INSTITUTO POLITÉCNICO DE BRAGANÇA Escola Superior Agrária

Université Libre De Tunis
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Evaluation of LED photostimulation in the fermentation process of table olives and its impact on physico-chemical and microbiological parameters

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Dissertation submited to Escola Superior Agrária de Bragança to obtain the Degree of Master in Food Quality and Safety under the scope of the double diploma with Université Libre de Tunis

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Bragança
2024

Acknowledgments

First and foremost, I would like to extend my heartfelt gratitude to everyone who played a part in this incredible journey. I am deeply grateful to my supervisors, Doctor Maria de Fátima Tomé Martins and Doctor Pedro Jorge Louro Cruigeira, for their unwavering support, guidance, and patience throughout my study and work in the laboratory. Their constant availability, kindness, and insightful suggestions have been invaluable, and I am immensely grateful for the knowledge and encouragement they provided me every step of the way. Their dedication to my growth, both academically and personally, is something I will always cherish.

I would also like to extend my sincere thanks to my supervisor at my home university, Université Libre de Tunis, Doctor Ichrak Charfi. Her continuous support, insightful advice, and encouragement have been vital to my journey. I am deeply appreciative of her mentorship and belief in my abilities.

My heartfelt gratitude goes to my family, who have always believed in me, stood by my side, and provided me with endless support and motivation. Without their constant encouragement and love, this achievement would not have been possible.

I am equally grateful to my colleagues from the Agro-industries Laboratory, who shared this journey with me. Their friendship, collaboration, and willingness to assist made my time in the laboratory a truly enriching experience. Special thanks to Doctor Elsa Cristina Dantas Ramalhosa for her guidance during the laboratory work and for her motivational words, advice, and continuous support, which helped me navigate through the challenges I encountered.

I would also like to acknowledge both institutions, the Polytechnic Institute of Bragança and Université Libre de Tunis, for providing me with this incredible opportunity to pursue a double diploma program. This mobility program has allowed me to broaden my horizons and gain invaluable experiences, and I am thankful to all the staff members from both universities who have made this journey possible.

Lastly, I want to thank all my friends for their unwavering support throughout this time. Your encouragement, understanding, and belief in me have been a constant source of strength, and I am deeply grateful to have you by my side.

The work presented had the financial support of the project "**OleaChain - Skills for sustainability and innovation in the value chain of traditional olive groves in the Northern Interior of Portugal**" (NORTE-06-3559 FSE-000188).

Abstract

The consumption of fermented foods has increased in recent years due to their potential health benefits. Table olives are a fermented vegetable product, obtained from the fruits of the olive tree (*Olea europaea* L.), and are considered an integral part of the Mediterranean diet. Recognized for their sensory, chemical, and nutritional characteristics, they have an autochthonous microflora of yeasts and lactic acid bacteria (LAB), considered a source of probiotics. Among the various methods of obtaining table olives, natural fermentation stands out. Carried out due to the action of microorganisms present in the fruits, it is an artisanal, spontaneous, and very slow process, requiring several months to remove the bitterness of the olives and obtain a quality product. This process depends on several factors, essentially the interactions resulting from the consortium of microorganisms inhabiting this environment. In the last decade, table olive producers have been improving the process by introducing some practices, that promote microbial activity, but the time required to obtain edible olives is still very long.

Photostimulation with LED light is a practice that has been explored in several areas, including food processing. Light acts as a promoter of the growth of microorganisms, interfering with bioprocesses. In this sense, this study evaluated the effect of LED photostimulation on the natural fermentation of Negrinha de Freixo black olives, focusing on physicochemical, microbiological, and sensory changes. The fermentation process was monitored under red LED light irradiation (630 ± 10 nm) to assess its impact on pH, titratable acidity, salt content, total phenolic compounds, texture, color, and microbial growth in both olives and brine. Results revealed that LED irradiation significantly influenced the fermentation dynamics, promoting higher microbial activity, particularly in yeasts and lactic acid bacteria (LAB), which accelerated the acidification process and improved microbial counts compared to non-irradiated samples.

The irradiated olives retained higher hardness and cohesiveness, indicating better structural integrity. In terms of color, there was an increased lightness, redness, and chroma in irradiated olives, enhancing the visual appeal of the final product. The total phenolic content in irradiated olive samples decreased, suggesting that LED light may help reduce bitterness. Sensory analysis indicated that irradiated olives had improved taste attributes, such as enhanced saltiness and reduced bitterness, along with fewer fermentation defects.

These findings suggest that LED photostimulation can optimize the natural fermentation process by enhancing microbial growth, preserving texture, and improving sensory qualities, potentially offering a sustainable approach to reducing fermentation time and enhancing the quality of table olives. The study supports the application of LED light as a promising strategy in the table olive industry, with implications for improving the efficiency and quality of the traditional fermentation process.

Keywords: Fermented food, table olive, LED irradiation.

Resumo

O consumo de alimentos fermentados tem aumentado nos últimos anos devido aos seus potenciais benefícios para a saúde. A azeitona de mesa é um produto vegetal fermentado, obtido a partir de frutos da oliveira (*Olea europaea* L.), sendo considerada parte integrante da dieta mediterrânica. Reconhecida pelas suas características sensoriais, químicas e nutricionais, apresentam uma microflora autóctone de leveduras e bactérias acido lácticas (BAL), sendo consideradas fonte de probióticos. Entre os vários métodos de obtenção de azeitona de mesa, destaca-se a fermentação natural. Realizada devido à ação de microrganismos presentes nos frutos, é um processo artesanal, espontâneo e muito lento, sendo necessários vários meses para a remoção do amargor das azeitonas e obtenção de um produto de qualidade. Este processo depende de vários fatores, mas essencialmente das interações resultantes do consorcio de microrganismos que habitam este ambiente. Na última década, os produtores de azeitona de mesa têm vindo a melhorar o processo introduzindo algumas práticas que promovem a atividade microbiana, porém o tempo necessário para obter azeitonas edíveis ainda é muito longo. O recurso à foto-estimulação com luz LED é uma prática que tem sido explorada em diversas áreas, incluindo no processamento de alimentos. A luz atua como um impulsionador do crescimento de microrganismos, interferindo em bioprocessos. Neste sentido, este estudo avaliou o efeito da fotostimulação por LED na fermentação natural de azeitonas pretas Negrinha de Freixo, ao nível das alterações físico-químicas, microbiológicas e sensoriais. O processo de fermentação foi monitorado sob irradiação de luz LED vermelha (630 ± 10 nm) para avaliar seu impacto no pH, acidez titulável, teor de sal, compostos fenólicos totais, textura, cor e crescimento microbiano em azeitonas e salmoura. Os resultados revelaram que a irradiação LED influenciou significativamente a dinâmica da fermentação, promovendo maior atividade microbiana, especialmente de leveduras e bactérias lácticas (LAB), o que acelerou o processo de acidificação e melhorou as contagens microbianas em comparação com as amostras não irradiadas. As azeitonas irradiadas mantiveram maior dureza e coesão, indicando melhor integridade estrutural.

A nível da cor verificou-se aumento na luminosidade, vermelhidão e croma nas azeitonas irradiadas, melhorando o aspeto visual do produto final. O conteúdo total de fenólicos nas amostras de azeitonas irradiadas diminuiu, sugerindo que a luz LED pode ajudar a reduzir o amargor. A análise sensorial indicou que as azeitonas irradiadas apresentaram melhores

atributos de sabor, como maior salinidade e menor amargor, além de menos defeitos de fermentação.

Os resultados obtidos sugerem que a fotostimulação por LED favorece o processo de fermentação natural ao melhorar o crescimento microbiano, preservar a textura e aprimoriar as qualidades sensoriais, oferecendo uma abordagem sustentável para reduzir o tempo de fermentação e melhorar a qualidade das azeitonas de mesa. O estudo apoia a aplicação da luz LED como uma estratégia promissora na indústria de azeitonas de mesa, com implicações para a melhoria da eficiência e qualidade do processo de fermentação tradicional.

Palavras-chaves: Alimento fermentado, azeitona de mesa, irradiação LED.

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Chapter 1

Framework and objectives

1. Framework and objectives

Table olives are considered one of the oldest fermented vegetables in the Mediterranean region. According to the International Olive Oil Council (IOOC) (2004), table olives are defined as "the product prepared from the sound fruits of varieties of the cultivated olive trees (*Olea europaea* L.) that are chosen for their production of olives whose volume, shape, flesh-to-stone ratio, fine flesh, taste, firmness, and ease of detachment from the stone make them particularly suitable for processing; treated to remove their bitterness and preserved by natural fermentation; or by heat treatment, with or without the addition of preservatives; packed with or without covering liquid."

In Tás-os-Montes region, table olives from the Negrinha de Freixo cultivar are recognized for their unique and differentiating characteristics, obtained through a natural, empirical, slow process without pre-treatment conducted by microorganisms present in the olives. Producers mention that it takes several months (6 to 9 months) to obtain the final product. The irradiation of LED light has favorable effects at the food fermentation level by promoting microbial metabolism.

Therefore, this study aims to achieve several objectives:

I - The aim is to assess the influence of LED irradiation at a wavelength of 630 nm on the microbial consortium present in the fermentation of Negrinha de Freixo table olives. The aim is to assess how LED irradiation affects the activity and composition of the microbial community by isolating and counting mesophilic aerobic microorganisms, yeasts, moulds, and LAB in brine and olives samples.

II - This research aims to determine the physicochemical parameters throughout fermentation, namely; color, texture, salt, pH, titratable acidity and total phenols compounds.

III - Evaluate the presence of pathogens such as *Salmonella*, *Listeria*, *Enterobacteriaceae*, and sulphite-reducing clostridia at the end of fermentation. Finally, the research aims to analyze the sensory value of the table olives at the end of the process. These parameters are crucial to understanding the evolution of the fermentation process and the impact of LED light irradiation on table olives' nutritional content and sensory characteristics.

Chapter 2

Introduction

2. Introduction

2.1Table Olives

Table olives are one of the most processed vegetable products and have great economic importance worldwide (Perpetuini et al., 2020). More than a fermented product, table olives are currently considered an important food source, being described as the food of the future (Bonatsou et al., 2018).

This fermented vegetable has several noteworthy characteristics. For one, table olives contain of various attractive nutritional elements, including monounsaturated and polyunsaturated fats, fiber, vitamins, and minerals. On the other hand, they present significant concentrations of well-known bioactive compounds, such as; hydroxytyrosol, tyrosol, oleuropein, and oleocanthal among others (Bautista-Gallego et al., 2011).

These compounds have several health benefits, including anti-inflammatory and cardiovascular protective effects. Furthermore, table olives may serve as carriers of beneficial microorganisms to consumers representing another intriguing aspect from a functional standpoint (Peres et al., 2012).

Olives, have a high content of phenolic compounds, responsible for the bitterness of the fruits, and must undergo treatment before they can be consumed. The treatment to make olives edible can be carried out using different processes, some of which are very well known and applied, such as the preparation of table olives through Greek or Natural Fermentation (natural olives) Sevillian or Spanish (green olives in brine), Californian (oxidized black olives). It should be noted that the natural fermentation of olives is the most common process and still the most used in the Trás-os-Montes region, for the preparation and/or production of table olives (Campus et al., 2018).

According to the International Olive Council (IOC, 2004) and the Codex Alimentarus (2013), different types of table olives can be found on the market, depending on the degree of ripeness, shape, size, and color. Are considered;

i) green olives, those obtained from fruits harvested during the ripening period, presenting a green to straw-yellow color and which have reached normal size;

ii) mixed olives, the fruits harvested before reaching full maturity, at the time of color change, which can vary between pinkish and brownish tones;

iii) black olives correspond to fruits harvested at the moment they have reached full ripeness or slightly before ripening, and their color may vary between black and red, to dark brown, passing through violet tones.

2.2 Negrinha de Freixo Table Olive

Portuguese Negrinha de Freixo olive, protected designation of origin (PDO) is the fruit of the olive tree (*Olea europeae sativa* Hoffg Link) of the Negrinha cultivar, with great agricultural importance as a table olive in Portugal [Commission Regulation (EC) No 1107/96]. Its geographical production area is restricted to the Trás-os-Montes region, in the municipalities of Vila Nova de Foz Côa, Freixo de Espada à Cinta, Torre de Moncorvo, Alfândega da Fé, Vila Flor, Mirandela, and Macedo de Cavaleiros (Figure 1). The cultivated area is 4178 ha and is largely irrigated.

