.4. Microbiological Analyses

3.4.1. Microbial Counts of Brine and Olives Samples through Fermentation
Microbial dynamics (yeasts, mesophilic aerobic, molds, and LAB)

Table 4
Nutritional composition (grams per 100 g dry matter) and energy value (kcal per 100 g dry matter) of non-irradiated and irradiated table olives after 122 days of fermentation.

Nutritional composition	Table olives	
	Non-irradiated	Irradiated
Ashes (%, d. m.)	3.2 ± 0.2^a	3.8 ± 0.2^b
Total fat (%, d. m.)	53.9 ± 1.6^a	52.2 ± 2.0^a
Protein (%, d. m.)	3.9 ± 0.2^a	3.8 ± 0.1^a
Total Dietary fiber	6.1 ± 0.5^a	6.2 ± 0.7^a
Carbohydrates (%, d.m.)	32.3 ± 1.2^a	33.5 ± 0.1^a
Energetic value (kcal/100 g d. m.)	423 ± 2^a	425 ± 1^a

Values are expressed as mean ± standard deviation. Different letters in the same line indicate significant differences ($p < 0.05$).

were assessed through the total count of microorganisms present in the brine (Fig. 3) and olives (Fig. 4).

Over the 122 days of fermentation, significant differences in microbial quantification were recorded between the two types of brine (Fig. 3). The irradiated brine had the highest microorganism counts. Among the groups evaluated, yeasts were the dominant group in the fermentation process (Fig. 3A). An increase in counts was observed after the first period of light radiation (47 days). In subsequent evaluations, yeast proliferation continued to increase, reaching a maximum of 7.0 Log_{10} CFU/mL at 122 days. This value was significantly different ($p < 0.001$) from the non-irradiated brine (5.7 Log_{10} CFU/mL). At the end of fermentation, the maximum counts of mesophilic aerobic were 5.38 Log_{10} (CFU/mL), and for LAB, 5.16 Log_{10} (CFU/mL), while in molds, they were only 3.70 Log_{10} (CFU/mL). At 47 days, the molds did not show significant differences in the irradiated brine compared to the non-irradiated brine (Fig. 3C). LAB counts were recorded from 47 days onwards, not being present at the start of fermentation. However, from this period, the LAB had a similar behavior to yeast (Fig. 3D). Regarding the fruit pulp, it was observed that the counts of the different groups of microorganisms were lower compared to the respective brines (Fig. 4). Nevertheless, there were significant differences between irradiated and non-irradiated olives. In the irradiated olives, the yeast counts varied between 3.65 and 3.92 Log_{10} (CFU/g) for the 77 and 122 days of fermentation. In the same period, the non-irradiated olives showed lower values ranging from 3.25 to 3.22 Log_{10} (CFU/g) (Fig. 4A). The mesophilic aerobic population did not show significant differences between the two brines during fermentation. Only at the end of the fermentation a considerable difference was observed. Still, not very substantial, with counts from 3.33 Log_{10} (CFU/g) in irradiated olives to 3.15 Log_{10} (CFU/g) in non-irradiated olives (Fig. 4B). On the other hand, molds showed increasing counts after 77 days of fermentation (Fig. 4C). The microorganisms that recorded the lowest counts were LAB, indicating minor variations between treatments (Fig. 4D).

