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Quality grade evolution and health claim fulfillment of olive oils under simulated domestic consumption: Kinetic modelling and shelf life predictions

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Abstract

Olive oil has always played a vital role in the Mediterranean diet, well renowned for its nutritional properties and related health benefits. This is largely attributed to its high concentration of monounsaturated fatty acids and bioactive minor compounds, such as polyphenols. This study aimed to assess how typical household consumption conditions influence the physicochemical and sensory properties of extra virgin olive oil (EVOO), as well as its compliance with the European Union (EU) health claim. The results of the analysis of ten bottles stored in darkness and exposed to light under simulated household conditions, demonstrated an increase in PV and extinction coefficients over the 9-week period, with a more pronounced rise in the light-exposed samples. In these samples, PV increased by 81.2%, and at 6 weeks it exceeded the legal EU thresholds for EVOO/VOO classification. In contrast, oils stored in darkness kept all physicochemical parameters within legal limits allowing EVOO or VOO classification, up to or beyond the seventh week, respectively. Also, by the eighth week, sensory analysis revealed the presence of rancid in light-exposed samples, with intensities ranging from 3.6 to 6.1, indicating advanced oxidation and rendering the oil unsuitable for consumption (lampante oil). Kinetic models were developed to predict the shelf life (SL) of commercial grade EVOO under household conditions, applying zero-, first-, and second-order kinetic models for PV, K_{232}, and K_{268} in both dark and light storage conditions. The models fitted well the experimental data (*R*-Pearson values ranging from 0.89 to 0.97), with PV emerging as the most sensitive SL indicator under these conditions. The estimated SL was approximately 15 days for light-exposed oils and 63 days for oils stored in darkness. The greatest variation in SL predictions, based on PV, K_{232}, or K_{268}, was observed in the light-exposed samples. Photo-oxidation led to a rapid increase in PV, in contrast to the slower rise in extinction coefficients, which are associated with secondary oxidation products that form when oxygen availability diminishes. Regarding the health claim, the content of hydroxytyrosol and tyrosol derivatives remained relatively stable throughout the 9-week period in both dark- and light-stored oils, ranging from 12 to 13 mg/20 g of oil, significantly exceeding the minimum required by the EU regulation. In conclusion, typical household conditions significantly impact the quality of olive oil. Storing olive oil in darkness can extend its SL by 3 to 4 times, compared to light-exposed conditions, in terms of maintaining its EVOO classification.

Keywords: Extra virgin olive oil, physicochemical parameters, oxidation, household conditions, estimation.

Resumo

O azeite sempre desempenhou um papel vital na dieta mediterrânica, bem conhecida pelas suas propriedades nutricionais e benefícios para a saúde. Este facto é largamente atribuído à sua elevada concentração de ácidos gordos monoinsaturados e de compostos menores bioativos, como os polifenóis. Este estudo teve como objetivo avaliar de que forma as condições típicas de consumo doméstico influenciam as propriedades físico-químicas e sensoriais do azeite virgem extra (AVE), bem como a sua conformidade com a alegação de saúde da União Europeia (UE). Os resultados da análise de dez garrafas armazenadas na escuridão e expostas à luz em condições domésticas simuladas demonstraram um aumento dos coeficientes de extinção e do IP ao longo do período de 9 semanas, com um aumento mais pronunciado nas amostras expostas à luz. Nestas amostras, o IP aumentou 81.2% e, às 6 semanas, excedeu os limiares legais da UE para a classificação de AVE/AV. Em contrapartida, os azeites armazenados na escuridão mantiveram todos os parâmetros físico-químicos dentro dos limites legais que permitem a classificação de AVE ou AV, até à sétima semana ou para além desta, respetivamente. Além disso, na oitava semana, a análise sensorial revelou a presença de ranço nas amostras expostas à luz, com intensidades que variam entre 3.6 e 6.1, indicando uma oxidação avançada e tornando o azeite impróprio para consumo (azeite lampante). Foram desenvolvidos modelos cinéticos para prever o tempo de vida útil (TVU) da classificação de AVE em condições domésticas, aplicando modelos cinéticos de ordem zero, primeira e segunda para IP, K_{232} e K_{268} em condições de armazenamento à luz e no escuro. Os modelos ajustaram-se bem aos dados experimentais (valores de R-Pearson variando de 0.89 a 0.97), com o IP emergindo como o indicador de TVU mais sensível nestas condições. A estimativa da TVU foi de aproximadamente 15 dias para os azeites expostos à luz e de 63 dias para os óleos armazenados no escuro. A maior variação nas previsões de TVU, com base no IP, K_{232} ou K_{268}, foi observada nas amostras expostas à luz. A foto-oxidação levou a um aumento rápido do IP, em contraste com o aumento mais lento dos coeficientes de extinção, que estão associados a produtos de oxidação secundários que se formam quando a disponibilidade de oxigénio diminui. Relativamente à alegação de saúde, o teor de hidroxitirosol e derivados de tirosol manteve-se relativamente estável ao longo do período de 9 semanas, tanto nos azeites armazenados no escuro como nos azeites armazenados à luz, variando entre 12 e 13 mg/20 g de azeite, excedendo significativamente o mínimo exigido pelo regulamento da UE. Em conclusão, as condições domésticas típicas têm um impacto significativo na qualidade do azeite. O

armazenamento do azeite no escuro pode prolongar a sua TVU 3 a 4 vezes, em comparação com as condições de exposição à luz, em termos de manutenção da sua classificação AVE.

Palavras-chave: Azeite virgem extra, parâmetros físico-químicos, oxidação, condições domésticas, estimativa.

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List of abbreviations

ALA: α-Linolenic Acid

ANOVA: Analysis of variance

ASLT: Accelerated Shelf-Life Testing

EC: European Commission Regulation

EFSA: European Food Safety Authority

EU: European Union

EVOO: Extra Virgin Olive Oil

FA: Free Acidity

HPLC: High-Performance Liquid Chromatography

HPLC-DAD: High-Performance Liquid Chromatography-Diode Array Detection

IOC: International Olive Council

LOO: Lampante Olive Oil

MS: mass spectrometry

MUFA: Monounsaturated Fatty Acids

ND: Not detected

NE: Not Evaluated

OS: Oxidative stability

PUFA: Polyunsaturated Fatty Acids

PV: Peroxide Value

PVDF: Polyvinylidene fluoride

R: Correlation coefficient

RPM: Revolutions per minute

SD: Standard deviation

SL: Shelf life

TPC: Total phenolic content

Tc: Temperature coefficient

TRUL: Time to Reach Upper Legal limits

TRLL: Time to Reach Lower Legal limits

VIS: Visible

VOO: Virgin Olive Oil

UV: Ultraviolet

Chapter 1. Introduction

1.1. Framework

Virgin olive oil is a key component of the Mediterranean diet, recognized for its outstanding sensory qualities and notable nutritional properties (Mancebo-Campos et al., 2022). It is commercially available as extra virgin olive oil (EVOO) or virgin olive oil (VOO), grades determined by the fulfillment of specific legal thresholds for physicochemical parameters such as free acidity (FA), peroxide value (PV), and extinction coefficients at 232 nm and 268 nm (K_{232} and K_{268}), alongside with sensory criteria that require the perception of fruitiness and the absence of detectable defects (Commission Delegated Regulation (EU) 2022/2104). Cold-pressed olive oils, are rich in unsaturated fatty acids containing, in lower amounts, bioactive compounds, including polyphenols, tocopherols, and carotenoids, which contribute to their nutritional value, antioxidant capacity, and potential health benefits (Perona and Botham, 2013; García-González et al., 2023; Cairone et al., 2021).

Among the bioactive compounds, polyphenols, particularly hydroxytyrosol and tyrosol derivatives, are associated to cardiovascular health benefits when consumed in sufficient amounts, emphasizing the importance of evaluating the phenolic profile to assess the oil's nutritional quality and its potential health impacts (Caporaso et al., 2015). The European Union (EU) and the European Food Safety Authority (EFSA) have recognized these health benefits, allowing a health claim stating that "*Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress*" (Regulation (EC) No 1924/2006; EFSA, 2011). However, this claim can only be made if the olive oil contains at least 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) per 20 g of olive oil (European Commission Regulation EU No 432/2012, 2012).

The composition of olive oil is influenced by numerous agronomic factors, which are broadly categorized into pre-harvest and post-harvest factors. Pre-harvest factors include olive cultivar, geographic location, environmental conditions, soil composition, tree age, irrigation practices, fruit ripeness at harvest, and harvesting methods. Post-harvest factors encompass fruit storage, leaf removal, fruit washing, extraction methods (e.g., fruit crushing and malaxation), oil storage, and household usage (Mele et al., 2018).

Due to its high content of unsaturated fatty acids and the presence of oxygen in the packaging headspace, olive oil is susceptible to oxidation, including auto-oxidation (storage in darkness) and photo-oxidation (storage under light). These mechanisms have been extensively

documented (Choe & Min, 2006; Kanavouras et al., 2006), along with the occurrence of enzymatic oxidation if specific enzymes are present (Li et al., 2014). Understanding olive oil oxidation requires consideration of both the oxidative processes and the antioxidant activity of minor components like phenols, phytosterols, carotenoids, tocopherols, and vitamins. Additionally, components such as chlorophylls, free fatty acids, and trace metals play essential roles in oxidation and antioxidant reactions (Psomiadou & Tsimidou, 2002a, 2002b). Polyphenols are also subject to degradation due to the presence of enzymes like polyphenol oxidase and peroxidase (Bertuccioli et al., 2005). Oxidation generates undesirable volatile compounds, including alkanes, alkenes, aldehydes, and ketones, leading to sensory defects such as rancidity (Angerosa, 2002; Kalua et al., 2005; Garcia-Oliveira et al., 2021). Consequently, the oxidation of fatty acids and polyphenols diminishes the quality and economic value of the oil, preventing its further commercialization as EVOO.

Olive oil degradation has been investigated under various storage conditions (e.g., time, temperature, dark or light exposure, packaging material) by monitoring the evolution of physicochemical parameters, sensory attributes and bioactive compound content, which were then applied to estimate shelf life (SL) using empirical or kinetic models (Li & Wang, 2018; Conte et al., 2020; Mancebo-Campos et al., 2022, 2023; Calligaris et al., 2022; Gagour et al., 2022; Martin-Torres et al., 2023). Two modeling approaches can be used to predict SL: time to reach the legal upper limit (TRUL) or time to reach the lower legal limit (TRLL), sometimes referred to as the time to lose the health claim (TLHC) (Mancebo-Campos et al., 2022, 2023).

However, the oxidation processes in bottled olive oil during storage differ significantly from those occurring in domestic use. In closed bottles stored in darkness the photo-oxidation is minimal, and since oxygen availability decreases over time, there is a shift from primary to secondary oxidation. Conversely, during domestic use, frequent bottle opening is responsible for a continuous oxygen supply, which together with the increase of the headspace due to the oil's removal, promotes primary oxidation and, if exposed to light, photo-oxidation. Therefore, oil oxidation during domestic use usually occurs at a faster rate than in unopened bottles, making it critical to evaluate this degradation.

To date, only three studies have assessed the impact of typical household consumption conditions on the physicochemical quality and polyphenol content related to the EU health claim (Rodrigues et al., 2018; Lolis et al., 2019; Klisović et al., 2022). Although these studies used different EVOOs and varied in terms of storage conditions (e.g., temperature, packaging

material, light exposure or protection from light), all consistently reported a decline in oil quality over time. The extent of this degradation varied across studies, with certain oils undergoing changes that affected their commercial classification, such as a downgrade from EVOO to VOO or lampante olive oil (LOO) (Lolis et al., 2019; Rodrigues et al., 2018). Remarkably, Klisović et al. (2022) observed no significant effect of domestic consumption routines on commercial grading, concluding that oil aging was the primary factor affecting degradation. Additionally, they found that the phenolic content related to the health claim remained almost constant and above the EU minimum specified levels over a one-month period under domestic conditions.