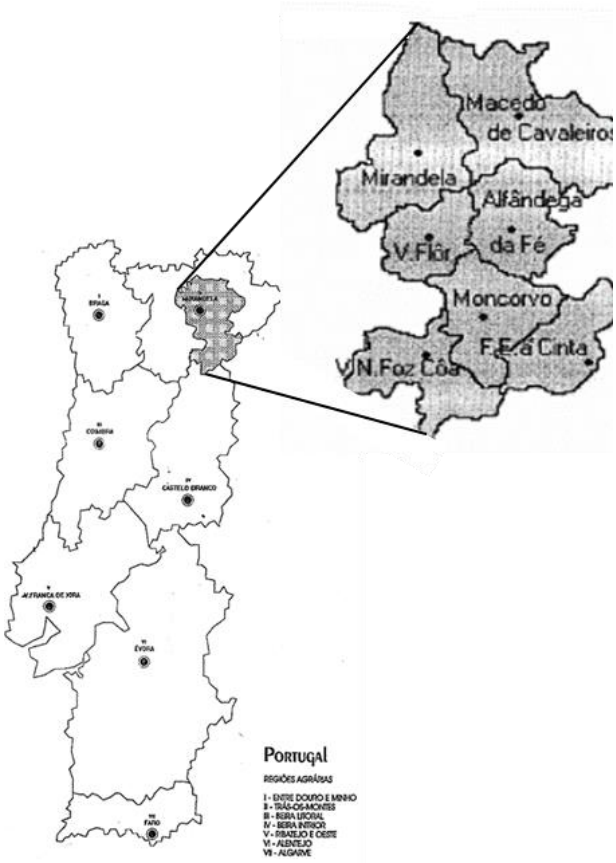


Figure 1: Geographic area of the PDO table olives Negrinha de Freixo (adapted from the specification notebook).

The climatic conditions of this region with very low precipitation, winters are cold and summers are hot and dry allow the production of quality table olives without the use of pesticides (DGADR, 2022), a product rich in valuable bio compounds with different beneficial properties for health. The Negrinha de Freixo variety olive has a rounded shape, a small pit that easily detaches when pitted, but has a low oil yield, generally not exceeding 15%.

The olives used in the production of this table olive can be of the green type, treated black, or mature olives, in brine, undergoing different types of preparation.

Table 1 describes the morphological characteristics of the black olive Negrinha de Freixo, as indicated in the specification's booklet (Ministério da Agricultura (s/d).

Table 1: Description of the morphological characteristics of the Negrinha de Freixo black table olive

Morphological Characteristics of the Negrinha de Freixo black olive	
Specifications Notebook of Negrinha de Freixo black olive - PDO (Protected Designation of Origin)	- Spherical shape ending in a slight beak - Weight from 3 to 5 grams - Volume from 3 to 5 cm^3 - The ripe olive is intense black - Fat yield is from 12 to 15% - Pulp percentage is about 83% - Caliber must be less than 400 fruits/kg

Table olives are obtained according to the natural fermentation procedure, where the fruit is placed directly in brine with a salt concentration that can vary between 6 and 10 % (m/v) (DGADR, 2022). The result is a unique product with different organoleptic, sensory, and textural characteristics.

2.3 Processing methods

The main objective of technological treatments to obtain table olives is to remove the natural bitterness of the fruits, mainly attributed to oleuropein, which is the most abundant phenolic compound in unprocessed olives. Among the various treatments, the most important worldwide are the Seville or Spanish (green olives), the Californian or American (oxidized black olives), and the Greek (natural fermentation) (Campus et al., 2018).

In the Sevillian and Californian processes, the bitterness of the fruit resulting from phenolic compounds is removed by treatment with sodium hydroxide (NaOH) solution. Depending on the region, different table olive production methods can be applied. The olive tree variety, climatic conditions, and all the empirical knowledge acquired over several generations are considered essential factors (Borges et al., 2013).

In the Trás-os-Montes region, the production of table olives has a great tradition, and this food is considered an integral part of local gastronomy. Natural fermentation (natural curing) is the most common and most applied process to table olives (Borges et al., 2013).

2.4 Natural fermentation

The natural fermentation of table olives is an archaic method, which is fundamentally based on curing in water/brine, the result of empirical knowledge, tradition, the intergenerational transfer of flavors, and the characteristics of the desired final product. It applies to green olives (harvested early in ripening), mixed olives, and black olives (harvested late in ripening) (Gandul-Rojas and Gallardo-Guerrero, 2020).

The removal of phenolic compounds present in olives is done slowly, without adding chemical compounds, through osmotic processes and enzymatic activity, resulting from the microflora present in the fruit skin (Gandul-Rojas and Gallardo-Guerrero, 2020).

Olives harvested manually from the tree, free from pests and diseases, are carefully transported, and washed in running water to remove surface dirt, selected by size or ripeness index. Then, olives are immersed in brine with a salt concentration ranging from 8% to 10% and fermentation occurs, where several microorganisms play an important role. However, depending on the climatic conditions of the production region, the concentration of salt added to the brine may be lower (Bleve et al., 2014).

Fermentation, which takes place over 8 to 12 months, is driven predominantly by a mixed population of lactic acid bacteria (LAB) and yeast. The table olives resulting from this process have unique and different organoleptic characteristics (Bautista-Gallego et al., 2011).

2.5 The consortia of microorganisms present in olive fermentation

The natural fermentation process of table olives involves a complex interaction of several microorganisms, each contributing to the transformation of olives into quality food products. The Enterobacteriaceae, LAB, and yeasts are the most relevant. The growth of Enterobacteriaceae members is only observed at the beginning of fermentation, reaching their maximum population between days three and four, disappearing after 7 to 15 days. The main genera found are *Citrobacter*, *Klebsiella*, and *Escherichia* (Hurtado et al., 2008).

Lactic acid bacteria are Gram-positive bacteria, which can be grouped into homofermentative or heterofermentative. The homofermentative bacteria produce lactic acid as the main product of glucose fermentation. The heterofermentative, in addition to lactic acid, forms other substances, such as carbon dioxide, acetic acid, and ethanol, from the fermentation of glucose. They have a complex nutritional requirement that grows only in nutrient-rich media (Abedi and Bagher-Hashemi., 2020).

Lactobacillus is the main isolated genus from the most diverse olive tree cultivars, in green or black olives and the predominant species are *L. plantarum* and *L. pentosus*, followed by *L. paraplantarum*. However, the presence of other species such as *Lactobacillus brevis*, *Lactobacillus casei*, and *Enterococcus* spp. was reported in the fermentation process of green olives not treated with NaOH (Randazzo et al., 2004).

These species play an important role in the olive fermentation process, due to their ability to produce organic acid and antimicrobial compounds. These compounds promote rapid acidification of the brine and inhibit the development of pathogenic microorganisms. Additionally, LAB produces several enzymes such as esterases, lipases, and β-glucosidases, which are involved in the hydrolysis of oleuropein present in olives (Arroyo-López et al., 2012).

Yeasts appear as dominant microorganisms throughout the process and have a dual function. They positively promote fermentation through the degradation of phenolic compounds and the production of vitamins that favor the development and growth of

LAB. However, they can cause the formation of gas pockets, softening of olive tissues, cloudiness of brines, and production of unpleasant flavors and odors (Arroyo-López et al., 2008).

The dominant yeasts belong to the genera; *Candida*, *Cryptococcus, Debaryomyces, Pichia, Rhodotorula, Saccharomyces, Zygosaccharomyces,* and, in small numbers, *Cyteromyces, Dekkera, Metschnikowia, Schwanniomyces, Sporobolomyces* and *Zygotorulaspora* (Sidari et al., 2019).

Microorganisms are important for the production of table olives and are influenced by factors such as the composition of the brine, acidity, salt concentration, olive variety, olive phenolic content, temperature, and oxygen availability (Penland et al., 2020).

Therefore, knowing the dynamics of the microbiota that occurs during the fermentation process is extremely important, as it allows us to understand more clearly the role of each microorganism, as well as justify the changes that occur throughout the entire fermentation process (Penland et al., 2020).

2.6 Impact of LED irradiation on food quality enhancement

LED (Light-Emitting Diode) light is a type of lighting that utilizes a semiconductor to emit light when an electric current passes through it. It is known for its energy efficiency, durability, and the ability to produce light in a variety of colors. The wavelength of the emitted radiation depends on the energy band between two consecutive levels of the semiconductor material. Therefore, the radiation emitted by LEDs can range from ultraviolet to infrared depending on the semiconductor materials used (Gayral, 2017). UV (Ultraviolet) light is a form of electromagnetic radiation not visible to the human eye. It is commonly used for sterilization, industrial curing, and other applications. The main difference between LED and UV light lies in their mechanisms of light production and their potential impact on human health (Prasad et al., 2020).

LED light is considered safer than UV light as it transmits rays of lower frequency and higher wavelengths, making it less hazardous upon prolonged exposure. Additionally, LED light does not generate heat in the same way that UV light does, and LED bulbs typically last longer than traditional UV bulbs, contributing to reduced maintenance costs (Prasad et al., 2020).

The LED emits light within the wavelength range of 420–780 nm, as illustrated in Figure 2. Based on this wavelength range, the light can be categorized into six distinct types:

red, orange, yellow, green, blue, and violet. Each different wavelength of light has a different role. (Yao et al., 2023).

For example, the light sources in the red spectrum specifically target the cytochrome C protein complex, stimulating proton pumping capacity. This stimulation leads to an immediate increase in cellular ATP production and availability. (Crugeira et al., 2022).

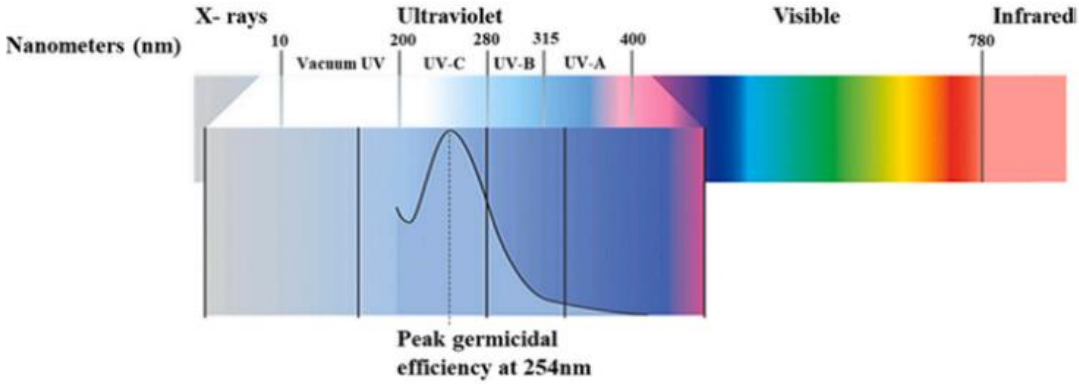


Figure 2: UV Spectrum (Patras et al., 2021).

The LED system is described in Figure 3. LEDs are made up of p-type and n-type semiconductor materials that form a p-n structure. The p-type carriers are electrons, while the n-type carriers are holes. When a positive bias is applied, which means an electric field is applied, the electrons and holes interact and combine, leading to the release of excited electrons in the form of light. This is the fundamental principle behind LED light. (Yao et al., 2023).

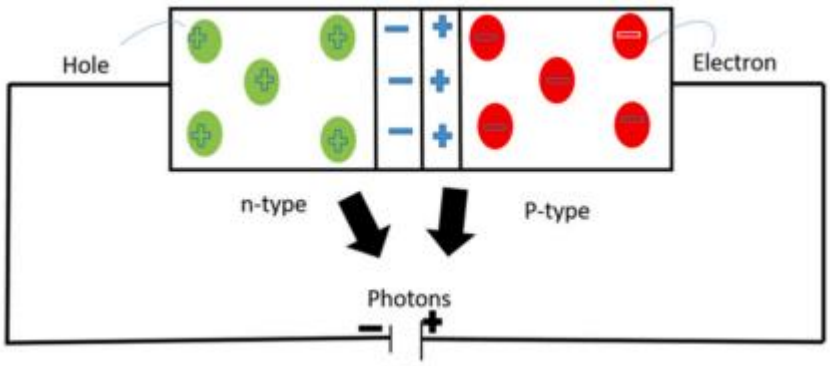


Figure 3: LED light emitting principal (Yao et al., 2023).

Research conducted by Poonia et al. (2022) investigated the application of light-emitting diodes (LEDs) in the preservation of various food items, focusing on the effects of specific wavelengths (ranging from 450 nm to 660 nm) on the production of bioactive

compounds. The study aimed to evaluate how LED illumination influences the accumulation of polyphenols, anthocyanins, flavonoids, and antioxidants in foods such as fruits and vegetables. The findings indicated that specific wavelengths of LED light can significantly enhance the levels of these beneficial compounds, thereby improving the nutritional quality of the food.

LEDs under controlled conditions in agricultural produce have been shown to increase the nutritional profile of various crops. For example, the combined effect of red and blue LED light has been reported to increase the accumulation of bioactive compounds in plants (Poonia et al., 2022). Red and blue LEDs have been found to stimulate the synthesis of bioactive compounds by influencing the activity of enzymes such as phenylalanine ammonia-lyase (PAL), which plays a crucial role in the induction of secondary metabolites in plants (Poonia et al., 2022). Additionally, blue and red LED light has been shown to promote plant growth, increasing the production of flavonoids, glycosides, and phenolic compounds (Poonia et al., 2022). LED technology is rapidly gaining popularity as a tool for growing greenhouse crops and preserving food, (Poonia et al., 2022). Other research found that red LED lights (620-645nm) increased the overall production of vinegar by 20% compared to other LED lights and non-LED conditions. Vinegar produced under red LEDs exhibited less cytotoxicity and high anti-inflammatory activity compared to commercial vinegar (Lim et al., 2022).