*3.4.2. Description of Isolates Obtained during the Fermentation Process*

The evolution and abundance of yeast and LAB species identified in the brine and olives throughout the fermentation process are shown in Fig. 5A and B, respectively. During fermentation, 992 strains were identified in the brines and 581 in the olives. The irradiated brine showed a greater abundance of microorganisms (446 yeast and 192 bacterial strains) than the non-irradiated brine (245 yeast and 109 bacterial strains) (Fig. 5A). The same trend was observed at the fruit level. A total of 216 yeast and 112 bacterial strains were found in the irradiated fruits, while 168 yeast and 85 bacterial strains were in the non-irradiated fruits (Fig. 5B). The main differences were found at 92 and 122 days of fermentation. The genera found through fermentation were *Candida, Pichia, Saccharomyces, Lactobacillus,* and *Leuconostoc*. In the brines, *Candida boidinii, Pichia membranifaciens* and *Saccharomyces cerevisiae*, and *Lactobacillus plantarum* and *Leuconostoc mesenteroides*, were the yeasts and LAB species identified. The same species were also identified in the fruit pulp. Among the yeast species, *S. cerevisiae* was the most abundant in brines (173 irradiated brines; 93 non-irradiated brines) and fruit pulp (77 irradiated olives; 67 non-irradiated olives).
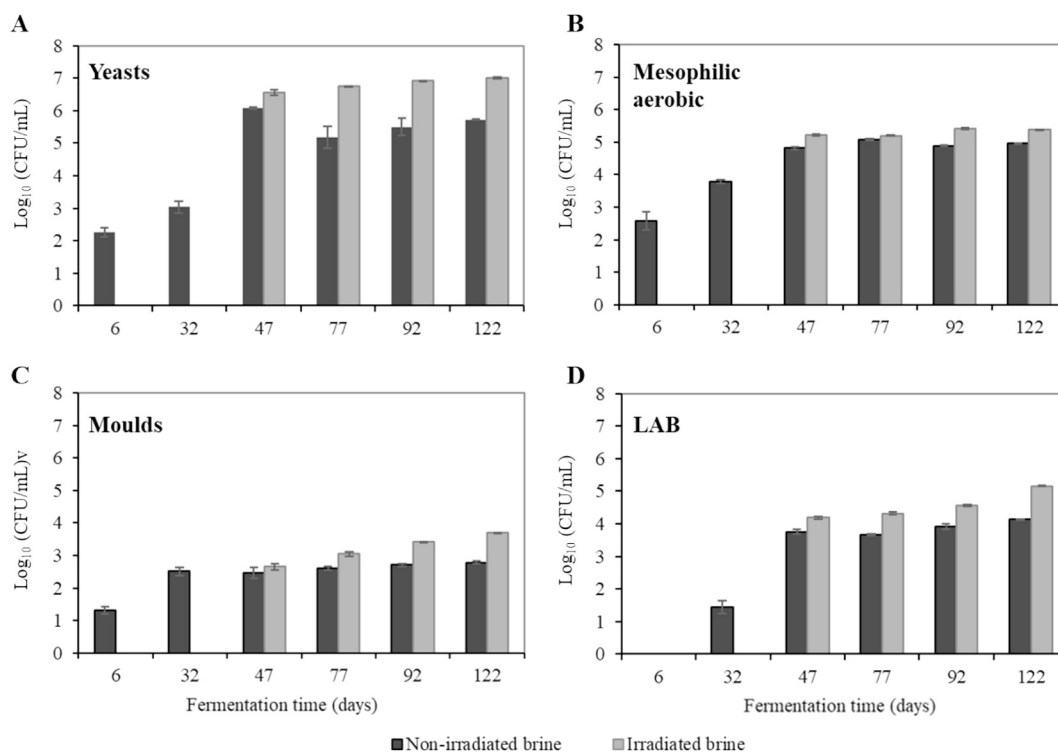
Fig. 3. Microbial counts of yeasts (A), mesophilic aerobic (B), molds (C), and lactic acid bacteria (LAB) evaluated in non-irradiated and irradiated brine after 6, 32, 47, 77, 92, and 122 days of fermentation. Each value is expressed as mean ± SD ($n = 3$). Asterisks indicate values that differ significantly between treatments, where *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.
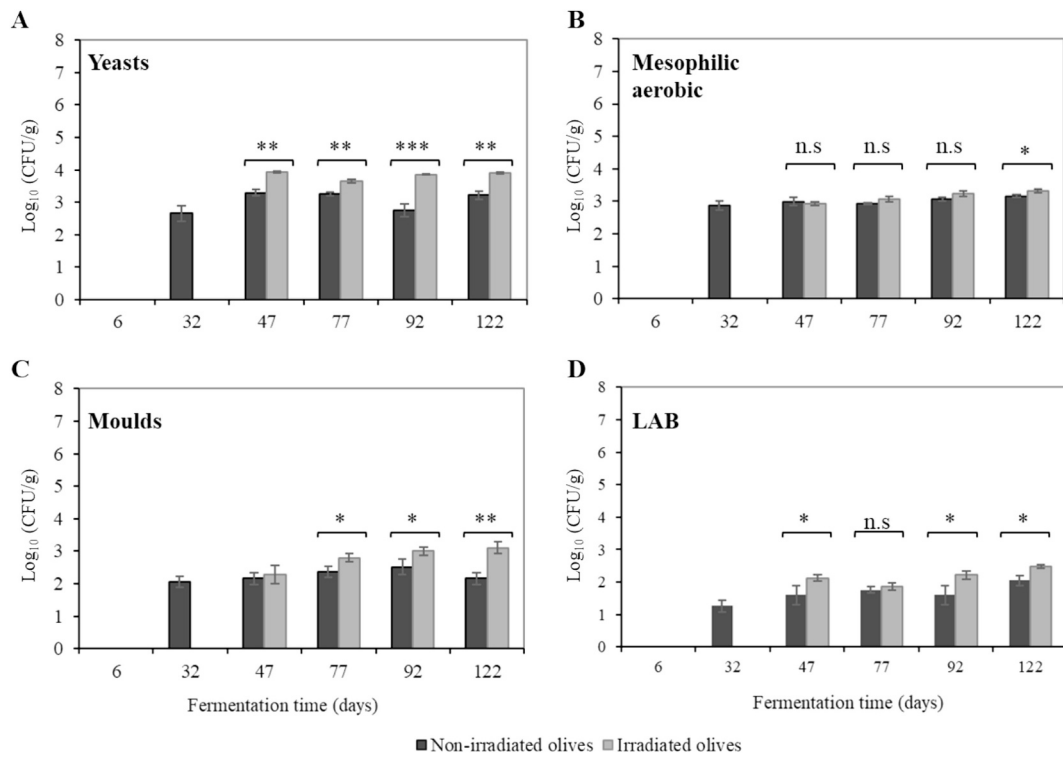
Fig. 4. Microbial counts of yeasts (A), mesophilic aerobic (B), molds (C), and lactic acid bacteria (LAB) evaluated in non-irradiated and irradiated olives after 6, 32, 47, 77, 92, and 122 days of fermentation. Each value is expressed as mean ± SD (n = 3). Asterisks indicate values that differ significantly between treatments, where *p < 0.05, **p < 0.01, and ***p < 0.001.
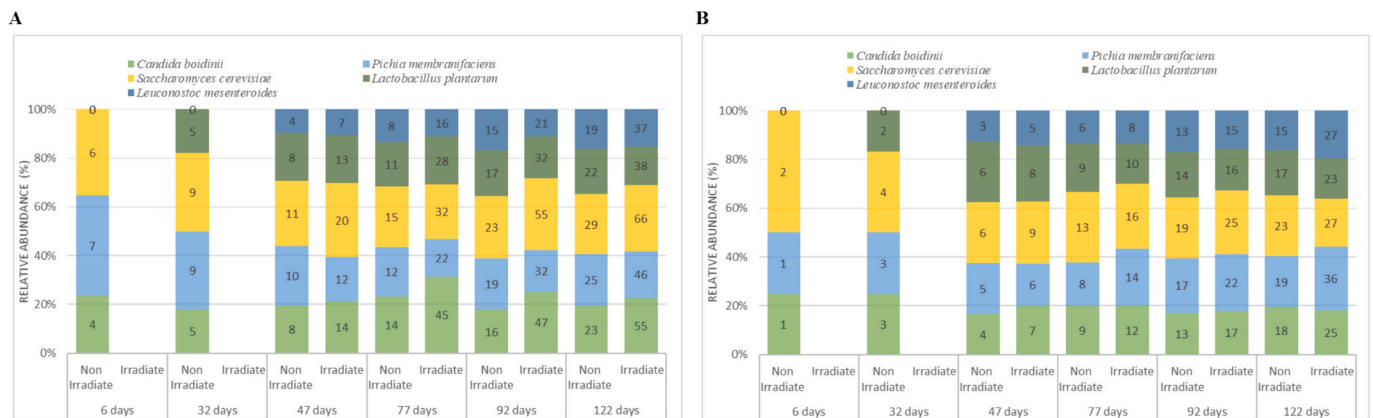
Fig. 5. Evolution and abundance of yeasts and lactic acid bacteria species in brine (A) and olives (B) after 6, 32, 47, 77, 92, and 122 days of fermentation exposed to LED light.