While these studies highlight the importance of proper storage conditions in limiting olive oil degradation, none have proposed predictive models for estimating the SL of olive oil under typical household conditions, an important concern for both consumers and olive oil producers.

1.2. Objectives

This study aimed to assess the degradation of extra virgin olive oil (EVOO) under simulated household consumption and storage conditions, with the objective of determining how long it could maintain its high commercial quality grade and/or a specific health claim. To achieve this, typical domestic practices were replicated, including daily opening and agitation of dark glass bottles, as well as the weekly removal of a predetermined volume of oil. These actions facilitated a continuous oxygen supply and increased the headspace within the bottles over a 9-week period, promoting auto-oxidation, specifically primary oxidation reactions. In addition, half of the bottles were stored in darkness, while the other half were exposed to light, allowing for an assessment of photo-oxidation's impact on oil degradation. The degradation of the EVOO was evaluated by monitoring four key physicochemical parameters: free acidity, peroxide value, and the extinction coefficients at 232 nm and 268 nm, alongside sensory analysis, throughout the 9-week study. A secondary aim was to investigate whether the household conditions studied would negatively impact on the hydroxytyrosol and tyrosol derivatives, which content is associated with the European Union's health claim regarding polyphenol protective role against oxidation of blood lipids. Lastly, the study envisaged to develop kinetic models of different reaction orders (zero-, first-, or second-order) to predict the shelf life of the EVOO in terms of both its commercial quality and its health claim status. Specifically, the goal was to establish TRUL (time to reach upper legal limit) models for quality

parameters and TRLL or TLHC (time to reach lower legal limit or time to lose health claim, respectively) models for polyphenol content, which could allow shelf life predictions based on the maximum or minimum legal thresholds, respectively.

Chapter 2. Theoretical background and literature review

2.1. Olive oil commercial quality grade, nutritional and health claims

2.1.1. Commercial grade categories

Specific regulatory standards established by the European Union (EU) and the International Olive Council (IOC) specify the quality grade categories of olive oil, which include Extra Virgin Olive Oil (EVOO), Virgin Olive Oil (VOO), and Lampante Olive Oil (LOO). An olive oil can be classified in one of these three categories depending on a set of physicochemical parameters that are related to the quality of the oil. As an example, an EVOO, the highest quality oil, must have a FA lower than 0.8 %, a PV lower than 20.0 mEq. O_2/kg of oil, a K_{232} lower than 2.50 and a K_{270} lower than 0.22. Limits for other parameters are established by the EU regulation, namely a maximum value of 35 mg/kg of oil for the fatty acid ethyl esters content (Commission Delegated Regulation (EU) 2022/2104, 2022). Additionally, according to the IOC regulations, a sensory analysis is required for establishing the oil's commercial grade, being set a median intensity greater than 0 (in a 10-point scale) for the fruity attribute and equal to 0 for any defect, which could rise to 3.5 for VOO. This analysis must be performed by an official sensory panel. When an oil is classified as LOO it cannot be consumed (IOC, 2015). In which concerns the sensory profile, besides the fruity attribute, other sensations are usually perceived in EVOO including bitter, pungent, and some specific flavor notes like herbaceous, artichoke, or almond (Fernandes et al., 2018).

2.1.2. Nutritional and health claims related to the olive oil's composition

A nutritional/health claim is defined as "*any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health*'' (European Commission Regulation EU No 432/2012, 2012). On the other hand a reduction of disease risk claim is defined as ''*any health claim that states, suggests or implies that the consumption of a food category, a food or one of its constituents significantly reduces a risk factor in the development of a human disease*'' (Asp and Bryngelsson, 2008).

Olive oil is recognized as a food with health-promoting properties, primarily attributed to its composition rich in unsaturated fatty acids and bioactive minor components, such as tocopherols and phenolic compounds (Pastor et al., 2021; de La Torre et al., 2021; Olmo-Cunillera et al., 2024). The high concentration of monounsaturated fatty acids (MUFAs),

particularly oleic acid, contributes significantly to its cardiovascular benefits and product stability, playing a key role at sensory level (Guasch-Ferré et al., 2020; Hijawi, 2021; Martínez-González et al., 2022). Vitamin E consists of eight lipid-soluble compounds including α-, β-, γ-, and δ-tocopherols plus α-, β-, γ-, and δ-tocotrienols. In olive oil, tocopherols are predominant, with α-tocopherol comprising up to 90% of the total tocopherol content (Jimenez-Lopez et al., 2020). This compound plays a critical role in protecting tissues from oxidative stress and contributes to the prevention of degenerative neuropathies (Baccouri et al., 2023; Reyes-Goya et al., 2024). Hydroxytyrosol and tyrosol derivatives are phenolic alcohols that can be found in olive oil, being associated with oil's health benefits, antioxidant properties, and characteristic sensory attributes, particularly bitterness and pungency (Baccouri et al., 2023; Passeri et al., 2023).

In the human body, phenolics act as potent antioxidants and anti-inflammatory agents, reducing oxidative damage and lowering risk factors for cardiovascular and age-related diseases (Guasch-Ferré et al., 2020; Riolo et al., 2022; Tarabanis et al., 2023; Roselli et al., 2020). The concentrations of these bioactive compounds in olive oil are influenced by several factors, including the genetic diversity of olive cultivars and are affected by local agro-climatic conditions, cultivation techniques, and the harvest time and oil extraction conditions (Figueiredo-González et al., 2022; Banco et al., 2022; Passeri et al., 2023).

Olive oil can be labeled with nutritional and health claims based on its composition. The European Food Safety Authority (EFSA, 2012) has approved several health claims associated with olive oil, including those related to the content of unsaturated fatty acids, hydroxytyrosol and tyrosol derivatives, and tocopherols (Table 1).

For example, the claim related to "*high concentration of unsaturated fatty acids*" requires that the oil contains at least 70% of its total fatty acids as MUFA (EFSA, 2012). In this case, the following sentence should be included "*Replacing saturated fats with unsaturated fats is beneficial for maintaining normal blood cholesterol levels, which supports cardiovascular health*". The claim states that replacing saturated fats with unsaturated fats helps to maintain normal blood cholesterol levels, which is associated with cardiovascular health benefits (Visioli et al., 2020; Riolo et al., 2022; Tarabanis et al., 2023). In the case of phenols, the claim is related to "*hydroxytyrosol and tyrosol derivatives*", stating that they contribute to the protection of blood lipids against undesirable oxidation. This health claim can only be used if their total content is at least 5 mg per 20g of olive oil (EFSA, 2012). As for tocopherols, a nutritional and

health claim of "high vitamin E content" can be used, which is related to the protection of cells against undesirable oxidation (EFSA, 2012).

Table 1. EFSA health claims with potential use in virgin olive oils (European Commission Regulation EU No 432/2012, 2012).

Nutrient or substance in the Food or Food Category	Declaration	Conditions of use of the claim
Monounsaturated and/or polyunsaturated fatty acids	Replacing saturated fats with unsaturated fats in the diet contributes to the maintenance of normal blood cholesterol levels. Replacing saturated fats with unsaturated fats in the diet has been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease.	The claim may be used only for food, which is high in unsaturated fatty acids, as referred to in the claim high unsaturated fat as listed in the Annex to Regulation (EC) No 1924/2006.
α-linolenic acid (ALA)	ALA contributes to the maintenance of normal blood cholesterol levels.	The claim may be used only for food which is at least a source of ALA as referred to in the claim source of omega-3 fatty acids as listed in the Annex to Regulation (EC) No 1924/2006. Information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 2 g of ALA.
Vitamin E	Vitamin E contributes to the protection of cells from oxidative stress.	The claim may be used only for food which is at least a source of vitamin E as referred to in the claim source of [name of vitamin/s] and/or [name of mineral/s] as listed in the Annex to Regulation (EC) No 1924/2006.
Olive oil polyphenols	Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress.	The claim may be used only for olive oil, which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g, oleuropein complex and tyrosol) per 20 g of olive oil. To bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 g of olive oil.

2.2. Lipid oxidation and its impact on the physicochemical composition and sensory profile of olive oil

The high concentration of unsaturated fatty acids in olive oil, even if being monounsaturated, naturally triggers enzymatic reactions that lead to its auto-oxidation. Fatty acids are hydrolysed by lipase through the lipoxygenase pathway, producing monohydroperoxides. These primary oxidation products are odorless and flavorless but are precursors of unpleasant odors and flavors that may arise in oils due to auto-oxidation reactions. Indeed, monohydroperoxides are converted into aldehydes by lyase, and subsequently into alcohols by alcohol dehydrogenase. Finally, alcohols are transformed into acetate compounds by alcohol acetyltransferase (Bertuccioli et al., 2005; Garcia-Oliveira et al., 2021). Moreover, polyphenolic compounds degrade due to the activity of polyphenol oxidase and peroxidase (Bertuccioli et al., 2005). These oxidation processes generate undesirable volatile compounds (e.g., alkanes, alkenes, aldehydes, and ketones), which are secondary oxidation products, leading to appearance of olfactory and/or gustatory-retronasal defects like rancidity (Angerosa., 2002; Kalua et al., 2005; Garcia-Oliveira et al., 2021).

Besides auto-oxidation, which involves free radical chain reactions with initiation, propagation, and termination steps, oils may suffer photo-oxidation if stored exposed to light (Choe and Min, 2006; Kanavouras et al., 2006). Auto-oxidation also depends on the type and content of unsaturated fatty acids, and the photo-oxidation requires the presence of photosensitizers and singlet oxygen quenchers. Additionally, oils can suffer from enzymatic oxidation (Li et al., 2014). The extent of the oxidation processes can be limited due to the antioxidant capacity of oil's phenolic compounds and tocopherols that can quench singlet oxygen by a charge transfer mechanism, decreasing the level of oils' photo-oxidation. Carotenoids can also inhibit photo-oxidation by quenching singlet oxygen and triplet excited states of photosensitizers.

The quenching mechanism of carotenoids is due to their low singlet energy state, which facilitates the acceptance of energy from singlet oxygen. The antioxidant activity of carotenoids can also be attributed to their capacity of acting as light filters (Psomiadou & Tsimidou, 2002b). However, carotenoids can also act as pro-oxidants by degrading hydroperoxides into hydroxyl- or epoxycarotenes. Chlorophyll pigments and derivatives can also promote pro-oxidation,

acting as photosensitizers due to the ability to transfer energy from light to triplet oxygen, producing thus singlet oxygen, which then reacts with unsaturated fatty acids.

Consequently, due to oxidation, olive oil undergoes a reduction in quality and economic value, due to the appearance of sensory defects or the rise of the physicochemical quality parameters above the legal thresholds, being not possible to commercialize it as EVOO. Depending on the severity of the detected defect(s) or the final physicochemical values, it may be downgraded to LOO, which is unsuitable for commercialization for food purposes in its unrefined form.

2.3. Olive oil storage under commercial or household consumption conditions

The oxidation of the lipid fraction in olive oil begins during its extraction, specifically during the malaxation process, and continues throughout the oil's commercial distribution, including its storage in various containers at producer or retailer warehouses, as well as during the domestic storage and period of use.

Following extraction and prior to consumption, the rate and extent of oxidation are influenced by multiple factors. These include the oil’s initial chemical composition, particularly the amount of unsaturated fatty acids, the type and concentration of minor compounds, the packaging material (e.g., glass and plastic bottles, tin containers, and bag-in-box), color and thickness, the volume of the headspace and its oxygen content (or the presence of an inert gas), storage temperature and time-period (i.e., oil’s age), as well as exposure to light or darkness during storage (Abbattista et al., 2020; Garcia-Oliveira et al., 2021; Macaluso et al., 2024; Martín-Tornero et al., 2023). Since the oil inside the containers is in a closed environment, the oxygen availability is limited, restricting the primary oxidation reactions and favoring secondary ones. The formation of secondary oxidation products is enhanced under these conditions which is results in a fast rise of the extinction coefficients values, especially of the K_{268} (Li & Wang, 2018; Conte et al., 2020; Mancebo-Campos et al., 2022, 2023; Calligaris et al., 2022; Gagour et al., 2022). Similarly, during domestic use, oxidation is influenced by factors such as temperature, duration of storage, packaging materials, and exposure to light or darkness.