Further study carried out by Jeong et al. (2018) investigates the effects of different LED lights on the fermentation of blueberry fruit powder by *Bacillus amyloliquefaciens* and *Lactobacillus brevis*. The main objective was to explore how LED light colors (green, red, blue, white) and sunlight influence the fermentation process, focusing on viable cell count, total phenolic content, and total flavonoid content. The study found that LED lights and sunlight affect the antioxidant, antibacterial, and cytotoxic properties of the fermented product (Jeong et al., 2018).

Similar studies have demonstrated how different LED light conditions (including various wavelengths and combinations) affect the production of bioactive compounds in *Cordyceps militaris* cultivated on brown rice. It was found that different LED wavelengths and their combinations significantly impact the production of cordycepin, mannitol, and adenosine (Chiang et al., 2017). Red light was optimal for biomass growth, while green, red, and blue lights were most effective for producing cordycepin, mannitol, and adenosine, respectively. The study concludes that combinations of LED lights are

more beneficial than single wavelengths for enhancing the content of these bioactive compounds in *Cordyceps militaris* (Chiang et al., 2017).

Therefore, specific wavelengths and combinations of LED light can be adapted to enhance the production of bioactive compounds in various food products, ultimately contributing to improved nutritional quality and health benefits(Poonia et al., 2022).

LED light interacts with biological systems by influencing various biochemical and metabolic processes, and its effects are highly dependent on the wavelength. Studies have shown that blue LED light (around 450 nm) stimulates the activity of enzymes such as phenylalanine ammonia-lyase (PAL), which plays a crucial role in the synthesis of phenolic compounds, leading to increased accumulation of flavonoids and anthocyanins (Poonia et al., 2022). Red LED light (around 640 nm) enhances photosynthetic efficiency by driving ATP and NADPH production, supporting biomass accumulation and the synthesis of secondary metabolites (Crugeira et al., 2022). Additionally, red LED light has been found to stimulate mitochondrial activity, enhancing cellular respiration and increasing ATP production, which improves metabolic functions and cellular growth (Crugeira et al., 2022). Furthermore, specific wavelengths, particularly red and blue, have been shown to regulate the expression of genes that control the biosynthesis of bioactive compounds, influencing color development, antioxidant activity, and overall nutritional quality (Chiang et al., 2017).

Hence, this study aims to assess the influence of LED irradiation on the fermentation process of Negrinha de Freixo table olives and its effects on physicochemical, microbiological parameters, and sensory value.

Table 2 describes several studies that report the impact of LED lighting on food processing. It presents a summary of various research studies on the effects of LED light treatment on different food items and shows how LED lighting influences factors like antibacterial activity, antioxidant levels, and physical-chemical properties. Each study demonstrates the potential of LED lighting to enhance food quality, shelf-life, and nutritional value during processing and storage.

Table 2: Impact of LED lighting on food processing

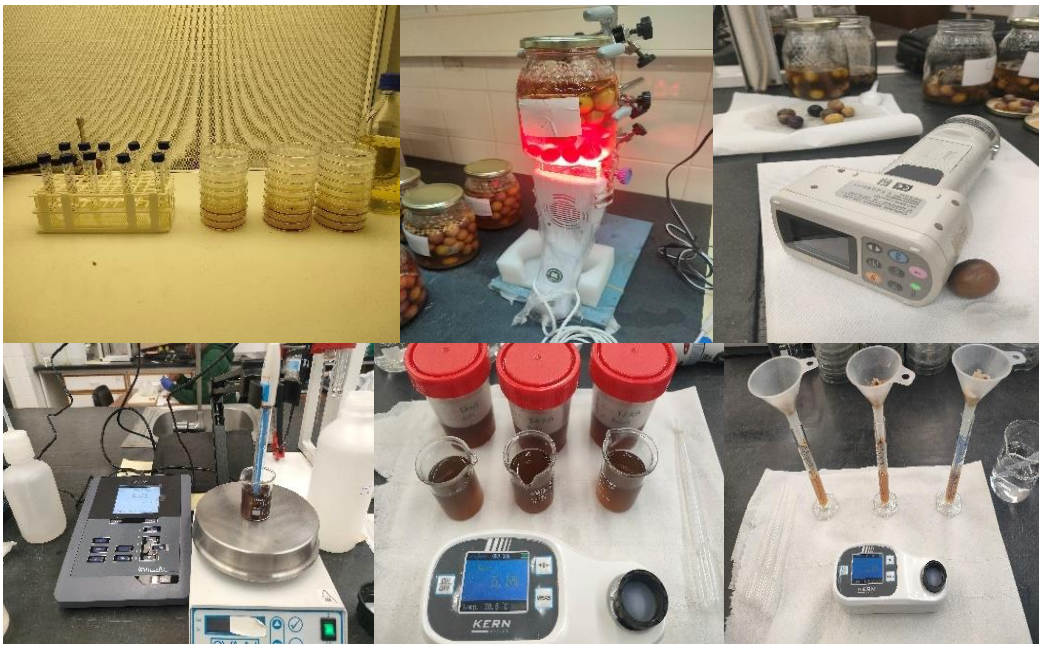
Example of food processed with LED lighting	Parameters measured	Main results	References
Purple sweet potato (*Ipomoea batatas* L.)	-Antibacterial activity -Antioxidant activity -Cytotoxic activity	The fermentation of purple sweet potato under various LED light sources produced bioactive compounds with significant antibacterial, antioxidant, and cytotoxic activities. These compounds were suggested to have potential applications in cosmetic and health-related products.	(Lee et al., 2018)
During storage of natural black table olives obtained using a starter culture.	-Total phenolic compounds -Antioxidant activity analysis -pH, Titratable acidity - Reducing sugar contents	The irradiation at different doses influenced the total phenolic content and antioxidant activity of the olives, with variations observed depending on the irradiation dose and storage duration. The study provides insights into how these processing methods can affect the quality and shelf-life of black table olives.	(Irmak et al., 2023)
The production of cordycepin, mannitol, and adenosine in solid-state fermented rice by *Cordyceps militaris*.	-Determination of ergosterol content -Determination of bioactive compounds	Different LED wavelengths and their combinations significantly affect the production of these bioactive compounds, with specific wavelengths promoting higher yields of certain compounds. This highlights the potential of LED light manipulation in enhancing the production of valuable compounds in *Cordyceps militaris* cultivation.	(Chiang et al., 2017)

Blueberry (*Vaccinium corymbosum* L.*)*	-Estimation of Bacterial (Viable Cell Count) -Total phenolic content -Total flavonoid content -Antibacterial activities -Antioxidant activity -Cytotoxicity assay	The LED light-mediated fermentation significantly affected the bioactive compounds in blueberry fruit, with variations in the antibacterial, antioxidant, and cytotoxic activities depending on the type of LED light used. This highlights the potential of using LED light in the fermentation process to enhance the production of beneficial compounds.	(Jeong et al., 2018)
Vinegar	-Total acidity measurement. -Cytotoxicity assay. -Nitric Oxide assay. -Pro-inflammatory Cytokine Production.	The red LED light significantly increased vinegar production compared to other colors and control (non-LED) conditions. Vinegar produced under red LED showed reduced cytotoxicity and enhanced anti-inflammatory properties. This research suggests potential applications of LED light manipulation in vinegar fermentation processes for improved yield and quality.	(Lim et al., 2022)
Skim milk	-Antioxidant assays - β-Galactosidase activity Measurement -Cholesterol assimilation analyses -Antibacterial activity determination -Proteolytic activity determination	The red laser exposure enhances the antioxidant, antimicrobial, and proteolytic activities of *Lactocaseibacillus casei,* improves lactose fermentation, and increases cholesterol assimilation, suggesting beneficial modifications in the fermentation process of skim milk.	(Mohamed et al., 2020)

Fresh meat	-Instrumental color measurements -Visual color evaluation. -Product internal temperature -Odor assessment. -Lipid oxidation analysis -Microbiological analysis	The LED lighting can influence meat color stability and shelf life, with variations observed across different types of meat products.	(Steele et al., 2016)
Tomato (S*olanum lycopersicum)*	-Physicochemical Quality Attributes (color, weight, firmness, total soluble solids content, pH, and titratable acidity -Bioactive Compounds (flavonoids, carotenoids, and phenolic compounds) -Total Antioxidant Compounds	The LED light treatments, particularly blue and red lights, significantly enhanced the bioactive compound content in tomatoes, including increases in phenolic acids, flavonoids, and carotenoids. The research suggests that specific LED lighting can improve the nutritional quality of tomatoes during storage.	(Martínez-Zamora et al., 2023)

Chapter 3

Experimental part



3. Experimental part

3.1 Sampling and fermentation condition

Black olives of the Negrinha de Freixo cultivar were supplied by a producer in Mirandela, Trás-os-Montes region (NE Portugal) in November 2023. The fruit was harvested by hand and transported in plastic containers to the agroindustry laboratory of the Polytechnic Institute of Bragança. The olives were then washed under running water and sorted by size. Fruit free from wounds or pests was selected and fermentation took place in transparent glass containers. Water and 7% NaCl salt were used to prepare the brine and 300 g of olives were weighed and placed directly into glass jars with 300 mL of brine (Figure 4).

Figure 4: Olives fermentation.

The fermentation process took place spontaneously over 70 days at an average temperature of 18 °C and under partially anaerobic conditions. Samples of brine and olives were collected over the fermentation process (20 days equilibration time; 35, 55, and 70 days). The samples were used to determine physicochemical parameters (texture, colour, salt, pH, titratable acidity, and total phenols) and microbiological parameters (total count of moulds, yeasts, mesophilic aerobic, and lactic acid bacteria).

3.2Irradiation process

Irradiations were carried out continuously using an LED device (Emilight, MMOptics, São Carlos, SP, Brazil) with a power of 100 mW, at wavelengths of 630 ± 10 nm, and depositing an energy density of 14 J/cm^2. An irradiation angle of 90◦ and a distance of 1 cm were used in the fermentation broth (Figure 5).

Figure 5: Irradiation process.

Two irradiations were carried out during the fermentation process: the first irradiation occurred at 21 days, and the second at 56 days of fermentation. Each irradiation persisted for 15 days, alternating with 20 days without irradiation (Figure 6).

The test was carried out in triplicate, and the irradiation was emitted without external lighting, thus avoiding interference in the process. The LED device was calibrated and energy absorption in the initial fermentation medium (7% saline (w/v)) 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 (Crugeira et al., 2022).

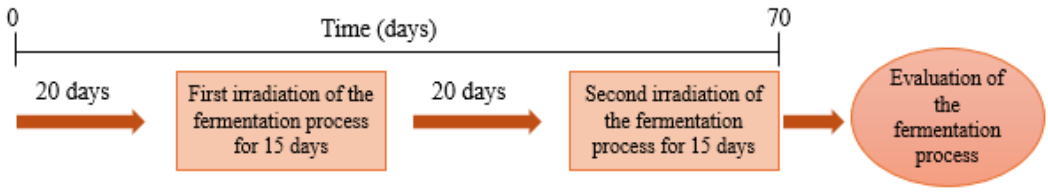


Figure 6: Description of irradiation time during the natural fermentation process of table olives.

3.3Evaluation of physicochemical parameters:

3.3.1 Color determination

The olives´ color was determined using a colorimeter, Minolta CR-400, on 10 fruits per sample. The color was assessed at 3 points on the olive to quantify spectral data and determine the sample's coordinates in the CIELAB color space, namely L*, a*, b*, C*, and h, to present this information in numerical terms. L* indicates luminosity, a* evaluates green/red tones (positive signal indicates red and negative signal indicates green), and b* corresponds to the coordinate assessing blue/yellow tones (positive signal indicates yellow and negative signal indicates blue). Saturation is represented by C*, with a higher value indicating greater intensity. h indicates hue or the actual color. Before measurement, the device was calibrated with a white standard. Figure 7 shows the procedure done for a sample.

Figure 7: Color determination.

3.3.2 Texture determination

Texture was assessed in fresh olives using a compression test, employing a texture analyzer (TA. XT Plus Texture Analyser) equipped with a 30 kg load cell. Each olive was taken directly from the jar, placed horizontally, and centered under the probe before measurement. Olive compression was performed using a flat cylindrical probe (P/36R, diameter 36 mm) at a speed of 5 mm/s and a penetration depth of 7 mm (Figure 8).

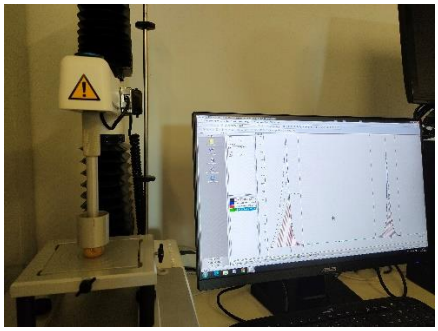


Figure 8: Texture determination.