3.5. Sensory Analysis

The sensory profile of both types of olives is represented in the spider graph (Fig. 6). The values of taste attributes (salty, bitter, acid) and kinesthetic sensations (hardness, fibrousness, crunchiness) are related to olives. In irradiated olives, the sensation of bitterness and acidity was slightly lower (3.43 and 3.01) than in samples of non-irradiated olives (4.18 and 3.08). On the contrary, the perception of salt (salty attribute) was greater in irradiated olives (5.68) than in non-irradiated ones (4.48). Regarding the kinesthetic perceptions of fibrousness and crunchiness, the values were quite similar between the samples. For both characteristics, the irradiated olives had indices of 5.65 and 4.65, respectively, while non-irradiated olives had indices of 5.98 and 4.90. On the other hand, the perception of hardness was higher in the fruits fermented under the effect of the LED light (6.63 - with irradiation and

6.05 - without irradiation). Samples of olives fermented with the LED light had negative sensory attributes (abnormal fermentation) lower than 3 (2.62). In contrast, non-irradiated olives presented an index value for abnormal fermentation equal to 3.63. The negative attributes perceived in non-irradiated olives were putrid (index = 1.5), zapateria (index = 2.1), and in the olives subjected to LED light were winey-vinegary (index = 2.4).

4. Discussion

Color is a critical parameter that affects consumers' choices. In the present study, when comparing both types of samples (irradiated and non-irradiated), significant differences were observed at 77 and 92 days of fermentation, suggesting slight modifications in the luminosity. However, after 122 days of storage, the significant difference was no
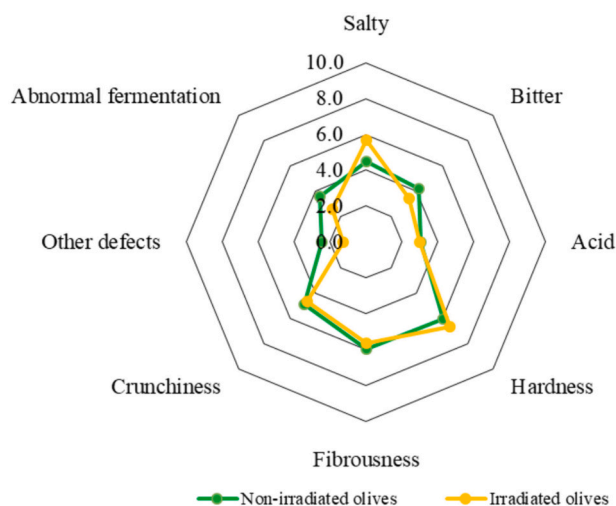
Fig. 6. Sensory profiles of non-irradiated and irradiated table olives after 122 days of fermentation.