However, during the consumption period, the container or bottle is regularly opened, often multiple times per day, with oil being removed at each use. This practice increases and renews the headspace within the container, thereby enhancing the availability of oxygen and promoting continuous oxygen exposure. The abundance of oxygen inside the oil's container during its domestic use accelerates primary oxidation reactions and thus a sharp rise of the PV is expected, leading to an increased rate of oxidation and, consequently, a faster decline in the oil's quality. Despite its significance to both consumers and olive oil producers, relatively few studies have systematically evaluated the degradation of olive oil quality under typical household consumption conditions (Rodrigues et al., 2018; Lolis et al., 2019; Klisović et al., 2022).

Two key studies provide valuable insights into the conditions impacting EVOO quality under household conditions. Firstly, Rodrigues et al. (2018) simulated typical household usage over a month, storing nine bottles of "Arbequina" EVOO at 16-20 °C in dark green glass under household lighting. While two bottles remained closed as controls, seven were opened, shaken daily, and sampled (5 mL daily, 110 mL weekly). Consequently, opened bottles exhibited significant quality degradation, with K_{232} and PV strongly correlating with time (R-Pearson ≥ +0.81), reflecting oxidative degradation and transition from EVOO to lampante oil after 28 days. This change, assisted by the development of rancid flavor, was confirmed through an electronic tongue and discriminant analysis, achieving 95% sensitivity in differentiating oils by usage duration.

Following this line of research, Lolis et al. (2019) extended this study by exploring the effects of packaging and temperature on EVOO stability, focusing on standard (22 °C) and abuse (37 °C) temperature conditions. Using oil from "Koroneiki" olives, they compared 3L bag-in-box multilayer pouches (low oxygen permeability) with traditional tinplated steel containers. Notably, bag-in-box packaging with minimal headspace (2%) demonstrated superior quality preservation, particularly under high temperatures, as it reduced oxygen exposure. In contrast, EVOOs in tin containers reached the acidity threshold for EVOO standards after 80 days at 22 °C and 60 days at 37 °C, while the bag-in-box packaging maintained the extra virgin quality grade for 120 and 100 days, respectively. Furthermore, PV and K_{232} limits were surpassed more quickly in tin containers, emphasizing the role of headspace and oxygen exposure in oxidation.

More recently, Klisović et al. (2022) investigated the stability of EVOO quality, health, and flavor properties under simulated household consumption also over one month. Their study used nine one-liter bottles of "Istarska bjelica" and "Buža" EVOOs, stored at 20°C (±2°C) and in darkness. Three bottles were kept closed as controls, while the remaining six were opened daily to simulate regular house use. Daily withdrawals of 20 g of oil simulated typical consumption habits, and weekly 100 mL samples were analyzed. Results revealed a significant PV increase in both cultivars over the one month, attributed to headspace oxygen exposure, however PV remained within legal limits. Additionally, a slight rise in K_{232} values was observed only in "Istarska bjelica", while other quality indicators (K_{268}, ΔK, and fatty acid composition) remained stable. Total phenolic content decreased by 12.3% for "Istarska bjelica" and 17.6% for "Buža", and volatile compounds dropped by 19%.

The study demonstrated that the phenolic content stayed above EFSA health claim requirements, confirming EVOO's health benefits during typical consumption. In contrast, Rodrigues et al., (2018), which found greater degradation under household lighting, the study of Klisović et al. (2022) isolated the effects of oxygen without light exposure. This suggests that light, in addition to lower initial TPC, accelerated oxidative degradation in Rodrigues's study. By isolating light's influence, Klisović's study attributed TPC reductions to natural oil aging rather than photo-oxidative effects, emphasizing that phenolic compound degradation is primarily influenced by the oil's initial chemical composition.

These three studies collectively underscore the critical importance of house use in maintaining EVOO quality standards. Moreover, the findings have practical implications, offering guidance to producers on packaging choices and to consumers on storage practices to retain EVOO's chemical, health and sensory qualities.

However, none of the previous research works have considered a predictive modeling approach to estimate the duration (i.e., the shelf life, SL) for which EVOO or VOO can retain its original commercial grade classification, or maintain the minimum legally required levels of unsaturated fatty acids, tocopherols, and/or specific phenolic compounds under typical household storage/consumption conditions, ensuring continued nutritional and health benefits as defined by EFSA health claims.

2.4. Olive oil shelf life

According to European Union legislation (EC Regulation No. 1169/2011), the shelf life (SL) of food products is defined as the “date of minimum durability” and must be indicated either by a "best before" date or a "use by" date. The "best before" date signifies the period during which a food product retains its specific characteristics, such as taste, aroma, appearance, and other product-specific qualities, provided it is stored under appropriate conditions and the packaging remains unopened. In contrast, the "use by" date pertains to food safety, indicating the date beyond which consumption may pose health risks (Moschopoulou et al., 2019).

For EVOO (or VOO), the term SL refers to the period during which the oil maintains its sensory attributes (e.g., perceived fruitiness, and absence of sensory defects) and values of chemical parameters (FA, PV, K_{232} and K_{268} among others) within the regulatory limits under standard storage conditions (Guillaume & Ravetti, 2016; Mancebo-Campos et al., 2023). Additionally, the SL concept may encompass the time during which certain nutritional or health claims, such as those related with the minimum contents of polyphenolics, vitamin E, or the relative abundance of unsaturated fatty acids, remain valid, and provided these EFSA claims are included on the EVOO label. The application of this SL framework may extend beyond commercial storage (i.e., prior to sale) to include the oil’s shelf life during domestic use under typical household conditions. Communicating accurate information regarding the oil’s expected post-opening SL is essential for fostering consumer confidence in the continued quality and nutritional/health benefits of EVOO.

Shelf life assessments can be conducted either through real-time studies or by using accelerated shelf life testing (ASLT). ASLT speeds the oxidation process, enabling the prediction of EVOO's SL through extrapolation (Calligaris et al., 2022). However, ASLT may introduce inaccuracies, as the extrapolated values can overestimate the actual SL of EVOO (Kaya et al., 1993). Nonetheless, data from real-time and ASLT methods can be integrated or used independently to develop predictive models for determining the SL of EVOO either during its commercial storage or its domestic use.

Chapter 3. Materials and methods

3.1. Olive oil samples and storage conditions

Ten amber glass bottles, each with a capacity of 750 mL of a monovarietal EVOO (cv. Cobrançosa), were obtained from a local Portuguese olive oil producer (Cooperativa de Olivicultores de Valpaços, Portugal), totaling 7.5 liters. The EVOO samples were assessed in this study under simulated conditions of domestic use. The bottles were stored in a laboratory setting (Bragança, Portugal) under two distinct lighting conditions at ambient temperature (18±2 ºC). Five of the bottles were stored in darkness, while the remaining five were exposed to non-controlled day/night parameters corresponding to the natural exposure to day light as well as artificial light. Each day, all bottles were opened for 10 minutes and shaken for 1 minute to allow oxygenation, simulating the frequent opening of bottles during domestic use. Additionally, 60 mL of oil was withdrawn weekly from each bottle to simulate typical domestic consumption, which progressively increased the headspace within the bottles, thus accelerating the oxidation process. The removed EVOO was subjected to physicochemical analyses, including free acidity (FA), peroxide value (PV), specific UV absorbances at 232 nm (K_{232}) and 268 nm (K_{268}), total phenolic content (TPC), and quantification of hydroxytyrosol and tyrosol derivatives after acid hydrolysis. Sensory analysis was also performed to evaluate the progression of oxidation and degradation of the olive oil during the experiment.

3.2. Olive oil quality parameters

3.2.1. Free acidity

Free acidity (FA) is an important quality parameter that can determine the quality grade of olive oil. This method involves adding the following solutions to a 100 ml Erlenmeyer flask: 50 ml of ether alcohol solution (1: 1 ratio of diethyl ether and ethanol 96%) and 3 drops of 2% phenolphthalein to 5.00 g of olive oil already weighed in the Erlenmeyer. After agitating the solution for a few seconds, titration with a solution of sodium hydroxide (NaOH, 0.1 N) should be gradually added to the solution until the color deviation is observed. This analysis was carried out in accordance with the methodology present in Commission Implementing Regulation (EU) 2022/2105 following the COI/T.20/Doc. No 34 (Determination of free fatty acids, cold method).

The mathematical formula for free acidity, expressed as a percentage (%) is:

$$FA\ (\%) = \frac{(V \times C \times M)}{(10 \times m)} \quad (3.1)$$

Where:

V: Volume of titrated potassium hydroxide solution (expressed in milliliters)

C: concentration of titrated sodium hydroxide solution (expressed in moles per liter)

M: 282 g/mol, molar mass of oleic acid (expressed in grams per mole)

m: Mass of the sample (expressed in grams)

3.2.2. Peroxide value

The peroxide value (PV) analysis was conducted following the guidelines provided in Commission Implementing Regulation (EU) 2022/2105, according to COI/T.20/Doc. No 35 (Determination of peroxide value), in which 1.20 g of olive oil was weighed in a 250 mL Erlenmeyer, to be mixed with 15 mL of glacial acetic acid, 10 mL of chloroform and 1 mL of a saturated solution of potassium iodide. Erlenmeyer flasks were capped with a parafilm and agitated for one minute and then stored in the dark for 5 minutes. Once the 5 min are over, 75 mL of distilled water and three drops of starch solution at 1.0 g/100 mL should be added. The titration was done with 0.01 N sodium thiosulfate solution that should be gradually added drop by drop until noticing a change from a dark color to a transparent solution.

The mathematical formula for peroxide value, expressed as milliequivalents of active oxygen per kilogram of oil (m.EqO_2/kg), is:

$$PV\left(\frac{mEq.O_2}{kg}\right) = \frac{V \times T \times 1000}{m} \quad (3.2)$$

where:

V: Volume of titrated sodium thiosulphate solution (mL)

T: Molarity of titrated sodium thiosulphate solution (mol/L)

m: Mass of the sample (g)

3.2.3. Extinction coefficients

In a 15 mL tube, 0.60 g of oil was weighed and then diluted with isooctane (2,2,4-trimethylpentane) to a total volume of 10 mL. A vortex mixer was used to ensure homogenization of the mother solution which was subjected to first and second dilutions with isooctane. The diluted solutions were placed in a cuvette for spectrophotometric analysis at appropriate wavelengths (232, 268 nm) using Shimadzu UV-VIS/UV-1280 spectrophotometer. Typically, a 1:25 dilution was used for reading 232 nm and 1:5 for readings at 264, 268 and 272 nm. A second dilution was performed when results fell outside the range of 0.100 to 0.800 while keeping the cuvette clean by washing it with isooctane and calibrating the instrument with auto-zero using a cuvette containing only isooctane. This analysis also followed the instructions given by Commission Implementing Regulation (EU) 2022/2105 according to COI/T.20/Doc. No 19 (Spectrophotometric investigation in the ultraviolet).

The mathematical formulas for extinction coefficients at different wavelengths (232 and 268 nm), are:

$$K_{232} = \frac{A_{233} \times D}{m \times 10} \quad (3.3)$$

$$K_{268} = \frac{A_{268} \times D}{m \times 10} \quad (3.4)$$

Where:

A_{232}, A_{268}: Absorbances at 232 nm and 268 nm, respectively

D: Dilution factor

m: Mass of sample (g)

3.3. Sensory analysis

The sensory analysis of the EVOO samples was conducted by a trained panel from Escola Superior Agrária de Bragança (Bragança, Portugal), following the guidelines proposed by European community regulation and international Olive Council (IOC, 2005) (Rodrigues et al., 2020). Samples were evaluated weekly to assess the changes in both olfactory and gustatory

sensations. Olfactory attributes included ripely or greenly fruity, apple, tomato, dry fruits, tomato leaves, cabbage, fresh grass, dry grass and harmony. Gustatory attributes distinct from olfactory ones comprised sweetness, bitterness, pungency. Additional attributes evaluated were complexity, persistence and rancidity, which can be perceived both olfactory and gustatory. The oil profile sensory sheet used a 10 cm unstructured scale, with intensity ratings ranging from 0 to 10 for each attribute. Weekly evaluations were conducted using standard blue-colored glasses, with samples maintained at approximately 28°C to optimize the release of flavors and aromas. The oil has been classified based on the median of defects and the median of the fruity attributes.