Throughout the fermentation process, sample collections were made, and for each defined time interval, 10 fruits were collected from each jar, and 10 readings were taken. All analyses were conducted at room temperature, with data acquisition and integration achieved using Texture Exponent TPA32 software and applying the Texture Profile Analysis (TPA) test, which involves two compression cycles.

Multiple texture parameters were quantified, such as hardness (maximum force obtained during the first compression), adhesiveness (negative work between the two cycles), elasticity (ratio of relative distances to the maximum peaks determined in the second compression to the first compression (Distance 2/Distance 1)), cohesiveness (ratio of areas relative to the second compression to the first compression (Area 2/Area 1)), and chewiness (Hardness × Cohesiveness × Elasticity).

3.3.3 pH determination in olives and brine

The pH of the samples was determined by direct measurement at room temperature using a pH meter (Hanna HI-8417). The device was calibrated before measurements began with respective buffer solutions (4.00±0.02 and 7.00±0.02 at 20°C). For the brine, a volume of 20 mL of solution was used, and the pH electrode and temperature probe were inserted directly into the sample. For the olives, an aqueous solution was prepared from 5 g of pulp previously crushed in 20 mL of boiled and cooled water. Readings were always taken in triplicate (Figure 9).

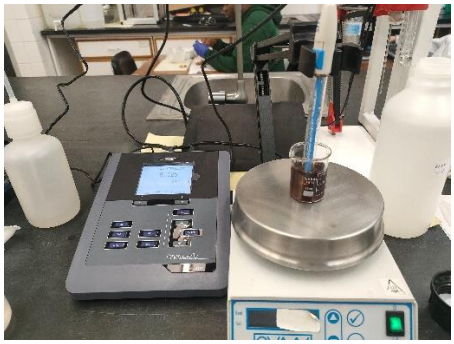


Figure 9: pH determination.

3.3.4 Determination of titratable acidity in olives and brine

The titratable acidity in fresh olives was carried out, through potentiometric titration, following Portuguese Standard NP 1421 (1997) (Figure 10).

Figure 10: Determination of titratable acidity.

A mass of 5 g of olive pulp was weighed into a round-bottom flask, to which 50 mL of previously boiled and cooled distilled water at room temperature was added. A reflux condenser was then attached, and the preparation was heated on a heating mantle for 30 minutes. After cooling to room temperature, the solution was transferred to a 100 mL flask test tube and the volume was completed with boiled water. After homogenization, the solution was filtered through gauze (two layers) into a beaker until a volume of 30 mL was obtained. A volume of 25 mL of the filtrate was measured, and titration was carried out with a 0.1 M sodium hydroxide (NaOH) solution (previously standardized) until reaching a pH of 8.1, using a pH meter (Hanna HI-8417) When necessary, the exact volume of NaOH solution required to reach a pH of 8.1 was calculated by interpolation. The titratable acidity of the olive was expressed in grams of lactic acid per 100 g of olive.

Regarding the brine, the determination of titratable acidity was performed using the same methodology described above, titrating directly 20 mL of brine solution. Acidity was expressed in grams of lactic acid per 100 mL of brine. Triplicates of each sample were always performed.

3.3.5 Determination of salt content in olive samples and brine

The determination of the salt content percentage of NaCl was carried out with a digital refractometer (KERN ORF-U), (Figure 11).

Figure 11: Determination of salt content.

To determine salt content in the brine, three drops of the solution were placed directly on the refractometer, then a black cover was placed to avoid interference from light, and the reading was taken. The salt content in olives was determined by using an aqueous solution resulting from macerating 5 g of pulp and 5 mL of previously boiled and cooled distilled water. The solution was filtered through a double-layer gauze. The filtrate was collected in a measuring cylinder, and the volume was adjusted to 6 mL. From this solution three drops were collected and read. 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 NaCl/100 mL brine).

3.3.6 Determination of total phenols in olives and brine

The brine samples were filtered using filter paper, and 100 µL of each sample was diluted in 25 mL of distilled water. Then, 0.5 mL of the diluted brine was transferred into test tubes in triplicate. To each tube, 0.5 mL of Folin-Ciocalteu reagent was added. After 3 minutes, 0.5 mL of 7.5% saturated sodium carbonate solution was introduced, followed by the addition of 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. A calibration curve with gallic acid of concentrations ranging from 0.02 to 0.4 mg/mL was previously prepared. The total phenols were extracted and determined following the protocol developed by (Ciafardini et al., 2019) with some adaptations.

For fruits, in a 15 mL falcon tube, 1.5 g of lyophilized olives were weighed, followed by the addition of 7.5 mL of acetone to remove the oil fraction. The samples were homogenized for 2 min and then centrifuged at 4000 R.P.M. for 15 min. The supernatant was removed, and 3 mL of methanol was added to the pellet, followed by homogenization and centrifugation. This procedure was repeated at least five times. The extracts were combined, the methanol was evaporated under a vacuum, and the residue dissolved in methanol to form extracts at a concentration of 25 mg/mL, then analyzed using a spectrophotometer at a wavelength of 725 nm. The analyses were performed in triplicate, and the results were expressed in mg equivalent gallic acid/ mL of brine or mg equivalent gallic acid/g of olive pulp, dry weight (d.w).

3.4 Evaluation of microbiological parameters

To understand the microbial dynamics and the influence of the irradiation throughout the fermentation process, microbiological parameters were determined in the brine and on the fruit surface. For this purpose, total microbial counts at 30°C (mesophilic aerobic), moulds, yeasts, and lactic acid bacteria were determined.

3.4.1 Sample Preparation for Analysis

Both brine and fruit samples were always processed under aseptic conditions, within a biological safety cabinet. For each evaluated time interval, a total of 9 brine samples and 9 table olive samples were collected.

From each jar, 25 mL of brine solution was removed and 25 g of fruits were weighed. The fruits were placed in Stomacher bags containing 225 mL of 0.015% (w/v) peptone water, followed by homogenization and incubation at 25°C for 10 minutes with slight agitation. Subsequently, successive decimal dilutions were performed in 9 mL of the same solution according to ISO 6887-6:2013 standard. Finally, the samples were inoculated into specific culture media for the respective microorganisms to be quantified.

The analysis of samples was conducted in triplicate, and the results were expressed in log CFU (colony-forming units) per mL or g, depending on the type of sample. The equation used for colony quantification was as follows:

$$N = \frac{\sum C}{V.(n_1 + 0{,}1 \times n_2)d}$$

Where:

N - number of colony-forming units (CFU);

ΣC - sum of colonies counted on two plates containing two successive dilutions of the sample;

n1 - number of plates selected from the first dilution;

n2 - number of plates selected from the second dilution;

d - dilution from which the first counts were obtained;

V - volume inoculated (mL).

3.4.2 Total count of mesophilic aerobic microorganisms

The total count of mesophilic aerobic microorganisms was performed according to ISO 4833-1:2013 standard, using Plate Count Agar (PCA, Himedia) as the culture medium. Inoculation was carried out by spreading 0.1 mL of suspension from each dilution onto the surface of the culture medium using a sterile spreader. After incubating the plates at 30°C for 48 hours, colony counting was performed.

3.4.3 Count of moulds and yeasts

The count of moulds and yeasts was carried out according to ISO 21527-1:2008 standard, using the plate count technique. The culture media used were *Sabouraud Dextrose Agar* (SDA, *Liofilchem*) and *Malt Extract Agar* (MEA, *Liofilchem*), supplemented with 0.1% (w/v) chloramphenicol. Inoculation was performed by spreading 0.1 mL of each decimal dilution onto the medium. The plates were incubated at 25°C for 3 days before reading.

3.4.4 Count of lactic acid bacteria

The count of lactic acid bacteria was conducted following the BS ISO 15214:1998 standard. Inoculation was performed by incorporating 1 mL of each dilution into *Man, Rogosa,* and *Sharpe* (MRS, *Himedia*) culture medium supplemented with 0.01% (w/v) cycloheximide at pH 5.7. The sample was incorporated into the culture medium through rotary movements, and after solidification, a second layer of medium was poured. Colony reading and counting were carried out after incubating the plates at 30°C for 72 hours.

3.4.5 Count of Enterobacteriaceae

The total count of Enterobacteriaceae was performed according to ISO 21528-2:2004 standard, using *Compact Dry* ETB plates (R-*Biopharm*), which consist of plates with chromogenic substrate and redox indicators. The culture medium contains glucose and selective agents for microbial differentiation and enumeration. Specific colonies are detected by red to violet coloration. 1 mL of each sample was applied to the center of the plate, and incubation was carried out at 37°C for 24 hours, followed by total colony counting.

3.4.6 Count of *Clostridium perfringens*

Selective isolation and enumeration of *Clostridium perfringens* followed the recommendations of ISO 7937:2004 standard. *Tryptone-Sulfite Cycloserine Agar* (TSC, *BioKar*) culture medium supplemented with an egg yolk emulsion and the antibiotic D-cycloserine 0.02% (w/v) was prepared. 1 mL of each dilution was inoculated and spread by incorporation into the culture medium. After solidification, a second layer of medium was applied. The plates were then incubated at 37°C for 48 hours. *Clostridium perfringens* is a sulfite-reducing *Clostridium* capable of reducing sodium sulfite to sulfide, forming a black precipitate of iron sulfide with ferric citrate. This compound will appear around the colonies. Thus, the growth of colonies with black coloration was considered a positive result.

3.4.7 Detection of *Listeria* spp. and *Salmonella* spp

The detection and enumeration of *Listeria* spp. were performed following the procedure defined by ISO 11290-1:2017, using the VIP *Gold Listeria* test, which is a one-step visual immunoassay for the detection of *Listeria* in food samples 25 g of olives were weighed and placed in a *Stomacher* bag with 225 mL of *Demi-Fraser* solution. Subsequently, homogenization was performed, and the sample was incubated at 30°C for 48 hours (sample enrichment process). After this phase, 1 mL of the solution was withdrawn and incubated at 100°C for 5 minutes (sample inactivation process). Finally, 0.1 mL of the inactivated solution was transferred to the test device, followed by a new incubation at room temperature for 10 minutes. The result was recorded, and the test was considered positive if two lines were present, one line in the test sample zone and another in the test verification zone.

The detection of *Salmonella* spp. was carried out according to ISO 6579:2002 standard, using the 1-2 Test kit (AOAC 989.13 official method). This is a rapid qualitative method for the detection of *Salmonella* in food. It is based on the observation of *Salmonella* immobilized in a medium with polyvalent antibodies (flagellar).

After incubating the sample at 35°C for 24 hours, 0.1 mL was collected and added to one side of the kit containing the iodine-iodide reagent. On the other side of the kit, the antibody was added. The result was observed after 24 hours. The presence of *Salmonella* is indicated by a white U-shaped band, the result of the binding between the antigen and the antibody.

3.5 Sensory analysis at the end of the fermentation process

The table olives were assessed after 70 days of fermentation by a trained panel from the Polytechnic Institute of Bragança. The panel was asked to evaluate the acceptability of the olives and express their preference for samples that were either exposed to LED light or not. The sensory evaluation focused on identifying negative attributes or defects (such as abnormal fermentation, putrid, butyric, and zapateria), gustatory attributes (bitterness, acidity, saltiness), and kinesthetic sensations (hardness, fibrousness, crunchiness). The assessment was recorded on a profile sheet (Figure 12) using an intensity scale from 1.0 (no perception) to 11.0 (extreme).

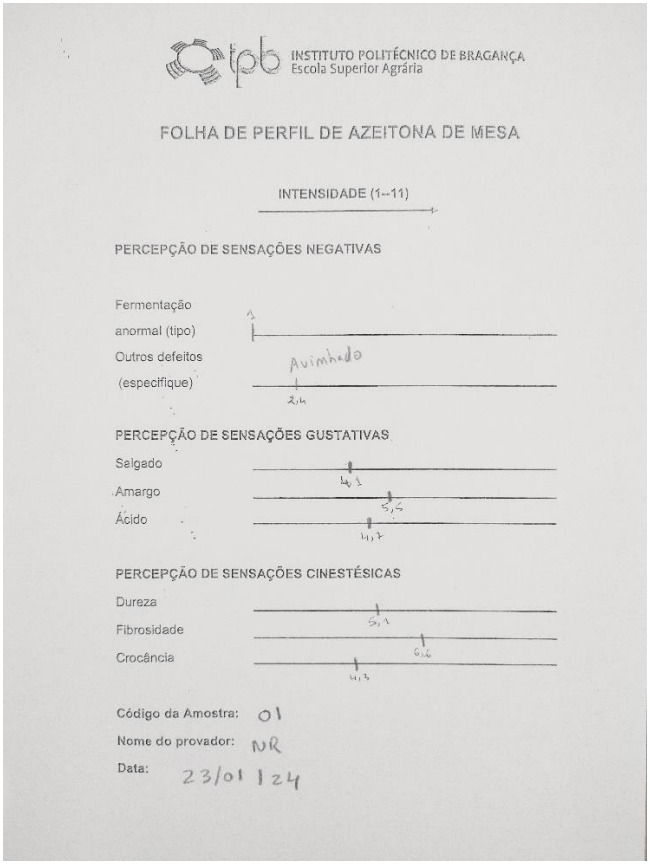


Figure 12: Sensory analysis profile sheet.