longer observed, indicating that the LED light did not irreversibly modify the sample's luminosity. To $a*$ values, a considerable increase occurred in irradiated olives compared to non-irradiated olives. This fact means that the fruits acquired a redness tone more quickly, and might be due to an increase in the enzymatic activity, leading to enzymatic browning, the occurrence of Maillard reactions, or changes in the olive pigments' color, such as carotenoids or chlorophylls. These phenomena must be studied in the future to better understand light's role on the olives' constituents. Although there was a certain decline in the values of the b* and C* coordinates in both samples, no significant differences were observed at the end of fermentation, and the fruits acquired a stable yellow color, as this parameter presented values higher than the a* coordinate, which is responsible for the greenish and reddish tones. During and at the end of the process, the irradiated olives always showed lower values than non-irradiated olives. In general, significant differences in the color of the olives subjected or not to light were observed, indicating that red LED irradiation could affect the color of the olives. In natural fermentation, the color change is associated with the acidification and salinity of the medium [30]. Nevertheless, there is no information in the literature about the degradation of pigments during the natural fermentation of green table olives under the effect of the red LED light. However, several authors have reported the effect of light on the accumulation of carotenoids and flavonoids [31,32] and the acceleration of chlorophyll degradation in unripe fruit [33]. Thus, these phenomena need to be studied in more detail in the future. It should be noted that even though significant differences were observed in terms of hue between the samples subjected to light, the h values were of the same order of magnitude and similar between the irradiated and non-irradiated samples, and the differences observed may not be detectable by the consumer. Regarding hardness, we found that the hardness value of irradiated olives was higher than non-irradiated olives, which is good, suggesting that the LED light did not cause the fruit to soften. On the contrary, after 47 days of fermentation, irradiated and non-irradiated olives' cohesiveness and chewiness values significantly differed. This fact coincides with the period of the first irradiation, where an energy density of 14 J/cm^2 was applied. However, no significant differences were determined in the remaining times. A loss of texture in naturally fermented olives is strongly influenced by the enzymatic activity [34], sodium content [35], and pH [36]. Overall, the results obtained in this study suggested that LED light did not play a relevant change in the texture profile of the olives when compared to non-irradiated counterparts.

The evolution of the pH profile was similar in the olives and the brine. However, with the application of LED light, a slightly faster decrease in the pH to the value considered safe by the commercial standards applied to table olives, i.e., a minimum of 4.3 [37], was observed compared with no LED light application. This decrease in pH allows the preservation of the olives against deterioration processes and growth inhibition of pathogenic microorganisms [38].

In the fruit pulp, titratable acidity changed less than in the brine, possibly due to the higher presence of LAB on the brine than in the fruit. This increase may be related to the production of organic acids, such as malic, lactic, acetic, succinic, and citric acids, by LAB and yeasts, as they absorb and metabolize sugars (e.g., glucose and fructose) provided by olives. These acids are often described in brines from green olives [8,36]. In this sense, the LED light may indirectly promote lactic acid production because a more significant growth was observed in the periods in which the samples were irradiated (areas indicated in yellow on the graph). It was found that the changes in pH reflected the titratable acidity values expressed as % of lactic acid; namely, the lower the pH, the higher the acidity.

In natural fermentation, the concentration of NaCl present in the brine/olive at the end of the process is essentially due to the diffusion of the sodium chloride and water through the epidermis of the fruit. This allows substances of different sizes (sugars/salt) to enter and exit until equilibrium. The olive cultivar, ripeness index, olive/brine ratio, and brine concentration are some of the factors that can influence this process [5]. In this study, the LED light did not appear to affect the solute's (NaCl) movement because no specific trend was observed in the irradiation periods.

Concerning the content of phenolic compounds, and according to some studies carried out in fermentations, their reduction after a certain period of exposure to light may be related to oxidation processes or bioconversion into bioactive compounds [39,40]. However, some of these compounds can diffuse to the exterior of the olives, explaining the slight increase in the total phenolics observed in the brine. The partial elimination of bitterness in natural olives is due to endogenous and exogenous mechanisms, such as enzymatic reactions, microbial metabolism, hydrolysis reactions [41], and membrane thickness, cultivar, and maturation index. These results indicated that prolonged exposure of olives to light (30 days) changed the total phenolic content of the fruits.

At the nutritional composition level, a high ash content in irradiated olives indicated that the fruit had more minerals (calcium, potassium, sodium, and other elements). In terms of energy, there were no significant differences between treatments. The nutritional composition of naturally fermented table olives is directly related to factors such as cultivar, ripening index, and processing conditions [42,43]. The results obtained for both (irradiated and non-irradiated) Negrinha de Freixo table olives were similar in some nutritional components to those obtained for other Portuguese olives, such as whole olives of Galega cultivar (13.5% d.m. of ash, 64.7% d.m. of total fat, 3.5% d.m. of protein, 5.3% d.m. of dietary fiber and 12.6% d.m. of carbohydrates) [44] and Cobrançosa cultivar after freeze-drying and three different maturation stages (13.6–15.0% d.m. of ash, 60.2–67.3% d.m. of total fat, 3.8–4.8% d.m. of protein and 15.0–20.0% d.m. of carbohydrates) [45].

In the present study, the microbiological analyses showed a decrease in microorganism counts at the olive level compared to the brine. This decrease may be related to the more significant presence of microorganisms on the surface of the fruit compared to its interior and the higher number of phenolic compounds present in the fruit pulp compared to brine, with antimicrobial properties, which can naturally inhibit the development of these microorganisms [46]. However, at the level of the brine and the fruit pulp, the influence of light was relevant in increasing the microbial load of different microorganisms. This fact may cause the acceleration and reduction time of natural fermentation. As described in the literature, fermentation time can last from 8 to 12 months, mainly driven by a mixed population of LAB and yeast [47]. According to some studies, eukaryotic and microbial prokaryotic cells can be modulated through the ability of photoreceptors to absorb

photons [48]. When light is emitted with a specific wavelength, power, energy density, and used mode of operation (pulsed/continuous), the induction of metabolic activities in the fermentative bioprocess occurs [48]. As the fermentation process developed in the presence of oxygen and the emission of LED light occurred at a wavelength of 630 ± 10 nm, the maximum absorption peak of the cytochrome protein complex, stimulation of this terminal enzyme of the respiratory chain may have occurred.