3.4. Total phenols content analysis

This method involves two major parts. The first part is the microextraction of the phenolic content compounds present in the olive oil, the microextraction should be performed three times. The analysis involves weighing 0.5 mL of the sample in a 2 mL Eppendorf tube and adding 1 mL of methanol-water (MeOH-H_2O) solution (80% v/v 80 mL methanol with 20 mL water). After vortexing all, the eppendorf was centrifuged for 5 min at 13.200 rpm. Once the centrifuge is complete, the supernatant should be carefully collected using a micropipette and transferred to a 15 mL plastic tube covered with aluminium foil. This step was repeated three times to obtain a combined supernatant that will be used for subsequent total phenols analysis (Pizarro et al., 2013).

For the determination of total phenol content, a mixture was prepared in a glass tube containing 1500 µL of distilled water, 100 µL of the phenolic extract, and 100 µL of Folin Ciocalteu reagent. This mixture was vortexed for 3 seconds and left to react for 3 minutes. After the initial reaction, 300 µL of 20% Na_2CO_3 solution was added to the tube. The sample was then stored in the dark for one hour. Finally, the absorbance of the solution was measured using a spectrophotometer at a wavelength of 765 nm. The procedure was carried out in duplicate.

3.5. Olive oil total hydroxytyrosol and tyrosol derivatives after acid hydrolysis

The quantification of total hydroxytyrosol and tyrosol derivatives in olive oil samples was performed using chromatographic analysis following the acid hydrolysis of secoiridoid compounds (Marx et al., 2021).

For phenolic compounds extraction, hydrolysis and analysis, 0.1g of each olive oil sample was weighed and combined with 0.006 mg of internal standard (40 μL of 0.15 mg/ml syringic acid solution in 80:20 v/v methanol/water). To this mixture, 2.5 mL of 2 M hydrochloric acid in methanol/water (80:20, v/v) was added and vortexed for 30 seconds. The solution was then incubated at 25°C for 6 hours, protected from light, with 30-second vortexing every 30 minutes to ensure thorough hydrolysis.

Following hydrolysis, 2.5 mL of acetonitrile/water (50:50 v/v) was added. The mixture was transferred to 2 mL Eppendorf tubes and centrifuged at 13.000 RPM for 10 minutes. Subsequently, 2 mL of the supernatant was removed and mixed with 2 mL of n-hexane. This mixture was vortexed for 30 seconds and centrifuged at 3500 RPM for 3 minutes.

The resulting polar extract was filtered through a 0.22 μm PVDF (Polyvinylidene fluoride) disposable filter. The filtered sample was then analyzed using HPLC-DAD with a C18 column ("Kinetex C18; particle size: 2.6 μm; pore size: 100 Å; LC length: 100 mm; internal diameter: 3.00 mm, Phenomenex").

Results were expressed as the sum of individual compounds in milligrams of hydroxytyrosol or tyrosol equivalents, per kilogram of olive oil (mg/kg). This method allows for the quantification of both free and bound forms of these phenolic compounds, providing a comprehensive measure of their presence in the olive oil samples(Tsimidou et al., 2019).

Since after hydrolysis only the tyrosol and hydroxytyrosol moieties are quantified, losing information on the molecular weight of the original molecules, the original bound forms were estimated using the correction factors proposed in the literature for hydroxytyrosol (correction factor = 2.2) and tyrosol (correction factor = 2.5) (Mastralexi et al.,2014; Tsimidou et al.,2019)

3.6. Oxidative stability

The stability of olive oil was assessed using the Rancimat method (Rancimat model 743 from Metrohm, Switzerland) following the methodology proposed by Rodrigues et al., (2019). The process starts with weighing 3.00g of oil in the tubes which were tested in duplicate. The methodology consists of bubbling filtered, cleaned and dried air through the samples at a speed of 20 L/h while heating them to 120±1.6°C. During this process, oxidation compounds formed in the oil are directed by the airflow into an aqueous solution. The analysis is completed when the conductivity value reaches 300µS/cm. The stability time is calculated by the software associated with the equipment, based on the tangents to the conductivity curve. This method effectively measures the oil's resistance to oxidation, providing useful information about oil's quality.

3.7. Olive oil Shelf life predictions using mathematical modelling strategies

The SL of the EVOO samples under simulated domestic consumption conditions was predicted using a kinetic approach. The physicochemical data collected during the 9-week time-period of the assay were used together with Eq. (3.6) to (3.8) to determine, by simple linear regression analysis (Excel, Data Analysis ToolPak), the kinetic reaction rates (k) at ambient temperature (20 ºC), for each parameter that showed a decreasing or increasing trend over time, for samples stored in dark or exposed to light. In each case, the quality of the regression was assessed by calculating the correlation coefficient (R-Pearson) of the fitted linear line. Finally, Eq. (3.9) to (3.11) were applied to predict the SL of the studied EVOO during the oil's domestic consumption for the two storage conditions considered in this study, taking into account the maximum or minimum levels established in the EU regulations for EVOO classification or EFSA claim for the polyphenolics content.

Two types of predictive models are commonly employed: empirical or kinetic models. Empirical models estimate the SL by considering stability parameters and often rely on the use of multivariate statistical tools and their accuracy is influenced by the database used, which should be as extensive as possible (Pagliarini et al., 2000). In contrast, kinetic models rely on estimating reaction rates (k) for key physicochemical parameters, assuming a specific reaction

order ($n \geq 0$). For that a relationship between the content/level of a specific parameter of interest (C) and time (t) is established, as indicated in Eq. (3.5).

The positive or negative sign (±) in the equation reflects the increasing or decreasing trend of the parameter over time, respectively.

$$\frac{dC}{dt} = \pm k \times C^n \tag{3.5}$$

The integration of Eq. (3.5), at a constant storage temperature, from the initial storage conditions ($t = 0$ and $C = C_0$) and the current conditions (t and C) will result in various models based on the assumed reaction order such as,

Zero-order reaction (n = 0):

$$\frac{dC}{dt} = \pm k \rightarrow integration: C - C_0 = \pm k \times t \tag{3.6}$$

First-order reaction (n=1):

$$\frac{dC}{dt} = \pm k \times C \rightarrow integration: ln\left(\frac{C}{C_0}\right) = \pm k \times t \tag{3.7}$$

Other-order reaction ($n \neq \{0,1\}$):

$$\frac{dC}{dt} = \pm k \times C^n \rightarrow integration: \frac{C^{-n+1}}{-n+1} - \frac{C_0^{-n+1}}{-n+1} = \pm k \times t \tag{3.8}$$

By employing experimental data (t, C) and using Eqs. (3.6) to (3.8), the kinetic reaction rates at a constant temperature can be estimated by linear regression analysis, being k equal to the linear slope.

Depending on the specific parameter being investigated, two distinct types of kinetic models can subsequently be employed to predict the SL, namely of olive oils (Mancebo-Campos et al., 2022, 2023):

- Time to reach the legal upper limit (TRUL), which is applied when the content/level of the parameter under study increases with the storage time (e.g., FA, PV, K_{232} or K_{268}).
- Time to reach the legal lower limit (TRLL), which is applied when the content/level of the parameter under study decreases with the storage time (e.g.,

polyphenols content related to the health claim ; also referred in the literature as the time to lose the health claim, TLHC).

The SL can be determined by solving Eqs. (3.6) to (3.8) with respect to time't', resulting in the SL. It is important to note that letter $C_{\text{legal limit}}$ represents the maximum or minimum legal content/level of the parameter being evaluated for TRUL or TRLL models, respectively:

Zero-order reaction (n = 0):

$$SL = \frac{C_{legal\ limit} - C_0}{\pm k} \tag{3.9}$$

First-order reaction (n=1):

$$SL = \frac{ln\left(\frac{C_{legal\ limit}}{C_0}\right)}{\pm k} \tag{3.10}$$

Other-order reaction (n≠{0,1}):

$$SL = \frac{\frac{C_{legal\ limit}^{-n+1}}{-n+1} - \frac{C_0^{-n+1}}{-n+1}}{\pm k} \tag{3.11}$$

Li and Wang (2018) provided a review of both empirical and kinetic models reported in the literature for predicting the SL of olive oils in unopened containers, under different commercial storage conditions. However, no information could be found regarding the use of similar modelling strategies for predicting the SL of olive oils under typical household consumption conditions.

3.8. Statistical analysis

The evaluation of the results from various analyses under dark and light storage conditions during the 9-week household consumption period was conducted using a full one-way analysis of variance (ANOVA). When significant differences were detected, Tukey's test was applied for multiple comparisons between the different weeks. To assess statistical significance between the two storage conditions (dark and light), an independent sample Student's test was applied. All statistical analyses were performed using RStudio (version 3.6.2), with the significance level set at α=0.05, all the data reported as the mean ± standard deviation (SD).

Chapter 4. Results and discussion

4.1. Olive oil physicochemical quality parameter

To evaluate the degradation of EVOO quality and changes in its chemical composition, various physicochemical quality parameters were analyzed. The results of these analyses, which provide valuable assessments into olive oil quality, are presented and discussed in this section.

The study carried out showed that FA of the olive oil remained almost constant over the 63-day duration of the simulated household consumption conditions for oils exposed to light or stored in darkness (Figure 1). The FA varied between 0.23 and 0.25%, remaining well below the maximum limit of 0.8% for EVOO classification as established by the European Commission regulation (EU) 2022/2105.

In which concerns the PV, an indicator of the extent of the primary oxidation, it was observed a more significant difference between the oils kept exposed to light and stored in darkness. Light-exposed oils showed a faster and more pronounced rise in PV over time. This finding underscored the accelerated oxidation in light-exposed samples probably related to the effect of photo-oxidation. As shown in Table A1 and Figure 2, The PV in light-exposed oils increased by 81% from 10.6±0.2 mEq O_2/kg to 56.73±0.95 mEq O_2/kg (as seen in Table A2).

Indeed, between days 28 and 35 of the study, the PV level of light-exposed oils exceeded the EU legal maximum limit of 20 mEq O_2/kg for EVOO or VOO classification (European Commission regulation (EU) 2022/2105) as it is shown in the Figure 2 So, within this time interval (28-35 days) the EVOO grade diminished to LOO grade, rendering the oil unsuitable for consumption. Contrary, light-protected oils exhibited a slower PV increase (of +44%, Table A1), remaining below the maximum legal limit, and thus retaining the EVOO classification.

This slower rate of oxidation can be due to the protective role of natural pigments in olive oil, such as chlorophyll and polyphenols, which function as antioxidants. The marked difference in PV between light and dark storage highlights the significant impact of photo-oxidation on the oxidation level of unsaturated fatty acids (Pristouri et al., 2010). The results demonstrate that light exposure, in combination with oxygen availability, greatly accelerates the formation of hydroperoxides, the primary products of lipid oxidation and thus PV.