3.6 Calculations and statistical treatment

The statistical analysis of the physicochemical parameters was performed using RStudio version 4.3.2. An analysis of variance (ANOVA) was conducted to determine if there were significant differences ($p < 0.05$) between samples. If significant differences were found, a Tukey's honestly significant difference (HSD) post hoc test was applied. The compact letter display (CLD) method was used to represent significant groupings visually. The analysis utilized various R packages, including datasets, ggplot2, multcompView, and dplyr.

Chapter 4

Results and discussion

4. Results and discussion

The profile of the fermentation process of Negrinha de Freixo black table olives under the effect of LED irradiation, in 7% NaCl brine was monitored by analysis of physicochemical and microbial parameters. Fermentation was completed after 70 days and the presence/absence of pathogenic microorganisms was determined. In addition, sensory analysis was carried out by a panel of tasters.

4.1 Physicochemical parameters

4.1.1 Analysis of the color characterization

Color is a parameter that influences the rejection of olives by the consumer. Table 3 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.

The lightness (L*) of non-irradiated olives remained stable, ranging from 42.07 to 44.37, with no significant differences. In contrast, irradiated olives exhibited a marked increase in lightness, particularly from day 55 to day 70, where L* values rose significantly from 42.55 to 49.74. This suggests that LED irradiation may promote a lighter appearance in olives after 70 days of fermentation.

Redness (a*) values showed distinct patterns between the two groups. Non-irradiated olives maintained consistent a* values (8.74 to 9.09) throughout fermentation. However, irradiated olives initially displayed higher redness (14.97 at day 35), which then decreased significantly to 10.71 by day 70. This indicates that LED irradiation initially enhances red coloration, but this effect diminishes over time, this may be linked to LED-stimulated anthocyanin synthesis and degradation, a phenomenon observed in other studies involving LED-treated fruit (Jiang et al., 2019).

Yellowness (b*) exhibited interesting trends in both groups. Non-irradiated olives showed a general decrease from 18.36 to 12.04, with significant fluctuations. Irradiated olives, conversely, started with lower b* values (11.75 at day 35) but increased significantly to 18.22 by day 70. This suggests that LED irradiation may initially suppress yellow pigments, possibly carotenoids, but ultimately enhances their expression in the later stages of fermentation (Hu et al., 2012).

Chroma (C*) values, indicating color intensity, increased in both groups but more prominently in irradiated olives. Non-irradiated olives showed a gradual increase from 13.99 to 17.27, while irradiated olives maintained consistently higher values (20.23 to 22.73) throughout the observed period. This indicates that LED irradiation results in more vivid coloration throughout the fermentation process.

Hue angle (h) values remained relatively stable in non-irradiated olives (52.48 to 47.85) but showed significant changes in irradiated olives. Initially lower in irradiated olives (35.38 at day 35), h values increased significantly to 53.68 by day 70, approaching those of non-irradiated olives. This suggests that LED irradiation initially shifts the hue towards red but gradually returns to values similar to non-irradiated olives by the end of fermentation. This may reflect the influence of LED light on pigment synthesis pathways, particularly involving anthocyanins and carotenoids (Gong et al., 2015).

The color results suggest that LED photostimulation plays a significant role in enhancing the color development of table olives during fermentation. The irradiated olives displayed higher lightness (L*), faster development of redness (a*), and yellowness (b*) compared to non-irradiated olives. This accelerated color change might be linked to pigment degradation and enzymatic activity under light exposure. However, despite these differences, the changes in hue (h) at the end of fermentation were within similar ranges between irradiated and non-irradiated samples, which may indicate that these color differences could be subtle to consumers. These changes likely result from the interaction of light with microbial fermentation processes and pigment synthesis, particularly anthocyanins and carotenoids, which are sensitive to red LED exposure (Martins et al., 2024; Jiang et al., 2019).

The red LED light irradiation during olive fermentation promotes a lighter color.. This effect is most pronounced in the later stages of fermentation, suggesting that LED treatment could be a valuable tool for modulating olive appearance to meet specific market preferences or to create novel product variations. However, further study of the effects of light on olive pigments should be carried out in the future. In natural fermentation, the color change is associated with the salinity and acidification of the medium. However, there is no information in the literature about the degradation of pigments during the natural fermentation of black table olives under the effect of the red LED light.

Table 3: Effects of red LED light on the color parameters in table olives fermented after 20, 35, 55 and 70 days.

		Fermentation time (days)			
Parameters	Treatment	20	35	55	70
L^*	Non-irradiated olives	42.07 ± 10.20^{A}	$43.12 \pm 8.95^{a,A}$	$43.56 \pm 10.57^{a,A}$	$44.37 \pm 8.47^{b,A}$
	Irradiated olives		$42.83 \pm 7.63^{a,B}$	$42.55 \pm 7.92^{a,B}$	$49.74 \pm 9.72^{a,A}$
a^*	Non-irradiated olives	8.74 ± 3.16^{A}	$9.02 \pm 4.41^{b,A}$	$8.34 \pm 3.35^{b,A}$	$9.09 \pm 2.34^{b,A}$
	Irradiated olives		$14.97 \pm 3.56^{a,A}$	$15 \pm 3.61^{a,A}$	$10.71 \pm 3.67^{a,B}$
b^*	Non-irradiated olives	18.36 ± 15.15^{A}	$13.73 \pm 7.08^{a,A,B}$	$11.77 \pm 7.1^{a,B}$	$12.04 \pm 6.57^{b,A,B}$
	Irradiated olives		$11.75 \pm 7.97^{a,B}$	$11.4 \pm 7.86^{a,B}$	$18.22 \pm 10.38^{a,A}$
C^*	Non-irradiated olives	13.99 ± 3.05^{AB}	$13.47 \pm 1.71^{b,B}$	$16.96 \pm 7.61^{b,A}$	$17.27 \pm 5.77^{b,A}$
	Irradiated olives		$20.23 \pm 4.37^{a,A}$	$20.33 \pm 4.4^{a,A}$	$22.73 \pm 7.01^{a,A}$
h	Non-irradiated olives	52.48 ± 17.55^{A}	$52.79 \pm 17.88^{a,A}$	$51.8 \pm 23.8^{a,A}$	$47.85 \pm 23.29^{a,A}$
	Irradiated olives		$35.38 \pm 20.16^{b,B}$	$34.31 \pm 19.46^{b,B}$	$53.68 \pm 24.06^{a,A}$

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 ($p < 0.05$).

4.1.2 Analysis of the texture profile

Table 4 provides detailed values for various texture parameters, including Hardness (N), Adhesiveness, Springiness (mm), Cohesiveness, and Chewiness (Nm^{-1}). These parameters are essential for understanding how the olive's texture evolves, and the results revealed significant changes during the fermentation process, highlighting the dynamic impact of fermentation on olive quality.

Non-irradiated olives showed a slight decrease in hardness from 30.28 N at day 20 to 25.21 N at day 70, with no significant differences throughout the fermentation period. Irradiated olives maintained higher hardness values, ranging from 32.38 N at day 35 to 29.15 N at day 70, also with no significant differences over time. Notably, irradiated olives had significantly higher hardness than non-irradiated olives at day 35, this may be attributed to the preservation of cell wall integrity and slower breakdown of pectins under the influence of LED light, as similar effects have been reported in studies involving other LED-treated fruits and vegetables (Gong et al., 2015). The consistency of hardness in irradiated olives suggests that LED does not accelerate the fruit to soften.

Both non-irradiated and irradiated olives showed fluctuating adhesiveness values with no significant differences either over time or between treatments. Non-irradiated olives ranged from -7.89 to -6.02, while irradiated olives ranged from -8.41 to -5.91. The range of values was similar for both groups, which suggests that the LED irradiation did not substantially affect this parameter, aligning with research on other fermented products where light exposure does not notably alter adhesive properties (Jiang et al., 2019).

Non-irradiated olives maintained stable springiness values (0.70 to 0.74 mm) with no significant differences throughout fermentation. Irradiated olives showed a slight increase in springiness from 0.69 mm at day 35 to 0.74 mm at day 70, with a significant difference between day 35 and day 70. This increase in springiness in irradiated olives may indicate enhanced structural resilience, potentially due to the influence of LED light on moisture retention and texture firmness (Moradi et al., 2021). Non-irradiated olives exhibited fluctuating cohesiveness values with no significant differences over time. Irradiated olives showed substantial changes in cohesiveness, increasing from 0.36 at day 35 to 0.51 at day 55, then decreasing to 0.45 at day 70.

This may reflect the effect of LED irradiation on the biochemical processes affecting protein cross-linking and cell wall stability, which are vital for maintaining texture during fermentation (Hu et al., 2012).

Both non-irradiated and irradiated olives demonstrated a significant decrease in chewiness over time. Non-irradiated olives decreased from 18.04 at day 20 to 9.01 at day 70, while irradiated olives decreased from 18.35 at day 35 to 10.42 at day 70. The most substantial decrease occurred between days 35 and 55 for both groups. This decline between days 35 and 55 is likely due to the breakdown of polysaccharides and other structural components during fermentation, although irradiated olives maintained slightly higher chewiness values, which could be attributed to the LED light's effects on slowing down enzymatic degradation (Gong et al., 2015). Red LED light irradiation during olive fermentation appears to influence several texture parameters, most notably hardness, cohesiveness, and chewiness. Irradiated olives generally maintained higher hardness values and exhibited more dynamic changes in cohesiveness compared to non-irradiated olives. Both groups showed similar trends in adhesiveness, springiness, and overall chewiness reduction, but irradiated olives tended to maintain slightly higher values in these parameters by the end of fermentation.

These findings suggest that LED irradiation could be a promising technique for modulating olive texture during fermentation, potentially allowing for better control over the final product characteristics. The maintenance of higher hardness and chewiness values in irradiated olives could lead to improved product quality and consumer acceptance, especially in markets where firmer olives are preferred.

Table 4: Effects of red LED light on the texture parameters in table olives fermented after 20, 35, 55 and 70 days.

		Fermentation time (days)			
Parameters	Treatement	20	35	55	70
Hardness (N)	Non-irradiated olives	30.28 ± 3.83^{A}	$26.39 \pm 4.08^{b,A}$	$25.95 \pm 5.5^{a,A}$	$25.21 \pm 5.38^{a,A}$
	Irradiated olives		$32.38 \pm 4.41^{a,A}$	$29.7 \pm 4.44^{a,A}$	$29.15 \pm 5.19^{a,A}$
Adhesivness	Non-irradiated olives	-7.89 ± 7.62^{A}	$-6.6 \pm 6.79^{a,A}$	$-7.75 \pm 5.32^{a,A}$	$-6.02 \pm 2.86^{a,A}$
	Irradiated olives		$-8.41 \pm 6.1^{a,A}$	$-5.55 \pm 9.33^{a,A}$	$-5.91 \pm 10.21^{a,A}$
Springiness (mm)	Non-irradiated olives	0.72 ± 0.06^{A}	$0.70 \pm 0.04^{a,A}$	$0.73 \pm 0.07^{a,A}$	$0.74 \pm 0.06^{a,A}$
	Irradiated olives		$0.69 \pm 0.06^{a,B}$	$0.75 \pm 0.05^{a,A,B}$	$0.74 \pm 0.05^{a,A}$
Cohesiveness	Non-irradiated olives	0.47 ± 0.06^{A}	$0.38 \pm 0.02^{a,A}$	$0.49 \pm 0.06^{a,A}$	$0.46 \pm 0.08^{a,A}$
	Irradiated olives		$0.36 \pm 0.04^{a,A}$	$0.51 \pm 0.07^{a,B}$	$0.45 \pm 0.04^{a,C}$
Chewiness (Nm^{-1})	Non-irradiated olives	18.04 ± 3.27^{A}	$17.13 \pm 3.42^{a,A}$	$9.99 \pm 1.62^{a,B}$	$9.01 \pm 1.55^{a,B}$
	Irradiated olives		$18.35 \pm 4.29^{a,A}$	$10.4 \pm 3.05^{a,B}$	$10.42 \pm 1.54^{a,B}$

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 ($p < 0.05$).