Consequently, the increase in its proton pumping capacity and the amount of cellular ATP available increased cell proliferation [49,50]. On the other hand, LED light can stimulate, in the red spectrum, flavoproteins, enzymatic cofactors, and their protein conformations, causing an increase in cellular concentration, RNA and DNA synthesis, and protein activation [51,52]. Light acts as a promoter through primary reactions in the respiratory chain, cell membrane, or enzymes performing anabolic or catabolic processes, thus forming a metabolic cascade that triggers cellular responses [52]. It should be noted that despite the presence of photosensitive pigments in olives, such as chlorophyll *a* and carotenoids, no microbial photoinactivation was identified in the irradiated fermentation and consequent death or reduction in microorganisms' concentration during the fermentation process. Even though the LED light was emitting at the wavelength of 630 ± 10 nm and the absorption peaks of chlorophyll a and b were between 450 and 475 nm and 650–675 nm, respectively, and carotenoids between 400 and 500 nm [53,54], results suggest that there was no interaction between the LED light and these pigments, that could have induced ROS formation and consequently the microorganisms' death.

The identification of microorganisms allowed us to verify the constant presence of the species: *Candida boidinii, Pichia membranifaciens* and *Saccharomyces cerevisiae*, and *Lactobacillus plantarum* and *Leuconostoc mesenteroides* throughout the fermentation process. Similarly, this study [6] described that these species were the most frequently isolated during the natural fermentation process of table olives (Negrinha de Freixo cv.). The yeasts are a fundamental group of microorganisms that can have positive or negative effects in natural fermentation. Species of the genera *Candida, Pichia,* and *Saccharomyces* are known to have many killer strains (toxic proteins or glycoproteins) against spoilage yeasts [55,56]. *C. boidinii* has been recognized to positively affect olive aroma by forming esters from free fatty acids [57]. *P. membranifaciens*, native to olives of Portuguese cultivars, has oleuropeinolytic, mycogenic, and antimicrobial activity [6,58]. Additionally, *S. cerevisiae* has antioxidant activity, which is helpful in protecting fruits from the oxidation of unsaturated fatty acids and the formation of peroxides [56]. Of the two LAB species identified, *L. plantarum* was the most abundant in the brine (111 irradiated brines; 63 non-irradiated brines) and fruit pulp (57 irradiated olives; 48 non-irradiated olives). These species have been identified in green olives of Geracese and Nocellara Etnea cultivars [59] and table olives of Cobrançosa cultivar subjected to natural fermentation [5]. LAB are recognized to improve fermentation, provide acidification to the brine, and produce bacteriocins that prevent the development of contaminating microorganisms [36]. *S. cerevisiae* and L. *plantarum* dominated the fermentation process, significantly increasing for the LED irradiated fermentation, especially after 47 days. This study showed that the LED light did not modify the type of microflora present in the fermentation; however, it increased its abundance, namely in species promoting desirable properties and activities impacting the quality and safety of the final product.

At the end of fermentation, the sensory analysis of the olives revealed that the irradiated ones had less bitterness and acidity than the non-irradiated olives, probably due to the lower phenolics found in the irradiated fruits. On the other hand, the perception of hardness was greater in fruits fermented under the effect of LED light, in line with what was previously observed about the absence of softening of the fruit pulp due to the action of LED light; additionally, non-irradiated fruits presented a sensation of putrid and zapateria due to the possible development of contaminating microorganisms.

The tasters identified the wine-vinegar attribute in the olives subjected to LED light, resulting from microbial changes during fermentation. The development of yeast and acetic bacteria promotes the production of ethanol, CO_2, and acetic acid, giving the sensation of wine or vinegar [60]. The wine-vinegar attribute is classified as unfavorabl [29], but when present in a slight content, this organoleptic sensation might be valued by consumers. An example of this appreciation is the Galega olive with Protected Geographical Indication (PGI) [61]. According to Lanza [60], the classification of olives might also be determined by the median of the defect that is predominantly perceived (DPP). From the results obtained in this study, the olives subjected to LED light were classified in the extra category: DPP ≤ 3.0.

5. Conclusion

In this study, the effect of red LED light on microbial growth and physicochemical parameters was evaluated for the first time in table olives fermentation. When applied to the green Negrinha de Freixo cultivar, the results suggested an increase in the fermentation process acceleration due to the proliferation of the desirable species in olives and brine. The irradiated olives showed less bitterness and acidity, higher hardness, and lower negative sensory attributes. Furthermore, during the LED light exposition, a more significant decrease in the olive's phenolics content was observed, accelerating their edibility. Thus, the LED light emission, applied during two fermentation periods (32 and 77 days), did not negatively affect the quality of the olives, which were classified as an extra category. LED light application during spontaneous fermentation is not a common practice for olive fermentation, which was herein tested with Negrinha de Freixo. So, this study showed, for the first time, that photostimulation through red LED light irradiation can be an innovative strategy to improve natural fermentation, reducing process time with potential economic advantages. However, a deeper investigation at the metabolic and chemical levels will be needed in the near future.

CRediT authorship contribution statement

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

References

[1] DGADR, Azeitona de Conserva Negrinha de Freixo DOP. Produtos Tradicionais Portugueses. https://tradicional.dgadr.gov.pt, 2022 (Accessed on 17th December 2023).