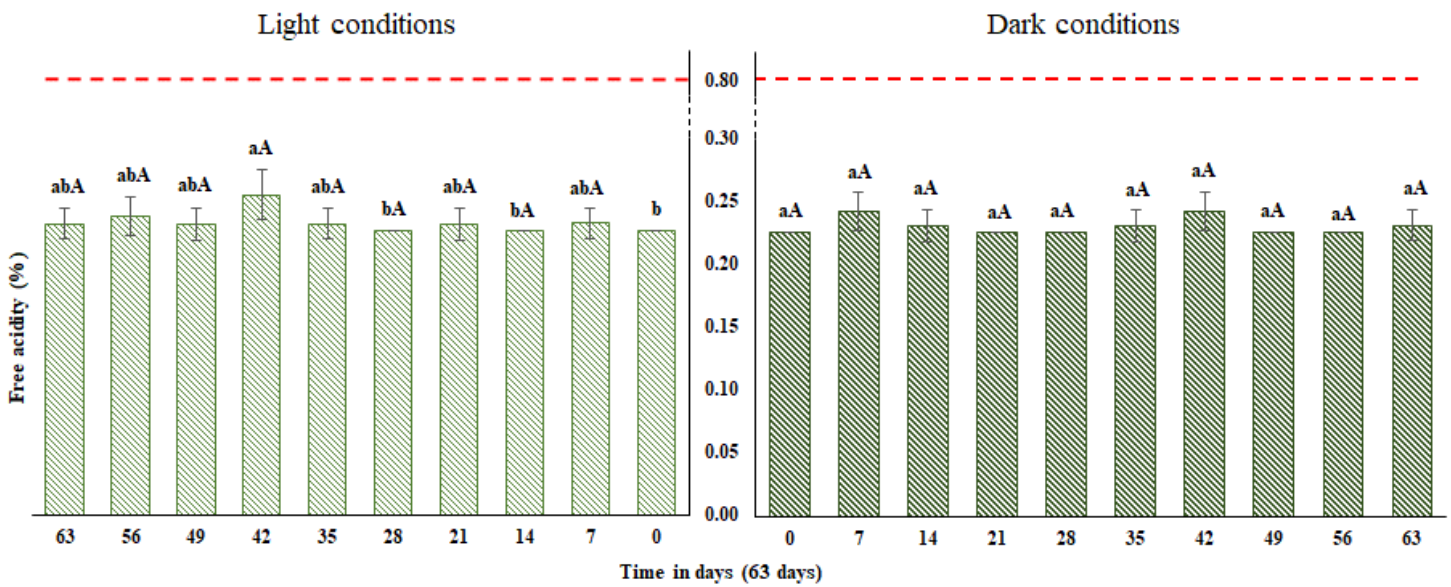

Figure 1. Time-evolution of free acidity in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness. Red dashed line represents the EU limit for EVOO classification (European Commission regulation (EU) 2022/2105).

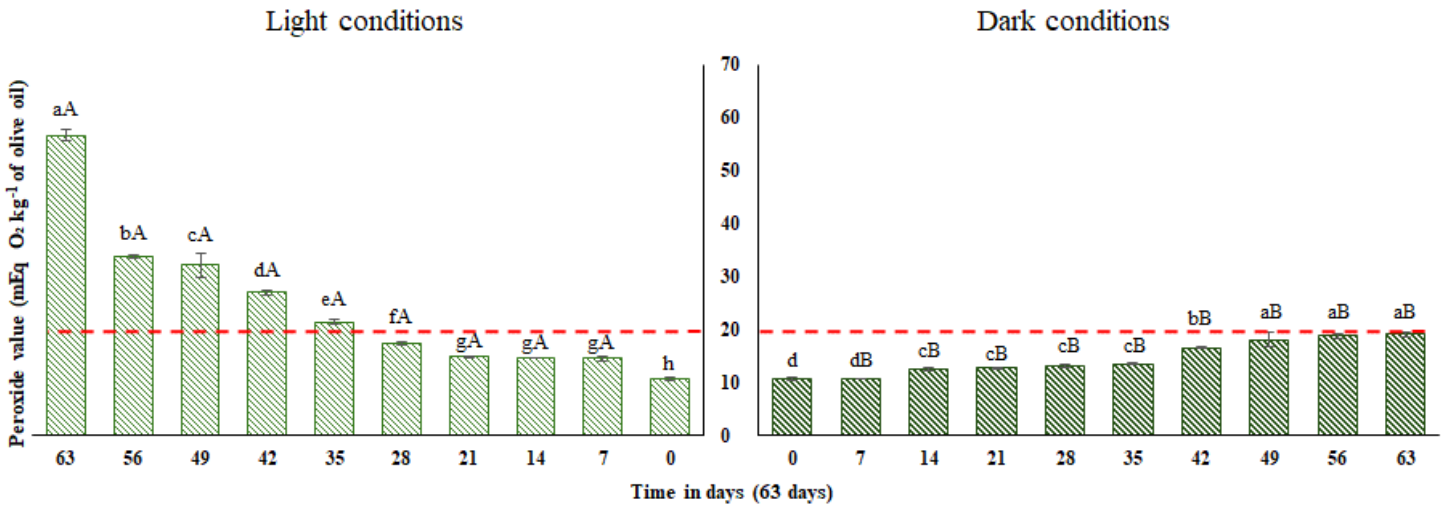

Figure 2. Time-evolution of peroxide value in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness. Red dashed line represents the EU limit for EVOO classification (European Commission regulation (EU) 2022/2105).

The oxidation of the bottled olive oil was further assessed by monitoring the time-evolution of the extinction coefficients K_{232} and K_{268}, which levels are related to the concentration of conjugated dienes (primary oxidation products), and secondary oxidation products (such as aldehydes and ketones), respectively (Balaky et al., 2020).

The values of K_{232} increased over time for both storage conditions, with a more pronounced rise for the samples kept in darkness compared to those exposed to light. It should be noted that for light-protected oils, at day 56, the K_{232} value exceeded the maximum limit allowed for EVOO classification, as established by the European Commission Regulation (EU) 2022/2105, being the oil's grade downloaded to VOO until the final of the study. Figure 3 shows the increase of K_{232} under both storage conditions, with a slightly higher increase for the samples kept in darkness (+30%) compared to those exposed to light (+26%) (Table A1), which was different from the observed PV trends.

These findings align with (Caponio et al., 2005), a study that showed higher K_{232} values in samples stored in darkness compared to those exposed to light. While photo-oxidation is known to significantly affect olive oil oxidation when exposed to light, the dark stored samples exhibited accelerated oxidation during light exposure during the analysis. This phenomenon, which can be called 'light shock', may be the explanation for the sudden oxidative stress experienced by samples that are not acclimated to light exposure.

Another possible explanation for the K_{232} trend of samples exposed to light was proposed by Esposto et al. (2017). Their study demonstrated that the decrease in health and sensory properties, as well as quality parameters such as extinction coefficients during light exposure, depends on the initial antioxidant capacity, especially the oleuropein derivative content. They hypothesized that "the higher the antioxidant capacity, the higher the resistance to oxidation." This finding suggests that olive oils exposed to light may develop resistance to oxidation due to their antioxidant capacity.

The increase for k_{232} samples stored in dark may be also more susceptible to rapid oxidation when suddenly exposed to light during analysis. It is important to note that further research and analysis are needed to fully understand and specify the mechanisms behind this reaction and its implications for olive oil quality assessment and storage practices.

Regarding K_{268}, as shown in Figure 4, in light and dark storage conditions a slight increase was observed over time during the oil's domestic simulated use. However, light-exposed samples exhibited a more pronounced increase (+28%), with K_{268} values rising from 0.16 ± 0.02 to 0.22 ± 0.00 by the end of study's period (Tables A1 and A2). Despite this increase over the 9 weeks, the K_{268} values did not exceed the maximum legal limit of 0.22 for EVOO classification set by the European Commission Regulation (EU) 2022/2105.

Overall, these results of the present study align with the findings of Rodrigues et al. (2018) and Klisović et al. (2022), that reported a similar increasing trend of all physicochemical parameters (FA, PV, K_{232} and K_{268}) during a 28-day of oil's simulated domestic use. The increasing trends of the physicochemical parameters are also in line with the main findings reported by Lolis et al. (2019), although some differences could be observed in the time of exceeding the maximum legal limits for FA, PV and K_{232}. These differences can be tentatively attributed to the different storage conditions studied, namely the study period, temperature and the type of packaging material (glass bottles versus bag-in-box containers).

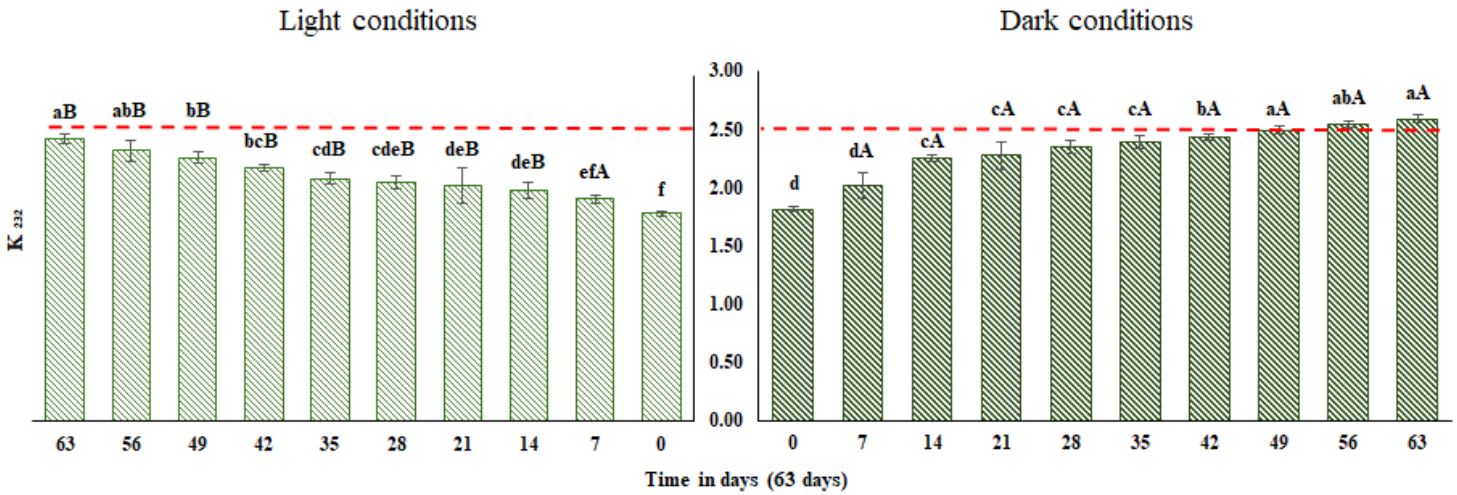

Figure 3. Time-evolution of the extinction coefficient K_{232} in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness. Red dashed line represents the EU limit for EVOO classification (European Commission regulation (EU) 2022/2105).

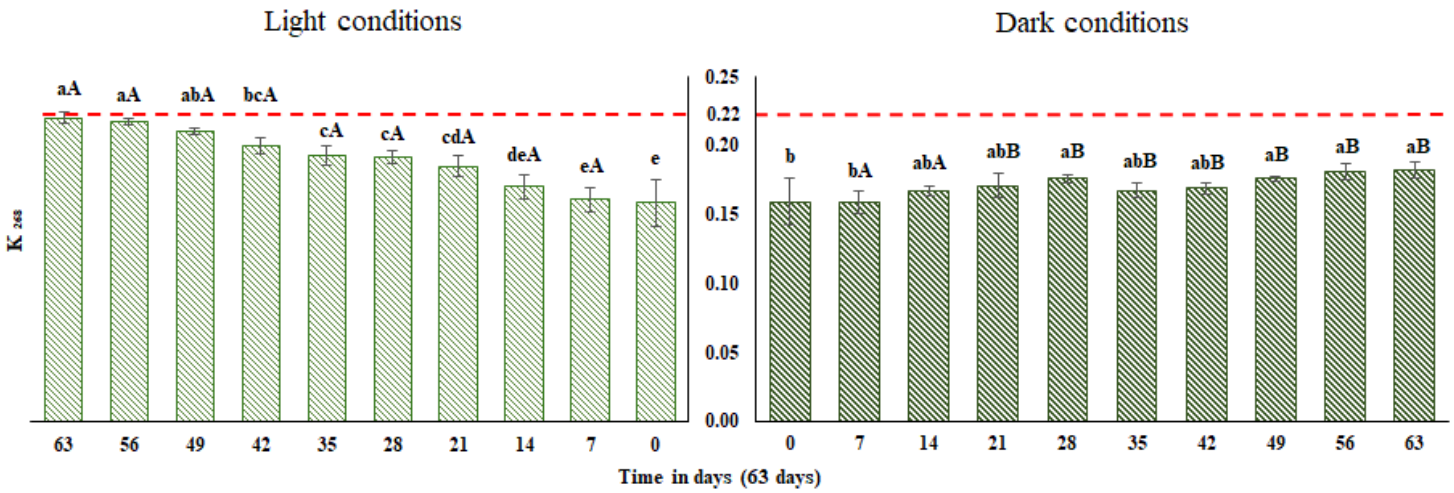

Figure 4. Time-evolution of the extinction coefficient K_{268} in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness. Red dashed line represents the EU limit for EVOO classification (European Commission regulation (EU) 2022/2105).