4.1.3 Evaluation of pH through the fermentation process

The pH results are presented in Figure 13, which outlines the changes in pH levels over the fermentation period for both non-irradiated and irradiated brine and olives. Throughout the observation days (0, 20, 35, 55, and 70), non-irradiated brine showed slight fluctuations in pH, ranging from 5.0 at the start, peaking at 5.3 on day 35, and returning to 5.0 by day 70. In contrast, irradiated brine experienced a more pronounced reduction in pH, starting at 5.1 and declining steadily to 4.5 by the end of the fermentation period. The pH of non-irradiated olives remained relatively stable, decreasing slightly from 4.7 to 4.5. Meanwhile, irradiated olives exhibited a more substantial pH decline, starting at 5.6 and dropping to 4.3 by day 70. This significant reduction in pH, especially in irradiated olives, suggests a favorable environment for preservation, as it helps inhibit the growth of pathogenic microorganisms and delays deterioration processes (Perricone et al., 2010). The pH value considered safe by commercial standards applied to table olives must be at least 4.3 (IOC, 2004).

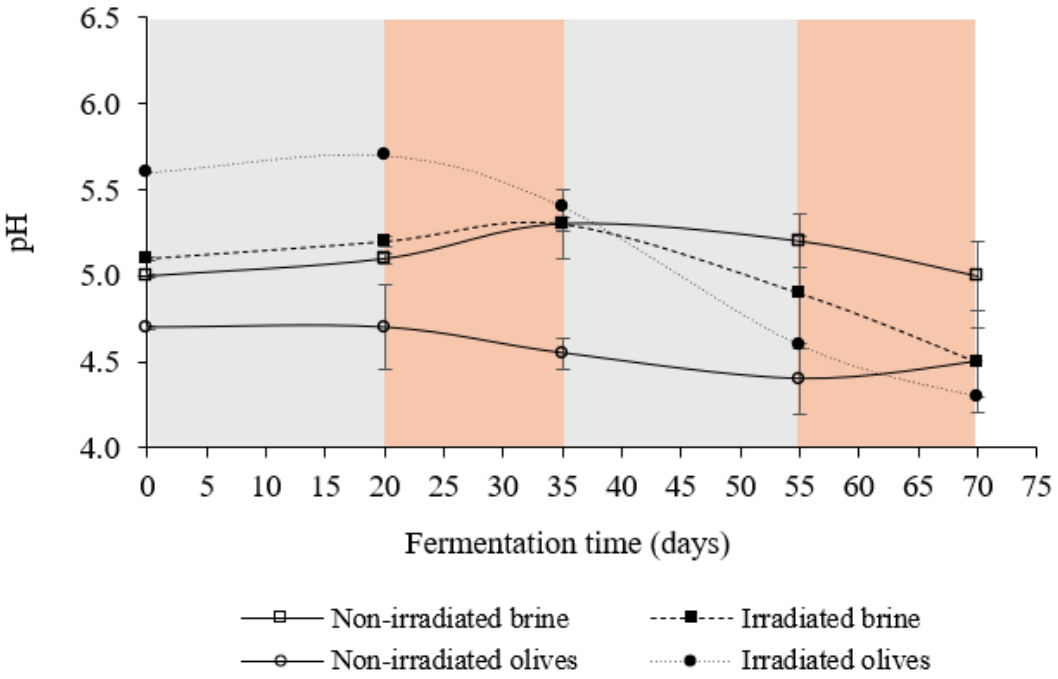


Figure 13: pH evolution during the fermentation process with and without LED light. Each irradiation persisted for 15 days (pink color), alternating with 20 days without irradiation (grey color). Data points are mean values of triplicate and standard deviation.

These results indicate that LED irradiation influences the acidification process in both brine and olives. The sharper decline in pH observed in irradiated samples indicates more rapid fermentation, likely due to the stimulation of microbial activity by the LED light. The pH decrease is a common indicator of lactic acid bacteria (LAB) activity, which is essential for olive fermentation. LAB converts sugars into lactic acid, contributing to the acidification of both the brine and the olives themselves. Studies, such as those conducted by Martins et al. (2024), have shown that red LED light can enhance the growth and metabolic activity of LAB, resulting in more rapid acidification during fermentation processes. This can explain the more substantial decrease in pH observed in irradiated brine and olives. Similar results were reported in the study by Moradi et al. (2021), which found that light exposure, particularly red LED light, accelerates microbial metabolic processes. This enhanced microbial activity under LED light conditions promotes faster lactic acid production, leading to more significant pH reductions in the irradiated samples. In non-irradiated samples, the slower pH reduction indicates a more gradual fermentation process, which aligns with traditional olive fermentation methods where LAB activity is not as accelerated. Furthermore, the slight but consistent pH decline in non-irradiated olives and brine indicates that the natural fermentation process occurs at a slower pace. These findings align with traditional olive fermentation studies, where pH decreases gradually over time due to LAB activity (Venturi et al., 2022).

Other relevant research further supports the notion that photostimulation can accelerate microbial metabolism. For instance, Jiang et al. (2019) demonstrated that LED irradiation promotes microbial growth and acid production in various food products, leading to faster fermentation and acidification. These results suggest that LED photostimulation significantly enhances the fermentation process by accelerating the acidification of both the brine and the olives.

4.1.4 Evaluation of titratable acidity through the fermentation process

The titratable acidity (TA) results are presented in Figure 14, illustrating the changes in acidity levels in both brine and olives throughout the fermentation process (0, 20, 35, 55, and 70 days). The results showed a progressive increase in the acidity of both brines until the 70th day of fermentation. For non-irradiated brine, the acidity increased gradually from 0.40% to 0.70%, while irradiated brine showed a more pronounced increase from 0.40% at day 0 to 1.00% at day 70. Similarly, the titratable acidity of non-irradiated olives

remained relatively stable, ranging from 0.14% to 0.13%, whereas irradiated olives displayed a slight increase in acidity from 0.10% to 0.16% over the same period.

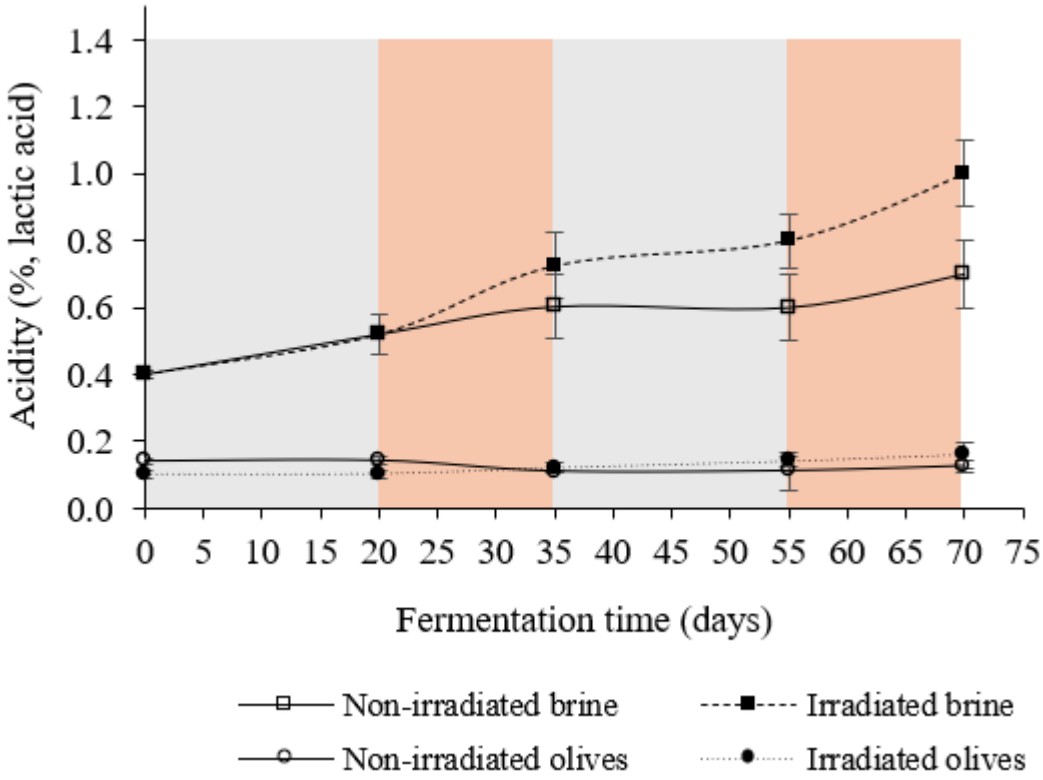


Figure 14: Titratable acidity evolution during the fermentation process with and without LED light. Each irradiation persisted for 15 days (pink color), alternating with 20 days without irradiation (grey color). Data points are mean values of triplicate and standard deviation.

The increased acidity observed in irradiated brine suggests that LED photostimulation promotes a faster accumulation of organic acids, such as lactic acid, which are key indicators of microbial fermentation activity. These acids are often described in brines from black olives (Martins et al., 2024). 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 pink 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. The more substantial increase in titratable acidity for irradiated brine, particularly at 35 days and 70 days, aligns with previous studies that have demonstrated the stimulating effect of red LED light on lactic acid bacteria (LAB) metabolism, enhancing the production of organic acids during fermentation (Martins et al., 2024). In the case of olives, titratable acidity remained relatively stable in both irradiated and non-irradiated samples, with irradiated olives

showing a slight increase by the end of the fermentation process. The slight rise in acidity in irradiated olives (from 0.10% to 0.16%) can be linked to the higher presence of LAB on the brine than in the fruit. This trend has been observed in other studies involving LED-treated food products, where photostimulation promotes microbial activity, thus accelerating acid formation (Jiang et al., 2019).

4.1.5 Evaluation of salt content through the fermentation process

Figure 15 presents the salt (NaCl) content results, illustrating the changes in salt levels for both brine and olives throughout the fermentation period (days 0, 20, 35, 55, and 70). Non-irradiated brine showed a gradual decrease in salt concentration, starting at 6.5% on day 0 and steadily declining to 4.5% by day 70. Similarly, irradiated brine followed a consistent downward trend, beginning at 6.5% and dropping to 4.0% by the end of the fermentation period.

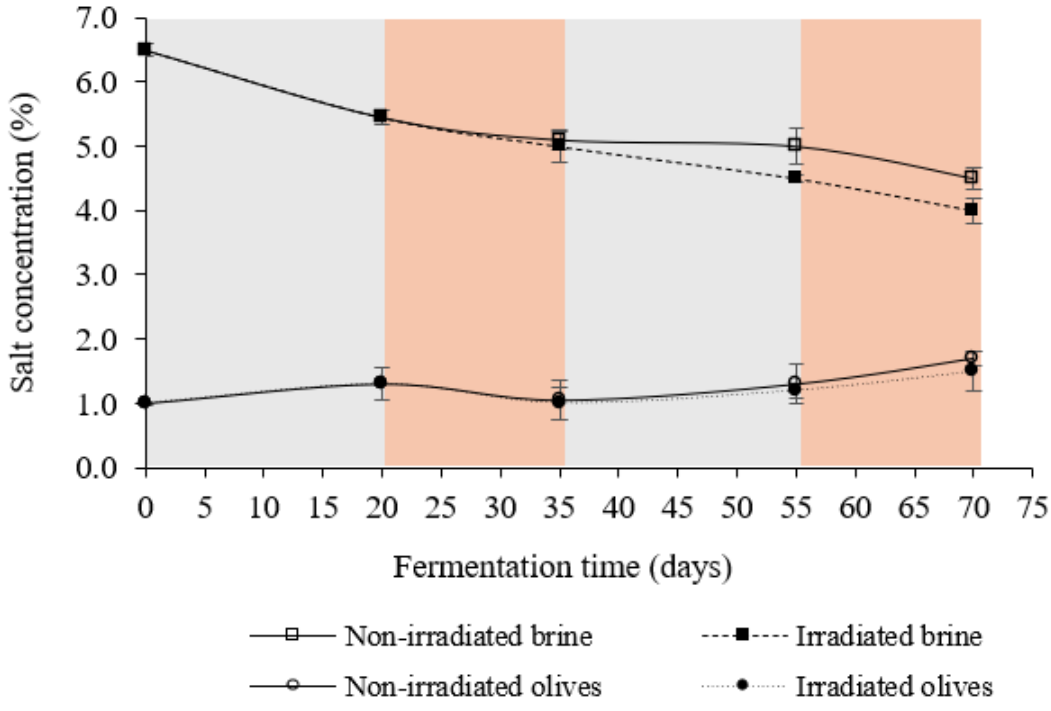


Figure 15: Salt concentration evolution during the fermentation process with and without LED light. Each irradiation persisted for 15 days (pink color), alternating with 20 days without irradiation (grey color). Data points are mean values of triplicate and standard deviation.

In the case of olives, non-irradiated samples exhibited slight variations, with salt content increasing from 1.0% at day 0 to 1.7% by day 70, despite minor fluctuations. In contrast, irradiated olives started at 1.0%, rose to 1.3% on day 20, and increased gradually, reaching 1.5% by the end of the fermentation period. However, these variations are not statistically significant. 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 (Reis et al., 2022).