[2] D. Campaniello, A. Bevilacqua, D.D. Amato, M.R. Corbo, C. Altieri, M. Sinigaglia, Microbial characterization of table olives processed according to spanish and natural styles, Food Technol. Biotechnol. 43 (3) (2005) 289–294. ISSN 1330-9862 (FTB-1389).

[3] A.H.S. Gómez, P.G. García, L.R. Navarro, Elaboration of table olives, Grasas Aceites 57 (1) (2006) 86–94. ENERO-MARZO. (ISSN: 0017-34).

[4] P. Johansen, L. Jespersen, Impact of quorum sensing on the quality of fermented foods, Curr. Opin. Food Sci. 13 (2017) 16–25, https://doi.org/10.1016/j.cofs.2017.01.00.

[5] P.J.M. Reis, T.G. Tavares, J.M. Rocha, F.X. Malcata, A.C. Macedo, Cobrançosa table olives: characterization of processing method and lactic acid Bacteria profile throughout spontaneous fermentation, Appl. Sci. 12 (19) (2022) 9738, https://doi.org/10.3390/app12199738.

[6] E.L. Pereira, E. Ramalhosa, A. Borges, J.A. Pereira, P. Baptista, YEAST dynamics during the natural fermentation process of table olives (Negrinha de Freixo cv.), Food Microbiol. 46 (2015) 582–586, https://doi.org/10.1016/j.fm.2014.10.003.

[7] S. Bonatsou, et al., Evaluating the probiotic potential and technological characteristics of yeasts implicated in cv. Kalamata natural black olive fermentation, Int. J. Food Microbiol. 271 (2018) 48–59, https://doi.org/10.1016/j.ijfoodmicro.2018.02.018.

[8] N.G. Chorianopoulos, I.S. Boziaris, A. Stamatiou, G.J.E. Nychas, Microbial association and acidity development of unheated and pasteurized green-table olives fermented using glucose or sucrose supplements at various levels, Food Microbiol. 22 (1) (2005) 117–124, https://doi.org/10.1016/j.fm.2004.04.010.

[9] A. Poonia, S. Pandey, Vasundhara, application of light emitting diodes (LEDs) for food preservation, post-harvest losses and production of bioactive compounds: a review, Food Prod. Process. Nutr. 4 (8) (2022) 10, https://doi.org/10.1186/s43014-022-00086-0.

[10] C.R. Martínez, L.S. Gómez-Pérez, A. Ordaz, A.L. Torres-Huerta, A. Antonio-Perez, Current trends of bacterial and fungal Optoproteins for novel optical applications, Int. J. Mol. Sci. 24 (19) (2023) 14741, https://doi.org/10.3390/ijms241914741.

[11] A.L.B. Pinheiro, et al., Effects of photo-stimulation with laser or LED on the composition of Xanthan gum produced in media containing distilled water or dialyzed or not produced water by means of Raman spectroscopy, J. Photochem. Photobiol. B 213 (2020) 112057, https://doi.org/10.1016/j.jphotobiol.2020.112057.

[12] P.J.L. Crugeira, et al., Effects of photostimulation on the catabolic process of xenobiotics, J. Photochem. Photobiol. B 191 (2019) 38–43, https://doi.org/10.1016/j.jphotobiol.2018.12.004.

[13] P.J.L. Crugeira, et al., Production and viscosity of Xanthan Gum are increased by LED irradiation of *X. campestris* cultivated in medium containing produced water of the oil industry, J. Photochem. Photobiol. B 226 (2022) 112356, https://doi.org/10.1016/j.jphotobiol.2021.112356.

[14] A.C. Kneuttinger, A guide to designing photocontrol in proteins: methods, strategies and applications, Biol. Chem. 403 (5–6) (2022) 573–613, https://doi.org/10.1515/hsz-2021-0417.

[15] European Union (EU), Regulation (EC) No. 1107/96 of 12 June 1996 on the registration of geographical indications and designations of origin under the procedure laid down in Article 17 of Council Regulation (EEC) No. 2081/92 with corrections published in the Official Journal of 13 November, 1996.

[16] J.A. Pereira, et al., Table olives from Portugal: phenolic compounds, antioxidant potential, and antimicrobial activity, J. Agric. Food Chem. 22 (54) (2006) 8425–8431, https://doi.org/10.1021/jf061769j.

[17] Norma Portuguesa (NP) 1421, Géneros alimentícios derivados de frutos e de produtos hortícolas, 1997. Determinação da acidez.

[18] G. Ciafardini, B.A. Zullo, Use of selected yeast starter cultures in industrial-scale processing of brined Taggiasca black table olives, Food Microbiol. 84 (2019) 103250, https://doi.org/10.1016/j.fm.2019.103250.

[19] P. Sáez-Plaza, M.J. Navas, S. Wybraniec, T. Michałowski, A.G. Asuero, An overview of the Kjeldahl method of nitrogen determination. Part II. Sample preparation, working scale, instrumental finish, and quality control, Crit. Rev. Anal. Chem. 43 (4) (2013) 224–272, https://doi.org/10.1080/10408347.2012.751787.

[20] AOAC, Total dietary fiber in foods, enzymatic-gravimetric method, in: Official Methods of Analysis of AOAC International, 17th ed. 985.29, 2003.

[21] EC, Commission Regulation, No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (Text with EEA relevance), 2005.