4.2. Sensory analysis

Tables 2 and 3 provide an overview of the olfactory and gustatory sensory profiles, respectively, of both light-exposed and light-protected oil during the 63-day period of simulated domestic use. In general, the intensity perceived by the sensory panel of positive attributes, especially those related with “fresh-fruity” sensations, decreased significantly in all oils over time, with a faster decline observed for light-exposed oils. Storage in dark preserved the positive attributes, avoiding the negative effect of photo-oxidation. It should be mentioned that attributes like tomato leaves, cabbage, and fresh grass became unperceived after 28-35 days of the assay.

Overall harmony of the oil decreased slightly but significantly in both conditions, with dark storage maintaining slightly higher scores. Oppositely, the intensity of “dry” and “ripe” sensations as well as sweetness increased with the duration of the simulated household consumption. For example, dry grass notes appeared only in later stages, with dark-stored samples showing a significant increase from day 42 to 63. Dry fruit sensations increased significantly, particularly in light-exposed samples. Ripe-fruity emerged only in later stages (from day 42 onwards), with dark-stored samples showing a significant increase over time.

Particularly, rancidity was detected only in light-exposed samples starting from day 56 onwards, showing a significant increase. The appearance of rancidity in this study aligns with the findings of (Rodrigues et al., 2018), which also detected rancid at day 28. In the study of (Klisović et al., 2022), no sensory defect was perceived. The descriptive sensory profile and main increasing/decreasing intensity trends, established in the present study align, in general, with those described by (Rodrigues et al., 2018) and (Klisović et al., 2022) that also reported the perception of "Olive fruitiness”, “Apple”, “Almond”, “Pungency”, “Bitterness”, with some different sensations being perceived in each study (e.g., banana, dry nuts and fresh herbs). These results underscore the great impact of storage time and domestic use conditions on the sensory profile of olive oil, with dark storage conditions providing in general a better capacity of preserving the intensity of positive attributes and delaying the onset of negative ones such as rancidity.

Table 2. Results of mean and SD of sensory analysis (olfactory sensations) assessed by a trained sensory panel during 63 days of household consumption conditions.

Olfactory sensations	**Storage conditions**	**Storage time**										
		0 day	**7 days**	**14 days**	**21 days**	**28 days**	**35 days**	**42 days**	**49 days**	**56 days**	**63 days**	**P-value**
Ripely fruity	**Light**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	5.59 ± 1.54^{aA}	6.11 ± 0.98^{aA}	N.E.	N.E.	0.5420
	Dark	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3.90 ± 1.32^{bA}	5.11 ± 0.44^{abA}	5.08 ± 0.39^{ab}	5.38 ± 0.24^{a}	0.0262
	P-value	----	----	----	----	----	----	0.0999	0.0711	----	----	----
Greenly fruity	**Light**	6.83 ± 0.57^{a}	5.75 ± 0.20^{bB}	5.59 ± 0.36^{bA}	5.61 ± 0.46^{bA}	4.57 ± 0.89^{cA}	3.34 ± 0.32^{dA}	N.D.	N.D.	N.E.	N.E.	<0.0001
	Dark		6.38 ± 0.29^{aA}	5.71 ± 0.27^{bA}	4.55 ± 0.16^{cB}	2.56 ± 0.33^{dB}	1.54 ± 0.21^{eB}	N.D.	N.D.	N.D.	N.D.	<0.0001
	P-value	----	0.0039	0.5710	0.0012	0.0014	<0.0001	----	----	----	----	----
Apple	**Light**	5.90 ± 0.37^{a}	5.25 ± 0.15^{bA}	4.45 ± 0.32^{cA}	4.27 ± 0.20^{cA}	3.26 ± 0.23^{dA}	3.43 ± 0.44^{dA}	2.42 ± 0.37^{eA}	2.56 ± 0.28^{eA}	N.E.	N.E.	<0.0001
	Dark		5.19 ± 0.47^{bA}	4.27 ± 0.30^{cA}	3.64 ± 0.32^{cdB}	3.42 ± 0.43^{dA}	2.68 ± 0.21^{eB}	2.59 ± 0.11^{eA}	2.53 ± 0.22^{eA}	2.35 ± 0.26^{e}	2.55 ± 0.29^{e}	<0.0001
	P-value	----	0.7910	0.3870	0.0062	0.4840	0.0092	0.3550	0.8560	----	----	----
Tomato	**Light**	6.73 ± 0.72^{a}	6.06 ± 0.07^{abA}	5.62 ± 0.23^{bA}	5.45 ± 0.29^{bA}	4.52 ± 0.24^{cA}	4.33 ± 0.41^{cA}	N.D.	N.D.	N.E.	N.E.	<0.0001
	Dark		6.32 ± 0.37^{abA}	5.59 ± 0.16^{bA}	4.56 ± 0.26^{cB}	3.20 ± 0.26^{dB}	2.64 ± 0.29^{dB}	N.D.	N.D.	N.D.	N.D.	<0.0001
	P-value	----	0.1640	0.8160	0.0009	<0.0001	<0.0001	----	----	----	----	----
Dry fruits	**Light**	2.83 ± 0.20^{d}	3.92 ± 0.54^{cA}	4.45 ± 0.30^{bcA}	4.47 ± 0.48^{bcA}	4.74 ± 0.43^{bcA}	5.05 ± 0.58^{bA}	6.19 ± 0.45^{aA}	6.25 ± 0.38^{aA}	N.E.	N.E.	<0.0001
	Dark	2.83 ± 0.20^{ef}	2.68 ± 0.39^{fB}	3.82 ± 0.15^{cdB}	3.86 ± 0.22^{cdB}	3.43 ± 0.27^{deB}	3.89 ± 0.53^{cdB}	4.29 ± 0.51^{abcB}	4.04 ± 0.28^{bcdB}	4.63 ± 0.27^{ab}	4.87 ± 0.43^{a}	<0.0001
	P-value	----	0.0031	0.0030	0.0310	0.0004	0.0109	0.0002	<0.0001	----	----	----
Tomato leaves	**Light**	5.33 ± 0.34^{a}	4.67 ± 0.78^{abA}	4.39 ± 0.30^{bA}	4.07 ± 0.17^{bcA}	3.33 ± 0.21^{cA}	N.D.	N.D.	N.D.	N.E.	N.E.	<0.0001
	Dark		4.67 ± 0.34^{bA}	3.86 ± 0.12^{cB}	3.77 ± 0.21^{cB}	2.81 ± 0.44^{dB}	2.47 ± 0.23^{d}	N.D.	N.D.	N.D.	N.D.	<0.0001
	P-value	----	1	0.0068	0.0390	0.0441	----	----	----	----	----	----

Cabbage	**Light**	6.32±0.56^{a}	5.41±0.35^{bA}	5.30±0.36^{bA}	4.42±0.28^{cA}	3.37±0.19^{dA}	N.D.	N.D.	N.D.	N.E.	N.E.	<0.0001
	Dark		5.48±0.40^{bA}	4.68±0.18^{cB}	4.08±0.43^{cdA}	3.47±0.29^{dA}	2.24±0.26^{e}	N.D.	N.D.	N.D.	N.D.	<0.0001
	P-value	----	0.7760	0.0089	0.1730	0.5330	----	----	----	----	----	----
Fresh grass	**Light**	5.09±0.17^{a}	4.49±0.36^{abA}	3.63±0.42^{bcA}	3.47±0.68^{cA}	3.30±0.52^{cA}	2.88±0.32^{cA}	N.D.	N.D.	N.E.	N.E.	<0.0001
	Dark		4.09±0.35^{bA}	3.64±0.12^{bcA}	3.30±0.48^{cA}	2.40±0.27^{dB}	2.25±0.35^{dB}	N.D.	N.D.	N.D.	N.D.	<0.0001
	P-value	----	0.1120	0.9600	0.6600	0.0087	0.0185	----	----	----	----	----
Dry grass	**Light**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	4.76±0.54^{aA}	4.90±0.24^{aA}	N.E.	N.E.	0.6140
	Dark	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2.48±0.23^{cB}	3.07±0.58^{bcB}	3.33±0.50^{b}	5.36±0.43^{a}	<0.0001
	P-value	----	----	----	----	----	----	<0.0001	0.0002	----	----	----
Harmony	**Light**	8.42±0.25^{a}	8.34±0.29^{abA}	8.27±0.21^{abA}	8.00±0.40^{abcA}	8.37±0.12^{abA}	7.81±0.22^{bcdB}	7.52±0.36^{cdA}	7.43±0.24^{dA}	N.E.	N.E.	<0.0001
	Dark	8.42±0.25^{ab}	8.16±0.15^{abcA}	8.09±0.20^{abcA}	7.83±0.12^{cA}	8.47±0.15^{aA}	8.25±0.07^{abcA}	7.84±0.40^{bcA}	7.72±0.46^{cA}	7.79±0.29^{c}	7.84±0.37^{bc}	0.0002
	P-value	----	0.2510	0.2110	0.3880	0.2680	0.0029	0.2220	0.2480	----	----	----
Rancidity	**Light**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3.60±0.72^{b}	6.10±0.48^{a}	0.0008
	Dark	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	<0.0001
	P-value	----	----	----	----	----	----	----	----	----	----	----

$^{a, b, c, d, e, f}$ Lowercase letters used to compare means of samples from different periods of time for the same analysis. Means that do not bear a common letter differ significantly.

$^{A, B}$ Uppercase letters used to compare means of samples from different storage conditions (dark or light) at the same period of time. Means that do not bear a common letter differ significantly.

N.D: Not detected.

N.E: Not evaluated, since rancid was detected with an intensity greater than 3.5, oils were no more evaluated

Table 3. Results of mean and SD of sensory analysis (gustatory sensation) assessed by a trained sensory panel during 63 days of household consumption conditions.