The salt (NaCl) content dynamics observed in this study indicate a decline in salt concentration in both non-irradiated and irradiated brine over the fermentation period, from an initial level of 6.5% down to 4.5% and 4.0%, respectively, by day 70. This consistent reduction suggests ongoing osmotic exchange, where salt moves from the brine into the olives. The decrease in brine salt concentration over time may also indicate water absorption by the brine, which could dilute its salt content, or salt precipitation due to changes in temperature and pH (Brenes et al., 2020).

For the olives, the salt content showed a modest increase across the fermentation period. Non-irradiated olives displayed a gradual rise from 1.0% to 1.7%, while irradiated olives increased from 1.0% to 1.5%. This pattern suggests a typical osmotic diffusion process, where the olives absorb salt from the surrounding brine, facilitated by the concentration gradient between the olives and the brine (Panagou et al., 2003). The slightly lower final salt concentration in irradiated olives might be attributed to the influence of LED photostimulation, which could have affected the permeability of the olive skin, leading to a less efficient salt uptake compared to non-irradiated olives.

Interestingly, the irradiation process did not lead to a significantly higher salt uptake in the olives, contrary to what might be expected if the LED light were enhancing cell membrane permeability. Previous research has suggested that LED light, particularly red light, can modify the properties of plant cell walls, potentially affecting their permeability (Jiang et al., 2019). However, the findings in this study did not show a marked difference, implying that the effect of LED photostimulation on the permeability of olive skins may be limited under the conditions tested. Further investigations are necessary to determine whether different light intensities, wavelengths, or exposure durations could produce more pronounced effects.

Overall, the results indicate that LED photostimulation may not significantly alter the osmotic behavior of olives during fermentation, though it is essential to explore other variables that could modulate salt uptake. Understanding the underlying mechanisms, such as the impact of LED light on cell wall structure and the diffusion of solutes, could offer valuable insights for optimizing the fermentation process.

4.1.6 Evaluation of total phenols through the fermentation process

The effect of the LED light was evaluated on the content of total phenolic compounds Figure 16. Significant differences were observed between treatments in the two fermentation periods. The first period stands out between 20 and 35 days, and the second between 55 and 70 days, corresponding to the irradiation periods. In non-irradiated brine, the total phenolic content increased steadily, starting at 0.45 (mg eq. gallic acid/ mL of brine) on day 0 and reaching 0.64 (mg eq. gallic acid/ mL of brine) on day 70. The irradiated brine displayed a similar upward trend, with slightly higher values than the non-irradiated brine in the later stages, increasing from 0.45 (mg eq. gallic acid/ mL of brine) at day 0 to 0.7 (mg eq. gallic acid/ mL of brine) at day 70. For olives, however, there was a marked decrease in phenolic content over time. Non-irradiated olives started with a high phenolic content of 9.03 (mg eq. gallic acid/ g olive, d.w) at day 0, but this value decreased significantly to 4.13 (mg eq. gallic acid/ g olive, d.w) by day 70. Irradiated olives also followed a downward trend, with phenolic content dropping from 9.03 (mg eq. gallic acid/ g olive, d.w) at day 0 to 3.35 (mg eq. gallic acid/ g olive, d.w) at day 70. Notably, irradiated olives had a steeper decline in phenolic content than non-irradiated ones, particularly at 35 days and 55 days.

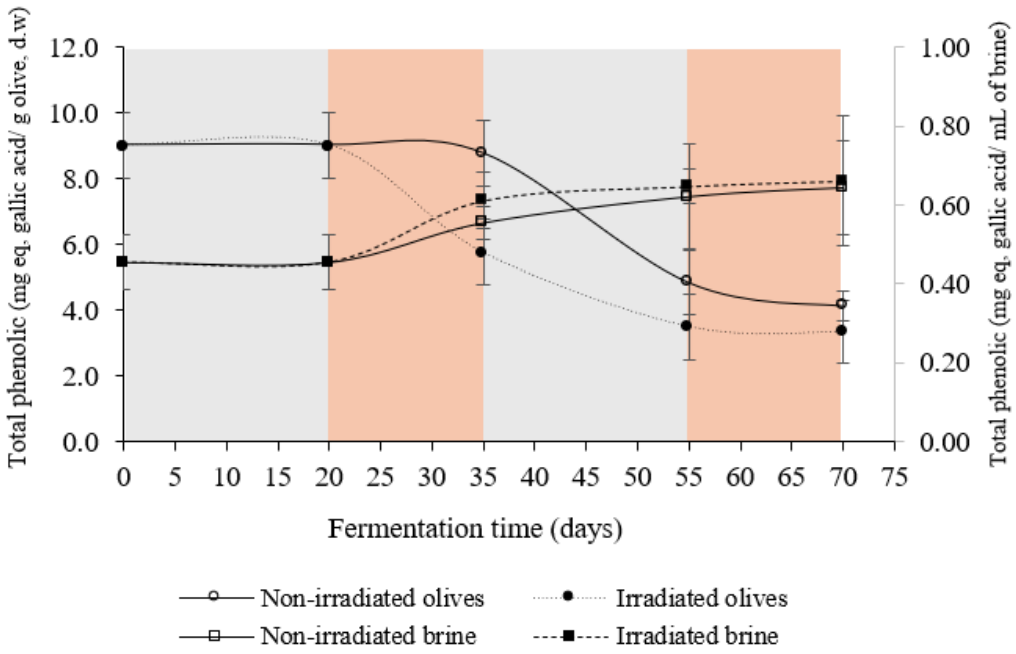


Figure 16: Total phenolics evolution during the fermentation process with and without LED light. Each irradiation persisted for 15 days (pink color), alternating with 20 days without irradiation (grey color). Data points are mean values of triplicate and standard deviation.

The steady increase in phenolic content in both non-irradiated and irradiated brine indicates the release of phenolic compounds from the olives into the brine over time. However, the slightly higher phenolic levels in the irradiated brine towards the end of the fermentation period may be attributed to the effect of LED irradiation, which likely promotes a more efficient extraction of these compounds into the surrounding brine. This is consistent with studies like Martins et al. (2024), which demonstrated that LED photostimulation enhances the release of phenolic compounds from green olive tissues into the liquid medium during fermentation.

Similar effects of LED irradiation on the release of phenolic compounds have been documented by Delgado-Adámez et al. (2021), who observed that photostimulation enhanced the diffusion of phenolics in fruit-based fermentations.

The decrease in phenolic content in olives, particularly in irradiated samples, indicates that fermentation, along with the action of LED light, degrades or transforms these compounds. As phenolic compounds are sensitive to oxidative processes, the exposure to red LED light might have accelerated the oxidation and degradation of phenolic compounds in the olive matrix, leading to a faster reduction in their levels compared to non-irradiated olives. According to Bhat et al. (2015) and Salar et al., (2016) the reduction in the content of phenolic compounds after a certain period of exposure to light may be

related to oxidation processes or bioconversion into bioactive compounds. This observation aligns with the findings of López-López et al. (2019), who reported that LED irradiation can promote oxidative reactions in phenolic compounds, thereby reducing their overall content in plant-based foods over time. Similarly, Venturi et al. (2022) found that phenolic compounds tend to decrease in concentration due to microbial enzymatic activity and chemical transformations throughout fermentation. The more pronounced decline in irradiated olives may reflect the combined effects of microbial fermentation and photostimulation, both of which accelerate phenolic degradation.

4.2Microbiological parameters

4.2.1 Microbial counts of brine and olives samples through the fermentation process

The microbial counts of yeast, mesophilic aerobic, bacteria, moulds, and lactic acid bacteria (LAB) in both brine and olives were assessed throughout the fermentation period (days 20, 35, 55, and 70). The results are presented in Figure 17 and 18. The irradiated brine had the highest microorganism counts. Among the groups evaluated, yeasts and LAB were the dominant groups in the fermentation process. The yeast counts in non-irradiated samples increased from 6.02 to 8.06 log CFU/mL over the fermentation period, while irradiated brine showed a significant increase, reaching 8.35 log CFU/mL by day 70. Mesophilic aerobic followed a similar trend, with non-irradiated brine maintaining high counts (7.38 to 8.17 log CFU/mL), while irradiated brine reached 8.38 log CFU/mL by day 70. Moulds counts in non-irradiated brine were stable (5.10 to 5.48 log CFU/mL), whereas irradiated brine exhibited an increase, peaking at 6.06 log CFU/mL by day 70. LAB counts in non-irradiated brine increased steadily from 2.02 to 5.43 log CFU/mL, while irradiated brine showed a substantial rise, reaching 6.41 log CFU/mL (Figure 17).

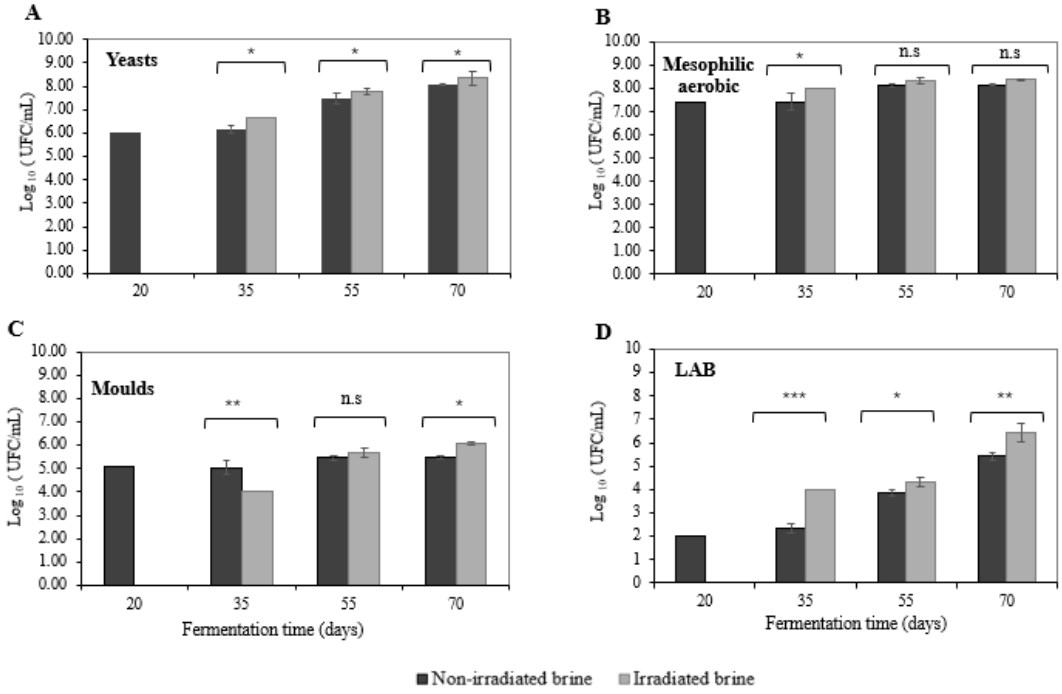


Figure 17:. Microbial counts of yeasts (A), mesophilic aerobic (B), moulds (C), and lactic acid bacteria (LAB) evaluated in non-irradiated and irradiated brine after 20, 35, 55, and 70 days of fermentation. Each value is expressed as mean ± SD (n = 3). Asterisks indicate values that differ significantly between treatments, where *p < 0.05, **p < 0.01, and ***p < 0.001

For olives, non-irradiated samples showed a gradual increase in yeast counts from 5.64 to 6.33 log CFU/g, whereas irradiated olives had higher counts, reaching 7.01 log CFU/g by day 70. Mesophilic aerobic bacteria counts increased in both groups, with irradiated olives showing higher values (7.60 log CFU/g at day 70). Mold counts fluctuated, with non-irradiated olives showing a gradual increase to 5.16 log CFU/g, while irradiated olives had lower initial counts but increased to 5.67 log CFU/g by day 70. LAB counts were significantly higher in irradiated olives, rising from 4.56 to 6.01 log CFU/g, compared to non-irradiated olives, which increased from 2.01 to 4.58 log CFU/g (Figure 18).