[22] ISO, Microbiology of food and animal feeding stuffs - horizontal methods for the detection and enumeration of Enterobacteriaceae - part 2: Colony-count method, 2004. ISO 21528-2:2004.

[23] ISO, Microbiology of food and animal feeding stuffs - horizontal method for the enumeration of Clostridium perfringens - Colony-count technique, 2004. ISO 7937, 2004.

[24] ISO, Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of Listeria spp. - Part 1: Detection method, 2017. ISO 11290-1, 2017.

[25] ISO, Microbiology of food and animal feeding stuffs - horizontal method for the detection of Salmonella spp, 2002. ISO 6579, 2002.

[26] D.J. Lane, 16S/23S rRNA sequencing, in: E. Stackebrandt, M. Goodfellow (Eds.), Nucleic Acid Techniques in Bacterial Systematics, J. Wiley, Sons, New York, 1991, pp. 115–175.

[27] G. Muyzer, E.C. de Waal, A.G. Uitterlinden, Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA, Appl. Environ. Microbiol. 59 (3) (1993) 695–700, https://doi.org/10.1128/aem.59.3.695-700.1993.

[28] T.J. White, T.D. Bruns, S. Lee, J. Taylor, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: M. Innis, D. Gelfand, J. Shinsky, T.J. White (Eds.), PCR Protocols: A Guide to Methods and Applications, 1990, pp. 315–322.

[29] IOC, Method for the Sensory Analysis of Table Olives COI/OT/MO/Doc. No 1/Rev. 2, International Olive Oil Council, Madrid, Spain, 2011.

[30] F.V. Romeo, Microbiological Aspects of Table Olives, In Olive Germplasm - The Olive Cultivation, Table Olive and Olive Oil Industry in Italy, InTech, 2012, https://doi.org/10.5772/51479.

[31] L. Zoratti, K. Karppinen, A.L. Escobar, H. Häggman, L. Jaakola, Light-controlled flavonoid biosynthesis in fruits, Front. Plant Sci. 5 (534) (2014) 1–16, https://doi.org/10.3389/fpls.2014.00534.

[32] L. Panjai, S. Röhlen-Schmittgen, J. Ellenberger, G. Noga, M. Hunsche, A. Fiebig, Effect of postharvest irradiation with red light on epidermal color and carotenoid concentration in different parts of tomatoes, J. Food Meas. Charact. 15 (2021) 1737–1746, https://doi.org/10.1007/s11694-020-00770-0.

[33] O. Livadariu, C. Maximilian, B. Rahmanifar, C.P. Cornea, LED technology applied to plant development for promoting the accumulation of bioactive compounds: a review, Plants 12 (5) (2023) 1075, https://doi.org/10.3390/plants12051075.

[34] J. Fernández-Bolaños, R. Rodríguez, C. Saldaña, A. Heredia, R. Guillén, A. Jiménez, Factors affecting the changes in texture of dressed ("aliñadas") olives, Eur. Food Res. Technol. 214 (2002) 237–241, https://doi.org/10.1007/s00217-001-0439-0.

[35] C. Fadda, A. Del Caro, A.M. Sanguinetti, A. Piga, Texture and antioxidant evolution of naturally green table olives as affected by different sodium chloride brine concentrations, Grasas Aceites 65 (1) (2014) e002, https://doi.org/10.3989/gya.037213.

[36] D.A. Anagnostopoulos, V. Goulas, E. Xenofontos, C. Vouras, N. Nikoloudakis, D. Tsaltas, Benefits of the use of lactic acid bacteria starter in green cracked Cypriot table olives fermentation, Foods 9 (1) (2019) 17, https://doi.org/10.3390/foods9010017.

[37] IOC, Trade Standard Applying to Table Olives (Resolution No. RES-2/91-IV/04) 19, 2004. http://www.internationaloliveoil.org/estaticos/view/222-standards (Accessed on 20th of December 2023).

[38] M. Perricone, A. Bevilacqua, M.R. Corbo, M. Sinigaglia, Use of Lactobacillus plantarum and glucose to control the fermentation of "Bella di Cerignola" table olives, a traditional variety of Apulian region (southern Italy), J. Food Sci. 75 (7) (2010), https://doi.org/10.1111/j.1750-3841.2010.01742.x. M430-6.

[39] R. Bhat, L.C. Suryanarayana, K.A. Chandrashekara, P. Krishnan, A. Kush, P. Ravikumar, Lactobacillus plantarum mediated fermentation of Psidium guajava L. fruit extract, J. Biosci. Bioeng. 119 (4) (2015) 430–432, https://doi.org/10.1016/j.jbiosc.2014.09.007.

[40] R.K. Salar, S.S. Purewal, M.S. Bhatt, Optimization of extraction conditions and enhancement of phenolic content and antioxidant activity of pearl millet fermented with aspergillus awamori MTCC-548, Resour. Effic. Technol. 2 (3) (2016) 148–157, https://doi.org/10.1016/j.reffit.2016.08.002.

[41] R.L. Johnson, A.E. Mitchell, Reducing Phenolics related to bitterness in table olives, J. Food Qual. 2018 (5) (2018) 1–12, https://doi.org/10.1155/2018/3193185.

[42] B. Lanza, Nutritional and sensory quality of table olives, in: I. Muzzalupo (Ed.), Olive Germplasm - the Olive Cultivation, Table Olive and Olive Oil Industry in Italy, InTech, Rijeka, Croatia, 2012, pp. 343–372, https://doi.org/10.5772/51723.