Gustatory sensation	Storage conditions	Storage time										
		0 day	7 days	14 days	21 days	28 days	35 days	42 days	49 days	56 days	63 days	P-value
Ripely fruity	Light	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	5.32 ± 0.28^{bA}	6.47 ± 0.34^{aA}	N.E.	N.E.	0.0004
	Dark	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3.30 ± 0.23^{bB}	5.57 ± 0.29^{aB}	5.60 ± 0.66^{a}	5.70 ± 0.84^{a}	<0.0001
	P-value	----	----	----	----	----	----	<0.0001	0.0020	----	----	----
Greenly fruity	Light	7.05 ± 0.48^{a}	6.40 ± 0.23^{aA}	5.23 ± 0.33^{bB}	5.09 ± 0.19^{bA}	4.77 ± 0.28^{bA}	3.38 ± 1.20^{cA}	N.D.	N.D.	N.E.	N.E.	<0.0001
	Dark		6.43 ± 0.22^{bA}	5.80 ± 0.20^{cA}	4.66 ± 0.30^{dB}	2.50 ± 0.28^{eB}	2.31 ± 0.21^{eA}	N.D.	N.D.	N.D.	N.D.	<0.0001
	P-value	----	0.8390	0.0107	0.0284	<0.0001	0.0859	----	----	----	----	----
Sweet	Light	1.45 ± 0.23^{d}	1.55 ± 0.19^{dA}	2.18 ± 0.26^{dA}	4.05 ± 0.64^{cA}	4.14 ± 0.35^{cA}	4.36 ± 0.72^{bcA}	5.36 ± 0.75^{abA}	5.44 ± 0.42^{aA}	N.E.	N.E.	<0.0001
	Dark	1.45 ± 0.23^{e}	1.68 ± 0.15^{eA}	2.17 ± 0.19^{eA}	3.25 ± 0.52^{dA}	4.14 ± 0.35^{cdA}	4.50 ± 0.27^{bcA}	5.44 ± 0.44^{abA}	5.76 ± 0.45^{aA}	6.23 ± 1.14^{ab}	6.28 ± 0.06^{a}	<0.0001
	P-value	----	0.2630	0.9460	0.0619	1	0.6950	0.8420	0.2810	----	----	----
Bitter	Light	5.74 ± 0.29^{a}	5.31 ± 0.30^{aB}	5.12 ± 0.61^{aA}	3.88 ± 0.80^{bA}	3.56 ± 0.39^{bA}	1.36 ± 0.16^{cB}	1.34 ± 0.46^{cA}	N.D.	N.E.	N.E.	<0.0001
	Dark		5.72 ± 0.14^{aA}	5.23 ± 0.14^{bA}	3.88 ± 0.19^{cA}	3.68 ± 0.14^{cA}	2.84 ± 0.31^{dA}	1.20 ± 0.40^{eA}	0.88 ± 0.10^{ef}	0.64 ± 0.18^{f}	0.73 ± 0.12^{ef}	<0.0001
	P-value	----	0.0251	0.7040	1	0.5360	<0.0001	0.6230	----	----	----	----
Pungent	Light	6.28 ± 0.26^{a}	6.06 ± 0.24^{aA}	5.79 ± 0.44^{aA}	5.53 ± 0.33^{aA}	4.54 ± 0.42^{bA}	2.22 ± 0.52^{cA}	0.77 ± 0.25^{dB}	N.D.	N.E.	N.E.	<0.0001
	Dark		6.14 ± 0.42^{aA}	5.86 ± 0.20^{aA}	4.16 ± 0.27^{bB}	3.63 ± 0.20^{bB}	2.78 ± 0.38^{cA}	2.06 ± 0.73^{dA}	0.92 ± 0.16^{eA}	0.71 ± 0.09^{e}	0.66 ± 0.12^{e}	<0.0001
	P-value	----	0.7210	0.7530	<0.0001	0.0024	0.0859	0.0055	0.0078	----	----	----
Apple	Light	5.28 ± 0.71^{a}	4.61 ± 0.14^{abA}	4.55 ± 0.38^{abA}	4.25 ± 0.27^{bcA}	3.84 ± 0.19^{bcdA}	3.58 ± 0.43^{cdA}	3.10 ± 0.30^{deB}	2.49 ± 0.35^{eB}	N.E.	N.E.	<0.0001
	Dark		4.84 ± 0.35^{abA}	4.31 ± 0.21^{bcA}	4.14 ± 0.50^{bcA}	4.02 ± 0.18^{bcA}	3.90 ± 0.30^{cA}	3.81 ± 0.32^{cA}	3.71 ± 0.58^{cA}	3.55 ± 0.41^{c}	3.50 ± 0.44^{c}	<0.0001
	P-value	----	0.2080	0.2540	0.6750	0.1530	0.2060	0.0065	0.0038	----	----	----

Tomato	**Light**	6.53±0.35^{a}	5.83±0.24^{aA}	5.21±0.11^{cB}	4.11±0.21^{dA}	3.92±0.32^{dA}	3.29±0.24^{eA}	N.D.	N.D.	N.E.	N.E.	<0.0001
	Dark		6.03±0.34^{abA}	5.55±0.21^{bA}	3.90±0.53^{cA}	3.69±0.19^{cA}	2.82±0.14^{dB}	N.D.	N.D.	N.D.	N.D.	<0.0001
	P-value	----	0.3150	0.0121	0.4330	0.2080	0.0047	----	----	----	----	----
Dry fruits	**Light**	3.09±0.16^{c}	3.76±0.15^{bcA}	3.82±0.30^{bcA}	3.97±0.47^{bA}	4.53±0.36^{bA}	5.37±0.58^{aA}	5.54±0.54^{aA}	5.56±0.42^{aA}	N.E.	N.E.	<0.0001
	Dark	3.09±0.16^{d}	3.62±0.45^{cdA}	3.71±0.43^{cdA}	3.44±0.62^{dA}	3.78±0.36^{cdB}	4.34±0.20^{bcB}	4.70±0.28^{abB}	4.85±0.21^{abB}	5.38±0.51^{a}	5.44±0.18^{a}	<0.0001
	P-value	----	0.5230	0.6540	0.1640	0.0109	0.0055	0.0147	0.0099	----	----	----
Tomato leaves	**Light**	5.99±0.77^{a}	4.12±0.18^{bB}	3.40±0.32^{bcA}	3.35±0.14^{cA}	2.27±0.28^{dB}	N.D.	N.D.	N.D.	N.E.	N.E.	<0.0001
	Dark		4.60±0.43^{bA}	3.74±0.27^{cA}	3.54±0.17^{cdA}	3.65±0.30^{cA}	2.78±0.24^{d}	N.D.	N.D.	N.D.	N.D.	<0.0001
	P-value	----	0.0481	0.1040	0.0885	<0.0001	----	----	----	----	----	----
Cabbage	**Light**	6.21±0.74^{a}	5.13±0.19^{bA}	4.62±0.31^{bA}	3.74±0.44^{cA}	2.19 ±0.32^{dA}	N.D.	N.D.	N.D.	N.E.	N.E.	<0.0001
	Dark		5.52±0.41^{aA}	4.47±0.17^{bA}	3.46±0.29^{cA}	2.46 ±0.29^{dA}	1.65±0.19^{e}	N.D.	N.D.	N.D.	N.D.	<0.0001
	P-value	----	0.0896	0.3660	0.2680	0.1990	----	----	----	----	----	----
Fresh grass	**Light**	5.79±0.61^{a}	4.26±0.24^{bB}	3.99±0.36^{bB}	3.63±0.26^{bcA}	3.09±0.46^{cA}	2.28±0.10^{dA}	N.D.	N.D.	N.E.	N.E.	<0.0001
	Dark		5.02±0.21^{bA}	4.50±0.24^{bA}	3.78±0.12^{cA}	2.33±0.29^{dB}	1.63±0.26^{eB}	N.D.	N.D.	N.D.	N.D.	<0.0001
	P-value	----	0.0007	0.0302	0.2740	0.0140	0.0008	----	----	----	----	----
Dry grass	**Light**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	4.74±0.45^{aA}	5.12±0.58^{aA}	N.E.	N.E.	0.2780
	Dark	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2.56±0.11^{cB}	4.51±0.38^{bA}	5.19±0.48^{a}	5.26±0.26^{a}	<0.0001
	P-value	----	----	----	----	----	----	<0.0001	0.0838	----	----	----

Harmony	**Light**	8.12±0.18^{ab}	7.99±0.21^{abA}	7.89±0.19^{bcA}	7.77±0.17^{bcA}	8.31±0.12^{aA}	7.89±0.16^{bcB}	7.90±0.21^{bcA}	7.60±0.20^{cA}	N.E.	N.E.	<0.0001
	Dark	8.12±0.18^{a}	7.70±0.27^{abA}	7.99±0.14^{aA}	7.76±0.17^{abA}	8.29±0.20^{aA}	8.12±0.07^{aA}	7.87±0.30^{abA}	7.21±0.67^{bA}	7.67±0.35^{ab}	7.64±0.36^{ab}	0.0002
	P-value	----	0.0943	0.3770	0.9290	0.8530	0.0179	0.8590	0.2500	----	----	----
Complexity	**Light**	7.21±0.21^{a}	7.36±0.25^{aA}	7.21±0.24^{aA}	7.14±0.17^{aA}	6.56±0.37^{abB}	5.81±0.38^{bcB}	5.59±0.44^{cA}	3.75±0.76^{dB}	N.E.	N.E.	<0.0001
	Dark		7.30±0.32^{aA}	7.19±0.29^{aA}	7.04±0.10^{abA}	7.31±0.47^{aA}	6.88±0.46^{abcA}	5.18±0.33^{eA}	5.59±0.92^{deA}	6.00±0.41^{cde}	6.22±0.50^{bcd}	<0.0001
	P-value	----	0.7510	0.9080	0.2800	0.0228	0.0040	0.1330	0.0089	----	----	----
Persistency	**Light**	8.18±0.50^{a}	8.34±0.23^{aA}	8.27±0.14^{aA}	8.12±0.27^{aA}	6.55±0.40^{bB}	6.82±0.36^{bA}	5.45±0.48^{cA}	3.73±0.58^{dB}	N.E.	N.E.	<0.0001
	Dark	8.18±0.50^{ab}	8.34±0.31^{aA}	8.23±0.18^{abA}	8.17±0.34^{abA}	7.37±0.45^{bcA}	6.94±0.47^{cdA}	5.15±0.37^{fA}	5.69±0.79^{efA}	6.00±0.51^{def}	6.20±0.38^{de}	<0.0001
	P-value	----	1	0.7040	0.8020	0.0158	0.6630	0.3050	0.0021	----	----	----
Rancidity	**Light**	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.E.	N.E.	----
	Dark	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	----
	P-value	----	----	----	----	----	----	----	----	----	----	----

$^{a, b, c, d, e, f}$ Lowercase letters used to compare means of samples from different periods of time for the same analysis. Means that do not bear a common letter differ significantly.

$^{A, B}$ Uppercase letters used to compare means of samples from different storage conditions (dark or light) at the same period of time. Means that do not bear a common letter differ significantly.

N.D: Not detected.

N.E: Not evaluated, since rancid was detected with an intensity greater than 3.5, oils were no more evaluated.

4.3. Oxidative stability

As illustrated in Figure 5, the oxidative stability (OS) determined by the Rancimat method decreased significantly over time in both light-exposed and light-protected oils. However, the decline was more pronounced in light-exposed samples, dropping from 9.1±0.15h at day 0 to 6.04±0.18h by day 63 as shown in Table A3. The decrease in OS for the light-exposed bottles was more than 3 h over the 63-day period, which contrasts with the finding of Rodrigues et al. (2018),with a decrease observed approximately one hour in OS over one month period, with no significant difference between the two studied conditions evaluated by those researchers (bottles left open after analysis and other closed). The difference in OS decrease between the two studies can be attributed to the different duration of both studies: 63 days compared to 30 days of Rodrigues et al. (2018). The opposite of samples kept in darkness maintained higher stability. These results showed the protective effect of dark storage conditions on olive oil's antioxidant properties, particularly maintaining higher oxidative stability(Marx et al., 2021).

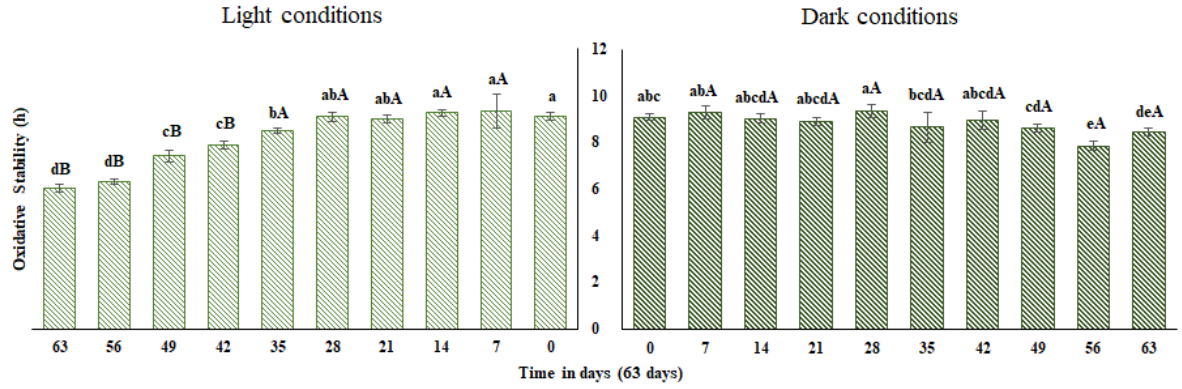

Figure 5. Time-evolution of the oxidative stability (Rancimat method) in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness. Different lowercase letters indicate a significant statistical difference between time periods within each light/dark condition.