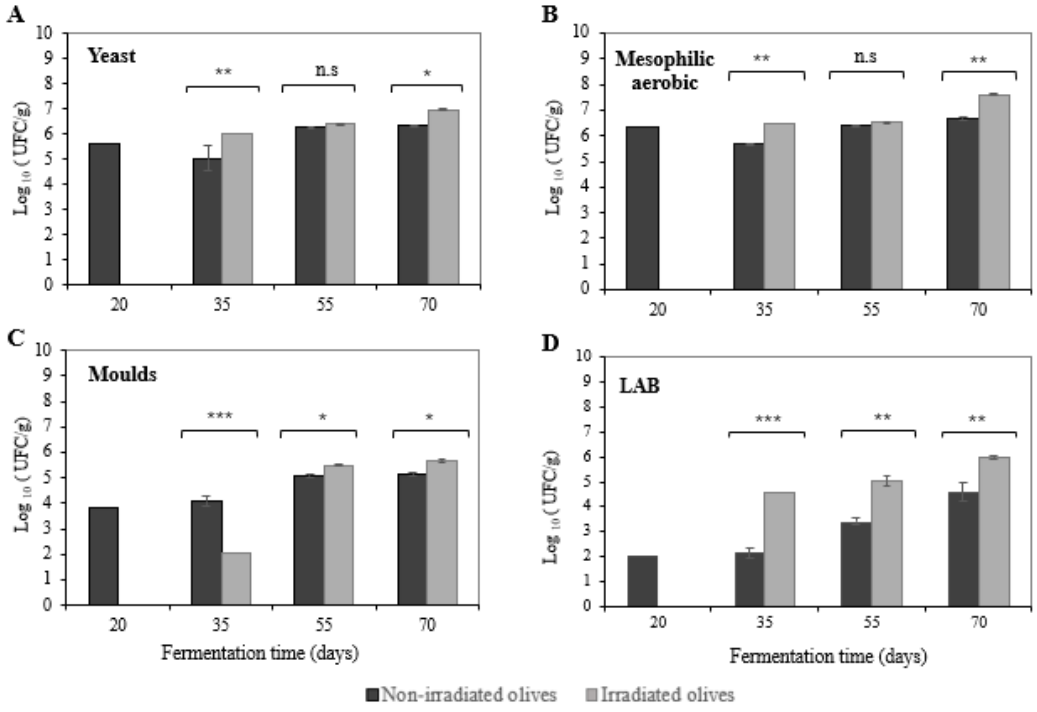


Figure 18: Microbial counts of yeasts (A), mesophilic aerobic (B), molds (C), and lactic acid bacteria (LAB) evaluated in non-irradiated and irradiated olives after 20, 35, 55, and 70 days of fermentation. Each value is expressed as mean ± SD (n = 3). Asterisks indicate values that differ significantly between treatments, where *p < 0.05, **p < 0.01, and ***p < 0.001

The observed microbial dynamics, particularly the enhancement of LAB and yeast populations, can be attributed to the photobiological effects of LED light, which stimulate microbial metabolism and improve fermentation efficiency. Studies have demonstrated that red LED light can modulate microbial activity by enhancing cellular respiration and metabolic pathways, thereby boosting the growth of beneficial microorganisms while inhibiting spoilage organisms like moulds in the early stages of fermentation (Martins et al., 2024; Delgado-Adámez et al., 2021).

The decrease in microbial counts in the olives compared to the brine could be due to the higher presence of microorganisms on the surface of the fruit compared to its interior and the greater concentration of phenolic compounds in the olive pulp. Phenolic compounds have well-known antimicrobial properties, which can naturally inhibit microbial growth. This aligns with the lower initial microbial counts observed in our non-irradiated olives compared to the brine, highlighting the selective inhibitory effects of these compounds on microorganisms present in the olive matrix (Martins et al., 2024).

LED light has a significant influence on increasing the microbial load during fermentation. The LED light at a wavelength of 630 ± 10 nm appears to stimulate metabolic activities in microorganisms by interacting with their photoreceptors, specifically cytochrome protein complexes, flavoproteins, and other enzymatic cofactors. This stimulation likely enhances cellular ATP production and promotes microbial proliferation, which is consistent with our findings where irradiated samples exhibited significantly higher counts of yeasts, LAB, and mesophilic aerobic compared to non-irradiated samples (Jeong et al., 2018; Martins et al., 2024).

The stimulation of cytochrome protein complexes and flavoproteins by red LED light facilitates enhanced proton pumping and ATP synthesis, thereby increasing the metabolic activity of the cells (Crugeira et al., 2015). This explains the accelerated microbial growth observed in the irradiated samples. Additionally, the increase in RNA and DNA synthesis, and protein activation, provide further insight into how LED light boosts cell proliferation during fermentation (Crugeira et al., 2015).

Also, despite the presence of photosensitive pigments in olives, such as chlorophyll and carotenoids, no microbial photoinactivation was observed. The emitted LED light wavelength (630 ± 10 nm) did not match the absorption peaks of these pigments closely enough to induce reactive oxygen species (ROS) formation, which could lead to microbial death. This finding is crucial as it suggests that while LED light enhances microbial activity, it does not adversely affect microorganisms through ROS-induced damage, thereby supporting the safe use of LED light for fermentation enhancement (Martins et al., 2024).

The microbial analysis results show that no adverse effects on microbial counts were detected due to LED irradiation. Instead, there was a notable increase in beneficial microorganisms, particularly yeasts and LAB, which play crucial roles in fermentation quality and product stability.

The LED light's ability to enhance microbial growth without inducing harmful photoinactivation aligns with the potential of LED photostimulation to shorten fermentation times, making the process more efficient and economically viable.

The use of red LED light irradiation positively affects the microbial dynamics during the fermentation process of olives, promoting the growth of beneficial microorganisms such as yeasts and LAB while avoiding microbial photoinactivation. This enhancement is likely due to the direct stimulatory effects of LED light on microbial metabolism, as evidenced by increased cellular ATP production, enhanced enzymatic activity, and greater overall microbial proliferation. These findings underscore the potential of LED photostimulation as an innovative tool for improving fermentation efficiency and product quality in table olives.

4.3Evaluation of the presence of pathogens at the end of fermentation

In this study, the microbiological quality assessment of both the brine and table olives revealed the absence of bacteria from the Enterobacteriaceae family (including Coliforms and *Escherichia coli*), as well as pathogenic bacteria such as *Clostridium perfringens*, *Listeria* spp., and *Salmonella* spp. Therefore, the table olives demonstrated excellent microbiological quality.

4.4Sensory analysis

The sensory analysis, conducted after 70 days of fermentation, are presented in Figure 19. Sensory attributes such as taste (salty, bitter, acidic) and kinesthetic sensations (hardness, fibrousness, crunchiness) were evaluated, along with defects and abnormal fermentation.

In terms of taste, non-irradiated olives were rated with lower saltiness (3.4), higher bitterness (5.26), and more acidity (4.53) compared to irradiated olives, which had saltier flavors (4.78), less bitterness (4.52), and reduced acidity (3.81). For kinesthetic sensations, irradiated olives had higher hardness (4.38 vs. 3.61) but lower fibrousness (5.53 vs. 7.86) and crunchiness (3.62 vs. 4.11) compared to non-irradiated olives. When assessing sensory defects, non-irradiated olives had significantly higher values (5.15 for other defects and 3.09 for abnormal fermentation) compared to irradiated olives (2.97 and 1.88, respectively). This suggests that LED photostimulation may increase firmness (hardness) but reduce the fibrous and crunchy sensations in olives. As described by Lanza (2013), the classification of olives can be determined by the median of the predominantly perceived defect (PPD). Based on the results obtained in this study, the olives subjected to LED light were classified in the extra category: DPP ≤ 3.0.

This suggests that LED photostimulation not only improves the overall texture and taste but also minimizes sensory defects and the occurrence of abnormal fermentation.

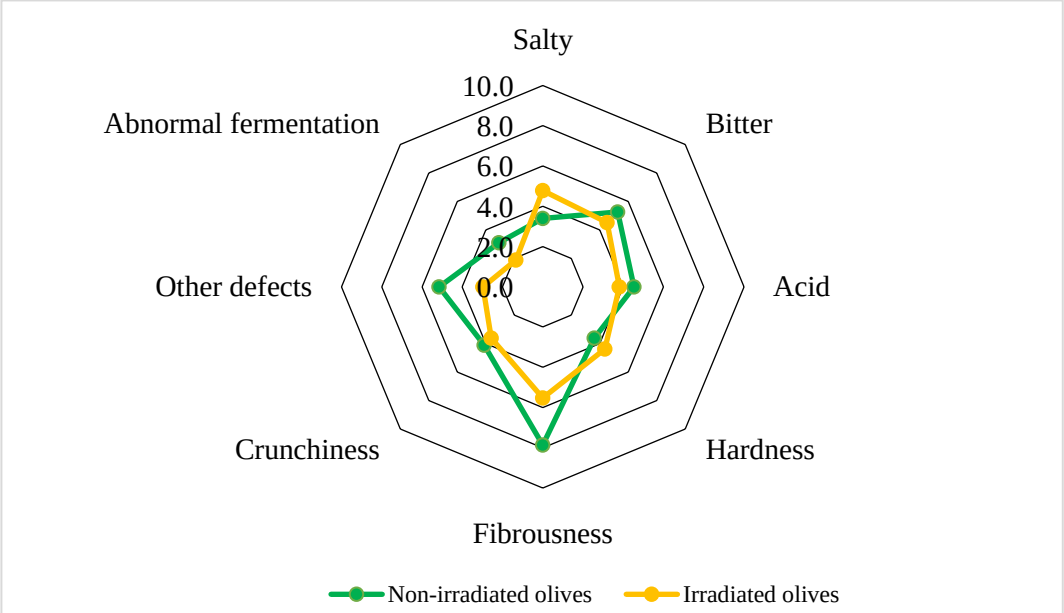


Figure 19: Sensory profiles of non-irradiated and irradiated table olives after 70 days of fermentation.

The enhancement of saltiness in irradiated olives can be attributed to the role of LED irradiation in promoting the diffusion of salt ions during fermentation, as Martins et al. (2024) observed. Similarly, the reduction in bitterness and acidity is consistent with findings by Delgado-Adámez et al. (2021), who reported that red LED light influences the biochemical pathways related to phenolic and organic acid metabolism, likely contributing to reduced bitterness and acidity in food products.

The increased hardness of irradiated olives is in line with the texture analysis results, confirming that LED irradiation enhances the firmness of olives. Studies like those of López-López et al. (2019) have shown that photostimulation can affect the structural integrity of plant cells, leading to increased hardness.

The lower sensory defect scores and reduced abnormal fermentation in irradiated olives are likely linked to the improved microbial stability and reduced spoilage caused by LED irradiation. Martins et al. (2024) noted that LED photostimulation can enhance beneficial microbial activity, such as lactic acid bacteria, while inhibiting spoilage microbes, thus improving the overall fermentation process and minimizing sensory defects.

The changes in sensory attributes observed in irradiated olives can be explained by the influence of LED photostimulation on microbial activity and metabolic pathways. Studies have shown that LED light promotes the growth of beneficial microbes like lactic acid bacteria (LAB), which play a critical role in reducing bitterness and acidity while enhancing saltiness (Martins et al., 2024; Delgado-Adámez et al., 2021). This is likely due to the fact that LAB can metabolize bitter phenolic compounds and organic acids, thus reducing their concentrations in the final product.

The improvement in hardness and reduction in sensory defects in irradiated olives can also be attributed to the structural effects of LED irradiation. Research by López-López et al. (2019) suggests that LED light may enhance cell wall integrity, making olives firmer, while also inhibiting spoilage microbes that cause sensory defects.

The sensory analysis after 70 days of fermentation reveals that LED irradiation has a positive effect on the taste and texture of olives, improving saltiness, and hardness, and reducing bitterness, acidity, and sensory defects. Additionally, the reduction in abnormal fermentation in irradiated olives further highlights the role of LED light in enhancing microbial stability and improving fermentation outcomes.

5 Conclusion

The evaluation of LED photostimulation on the fermentation of Negrinha de Freixo black olives provided significant insights into the impact of red LED light on the physicochemical, microbiological, and sensory characteristics of both olives and brine throughout the fermentation process. The findings suggest that LED irradiation can play a pivotal role in modulating fermentation dynamics, enhancing microbial growth, and influencing the quality of the final product.

The pH analysis indicated that irradiated samples experienced a more pronounced decrease in pH over time compared to non-irradiated samples, suggesting a more robust microbial activity, particularly by lactic acid bacteria (LAB), which are known to acidify the environment as fermentation progresses. This aligns with the increased microbial counts observed in irradiated samples, where yeasts and LAB showed significant proliferation, likely stimulated by the red LED light, enhancing metabolic activities through photoreceptor activation.

Titratable acidity measurements corroborated the pH findings, with irradiated brine exhibiting a notable increase in acidity, reflecting an intensified fermentation process. The red LED light's ability to enhance microbial metabolism contributed to this accelerated acidification, making the fermentation process more efficient. The texture analysis further supported these findings, with irradiated olives maintaining higher hardness and cohesiveness values, indicating that LED irradiation positively influences texture retention, a critical sensory attribute.

The total phenolic content in irradiated olive samples decreased, suggesting that LED light may help reduce bitterness.

Sensory analysis at the end of the fermentation period revealed that irradiated olives were perceived as having improved sensory qualities, particularly in terms of saltiness and reduced bitterness and other defects, demonstrating the influence of LED light on enhancing the sensory profile of the olives. The reduction in undesirable sensory attributes in irradiated olives supports the hypothesis that LED photostimulation can improve the overall quality of fermented table olives.

The results of this study are consistent with existing literature, which indicates that LED irradiation can stimulate microbial growth and metabolic activities without causing photoinactivation, thereby enhancing the fermentation process (Martins et al., 2024). The findings suggest that LED photostimulation represents an innovative and effective

strategy to optimize the natural fermentation of table olives, potentially reducing fermentation time and improving the quality of the final product. This approach could be highly beneficial for the table olive industry, offering a sustainable and efficient method to enhance product quality while maintaining traditional fermentation processes.

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