[43] J. Rocha, N. Borges, O. Pinho, Table olives and health: a review, J. Nutr. Sci. 9 (57) (2020) 1–16, https://doi.org/10.1017/jns.2020.50.

[44] P. Pires-Cabral, T. Barros, P. Nunes, C. Quintas, Physicochemical, nutritional and microbiological characteristics of traditional table olives from southern Portugal, Emir. J. Food. Agric. 30 (7) (2018) 611–620, https://doi.org/10.9755/ejfa.2018.v30.i7.1747.

[45] N. Rodrigues, C. Oliveira, S. Casal, J.A. Pereira, E. Ramalhosa, Table olive flours: an ingredient rich in bioactive compounds? Appl. Sci. 12 (3) (2022) 1661, https://doi.org/10.3390/app12031661.

[46] R. Malheiro, P. Mendes, F. Fernandes, N. Rodrigues, A. Bento, J.A. Pereira, Bioactivity and phenolic composition from natural fermented table olives, Food Funct. 5 (12) (2014) 3132–3142, https://doi.org/10.1039/C4FO00560K.

[47] P. Conte, C. Fadda, A. Del Caro, P.P. Urgeghe, A. Piga, Table olives: an overview on effects of processing on nutritional and sensory quality, Foods 9 (4) (2020) 514, https://doi.org/10.3390/foods9040514.

[48] S.Y. Jeong, P. Velmurugan, J.M. Lim, B.T. Oh, D.Y. Jeong, Photobiological (LED light)-mediated fermentation of blueberry (Vaccinium corymbosum L.) fruit with probiotic bacteria to yield bioactive compounds, LWT Food Sci. Technol. 93 (2018) 158–166, https://doi.org/10.1016/j.lwt.2018.03.038.

[49] T.I. Karu, in: T.I. Karu (Ed.), The Science of Low Power Laser Therapy, Gordon, Breach Science, 1998.

[50] S.S. Chiang, Z.C. Liang, Y.C. Wang, C.H. Liang, Effect of light-emitting diodes on the production of cordycepin, mannitol and adenosine in solid-state fermented rice by Cordyceps militaris, J. Food Compos. Anal. 60 (2017) 51–56, https://doi.org/10.1016/j.jfca.2017.03.007.

[51] T.I. Karu, S.F. Kolyakov, Exact action spectra for cellular responses relevant to phototherapy, Photomed. Laser Surg. 23 (4) (2005) 355–361, https://doi.org/10.1089/pho.2005.23.355.

[52] P.J.L. Crugeira, et al., Photobiological effect of laser or LED light in a thermophilic microbial consortium, J. Photochem. Photobiol. B 181 (2018) 115–121, https://doi.org/10.1016/j.jphotobiol.2018.03.006.

[53] J. Widomska, R. Welc, W.I. Gruszecki, The effect of carotenoids on the concentration of singlet oxygen in lipid membranes, BBA-Biomembranes. 1861 (4) (2019) 845–851, https://doi.org/10.1016/j.bbamem.2019.01.012.

[54] T.C.L. de Carvalho, C.A. Nunes, Smartphone-based method for the determination of chlorophyll and carotenoid contents in olive and avocado oils: an approach with calibration transfer, J. Food Compos. Anal. 104 (2021) 104164, https://doi.org/10.1016/j.jfca.2021.104164.

[55] F.N. Arroyo-López, et al., Potential benefits of the application of yeast starters in table olive processing, Front. Microbiol. 161 (3) (2012) 1–4, https://doi.org/10.3389/fmicb.2012.00161.

[56] A. Hernández, A. Martín, E. Aranda, F. Pérez-Nevado, M.G. Córdoba, Identification and characterization of yeast isolated from the elaboration of seasoned green table olives, Food Microbiol. 24 (4) (2007) 346–351, https://doi.org/10.1016/j.fm.2006.07.022.

[57] J. Bautista-Gallego, F. Rodríguez-Gómez, E. Barrio, A. Querol, A. Garrido-Fernández, F.N. Arroyo-López, Exploring the yeast biodiversity of green table olive industrial fermentations for technological applications, Int. J. Food Microbiol. 147 (2) (2011) 89–96, https://doi.org/10.1016/j.ijfoodmicro.2011.03.013.

[58] T. Silva, et al., Characterization of yeasts from Portuguese brined olives, with a focus on their potentially probiotic behavior, LWT Food Sci. Technol. 44 (6) (2011) 1349–1354, https://doi.org/10.1016/j.lwt.2011.01.029.

[59] C.L. Randazzo, A. Ribbera, I. Pitino, F.V. Romeo, C. Caggia, Diversity of bacterial population of table olives assessed by PCR-DGGE analysis, Food Microbiol. 32 (1) (2012) 87–96, https://doi.org/10.1016/j.fm.2012.04.013.

[60] B. Lanza, Abnormal fermentations in table-olive processing: microbial origin and sensory evaluation, Front. Microbiol. 4 (2013) 1–7, https://doi.org/10.3389/fmicb.2013.00091.

[61] DAGDR, Azeitona Galega da Beira Baixa. https://tradicional.dgadr.gov.pt/pt/cat/azeites-eazeitonas/1030-azeitona-galega-da-beira-baixa, 2022 (Accessed on 2nd June 2023).