4.4. Total Phenols Content

The total phenols content (TPC) decreased over time for both light-exposed and light-protected oils over the duration of the simulated domestic use as illustrated in Figure 6, being the decline more pronounced in light-exposed samples. This indicates that phenolic compounds, which are major contributors to olive oil antioxidant activity, are degrading more rapidly when exposed to light, highlighting the probable negative impact of photo-oxidation. The dark-stored samples also showed a decrease trend in the content of total phenols, but at a slower rate suggesting that these compounds are more stable in the absence of light. This decrease in phenolic content could partially explain the reduction observed on the oxidative stability of the oils over time. These findings align closely with those reported by (Rodrigues et al., 2018), and (Klisović et al., 2022) who also noted a decrease in total phenolic content of 12.3% and 17.6% in two monovarietal EVOOs during domestic simulated consumption over a 28-day period.

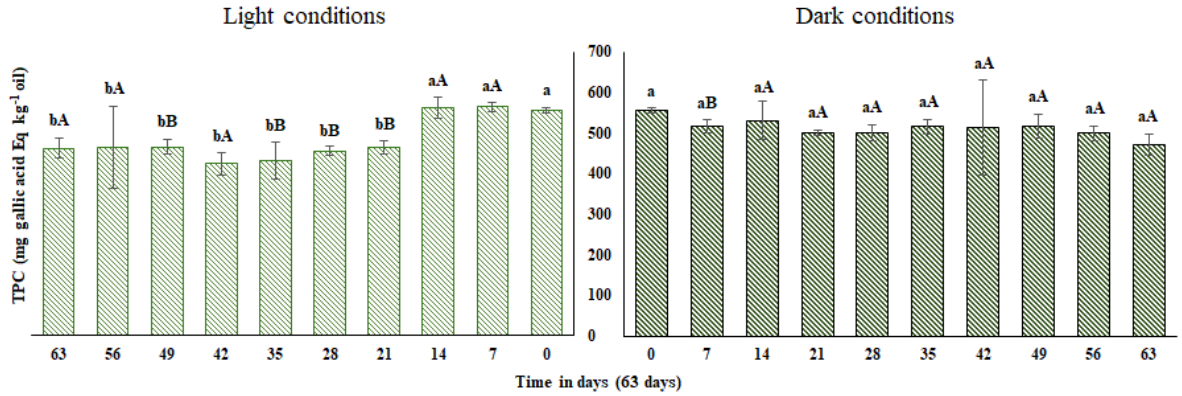

Figure 6. Time-evolution of the total phenols content in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

4.5. Olive oil total content of hydroxytyrosol and tyrosol derivatives after acid hydrolysis

In which concerns the health claim fulfillment, the total content of hydroxytyrosol and tyrosol derivatives after acid hydrolysis was monitored over the 63-days of the study. The results revealed a remarkable stability over the studied time-period for both light-exposed and light-protected oils, as shown in Table 4. This content remained consistently above the European Commission's legal limit of 5 mg/20 g of olive oil, ranging from 12.1 to 13.1 mg/20 g, for the two lighting conditions evaluated. So, under the studied simulated household consumption and storage conditions, the olive oil could maintain a health claim label. This contrasts with the increasing levels of PV, K_{232} and K_{268}, suggesting that unsaturated fatty acids were more susceptible to degradation than polyphenols.

These results align with those reported by (Klisović et al., 2022) who investigated olive oil quality during one month of simulated domestic consumption. Despite some slight methodological differences in which concerns the simulated domestic use conditions (frequency of bottle opening and amount of oil removed), the stability of the content of the polyphenols related to the health claim was observed in both studied, which remained above the minimum level established by the European Union Regulation (432/2012) for this claim "*olive oil polyphenols contribute to the protection of blood lipids from oxidative stress, provided it contains a minimum of 5 mg of polyphenols per 20 g of oil.".*

Table 4. Mean results and SD of health claim of phenolic content derived from hydroxytyrosol and tyrosol during the 63-day period of house use consumption.

Hydroxytyrosol and tyrosol derivatives estimation	Storage conditions	Storage time										
		0 day	7 days	14 days	21 days	28 days	35 days	42 days	49 days	56 days	63 days	P-value
Health claim (5mg/20g of olive oil)	Light	12.5±0.37^{a}	12.9±0.52^{aA}	13.1±0.56^{aA}	12.8±0.47^{aA}	12.6±0.16^{aA}	12.4±0.44^{aA}	12.1±0.63^{aA}	12.4±0.88^{aA}	12.2±0.5^{aA}	12.4±0.65^{aA}	0.1380
	Dark	12.5±0.37^{a}	12.8±0.19^{aA}	12.7±0.24^{aA}	12.4±0.3^{aA}	12.2±0.37^{aB}	12.1±0.36^{aA}	12.1±0.67^{aA}	12.6±0.29^{aA}	12.3±0.38^{aA}	12.2±0.36^{aA}	0.0532
	P-value	----	0.5730	0.1310	0.1970	0.0478	0.3370	0.9400	0.7160	0.8040	0.5720	----

[a] Lowercase letter used to compare means of samples from different periods of time for the same analysis. Means that do not bear a common letter differ significantly.

[A, B] Uppercase letters used to compare means of samples from different storage conditions (dark or light) at the same period of time. Means that do not bear a common letter differ significantly.

4.6. Kinetic models

The kinetic modeling of olive oil degradation provides significant insights into the behavior of key quality parameters under different storage conditions, such as storing oil bottles in darkness or exposed to light with increasing headspace oxygen. These models not only provide a quantitative measure of degradation speed under different storage conditions using the reaction rate (k) but also serve as powerful tools for estimating the shelf life of olive oil.

Reaction rate values (k) were determined using equations (3.5) to (3.8) by linear regression analysis, revealing that k values are generally greater for light-exposed oils compared to light-protected oils for the same parameter. Higher k values were observed for PV and K_{268} parameters in oil samples exposed to light compared to samples kept in darkness, indicating faster increases in PV and K_{268} in light-exposed samples. This emphasizes that light exposure accelerates degradation processes, particularly for PV and K_{268}. In contrast, for the K_{232} parameter, k values are lower in light conditions compared to dark conditions.

No TRUL or TRLL models could be developed for FA and the health claim related content of polyphenols, as these parameters remained constant over time. Kinetic models were only established to estimate SL for parameters showing changes over time and having legal limits, like PV and extinction coefficients. These kinetic models (zero-, first- and second orders) fitted well with the experimental data ($0.88 \leq R \leq 0.99$).

So, SL were predicted using TRUL models, which provide valuable insights into the expected longevity of the EVOO classification of the studied olive oils under different storage conditions using different kinetic orders equations, namely Eqs. (3.9) to (3.11). The SL predictions varied depending on the parameter and storage conditions, as well as on the model's order. When comparing predicted with experimental SL, some differences were observed for the different models and parameters, being the SL sometimes underestimated and other times overestimated.

PV was the quality parameter that led to the lowest SL values, predicted as the time to reach the legal upper limit (20 mEq. O_2/kg oil). Experimental SL based on PV data was set between 28 and 35 days for light-exposed samples, while kinetic models predicted a PV-based SL of 15 to 41 days. The first-order model provided the most conservative estimate of 27 days (table5), aligning closest with the experimental SL, compared to the zero-order model, which underestimated the SL at 15 days, and the second-order model, which overestimated it at 41

days. For dark storage conditions, all kinetic orders predicted SL of the EVOO classification ranging between 61 and 63 days, with the zero-order model appearing to give better agreement for dark-stored samples.

For the extinction coefficients, kinetic models overestimated the SL based on K_{268} in dark conditions, but for light-exposed samples, all orders provided closer predictions to the experimental results. Predictions based on the K_{232} data for light-exposed samples overestimated the SL compared to dark-stored ones, with the zero-order kinetic model (64 days) being the model that allowed to obtain the SL prediction to be closer to the experimental shelf life for samples kept in dark.

For light-protected oils, the SL of the EVOO classification was between 49 and 56 days, after which it declined to VOO status. Among the different models and parameters, the second-order kinetic model for PV and K_{232} provided better alignment with experimental results for dark-stored samples, predicting a shelf life of 61 and 64 days, respectively.

Finally, it could be stated that the kinetic models allow a way to mathematically describe olive oil degradation under simulated household conditions. It provides practical insights for consumers and producers while highlighting the complex interplay between storage conditions, chemical parameters, and degradation kinetics in olive oil. Future research could focus on refining these models to account for the multifaceted nature of olive oil degradation.

Table 5. Comparison of kinetic models results for peroxide value and extinction coefficient changes predicting olive oil shelf life.

	Zero order (Light)			Zero order (Dark)		
	PV	**K_{232}**	**K_{268}**	**PV**	**K_{232}**	**K_{268}**
R	0.8948	0.9854	0.9868	0.9691	0.9410	0.8861
k	0.5870±0.1024 **(mEq.O_2/kg.day)**	0.0093±0.0005 **(day^{-1})**	0.00103±0.00005 **(day^{-1})**	0.1486±0.0134 **(mEq.O_2/kg.day)**	0.0108±0.0014 **(day^{-1})**	0.00034±0.00006 **(day^{-1})**
SL (days)	15	77	59	63	64	178
	First order (Light)			**First order (Dark)**		
	PV	**K_{232}**	**K_{268}**	**PV**	**K_{232}**	**K_{268}**
R	0.9595	0.9892	0.9806	0.9754	0.9225	0.8852
k	0.0233±0.0021 **(day^{-1})**	0.0043±0.0002 **(day^{-1})**	0.0054±0.0003 **(day^{-1})**	0.0102±0.0008 **(day^{-1})**	0.0048±0.0007 **(day^{-1})**	0.0020±0.0004 **(day^{-1})**
SL (days)	27	79	60	62	67	161
	Second order (Light)			**Second order (Dark)**		
	PV	**K_{232}**	**K_{268}**	**PV**	**K_{232}**	**K_{268}**
R	0.9777	0.9860	0.9798	0.9766	0.9019	0.8840
k	0.00108±0.00008 **(kg/((mEq.O_2)day)**	0.0020±0.0001 **(day^{-1})**	0.0293±0.0021 **(day^{-1})**	0.00071±0.00005 **(kg/((mEq.O_2)day)**	0.0022±0.0004 **(day^{-1})**	0.0119±0.0022 **(day^{-1})**
SL (days)	41	74	60	61	70	147

Chapter 5. Conclusions and future perspectives

The quality of olive oil is influenced by various factors, both during oil extraction and storage. This study focused on the impact of simulated household consumption conditions on olive oil quality over a 9-week period. Ten bottles of extra virgin olive oil were divided into two groups; five stored in darkness and five exposed to light, with daily opening and agitation to simulate typical household use.

Laboratory analyses revealed significant changes in olive oil quality parameters and sensory profiles. Notably, the peroxide value, indicating primary oxidation products, exceeded the EU legal limit for light-exposed bottles after 63 days of storage. This underscores the significant impact of storage conditions on olive oil quality. Other quality parameters, such as extinction coefficients, showed an increasing trend, indicating the formation of secondary oxidation products. Conversely, total phenols content decreased over time, suggesting a loss of health-promoting properties due to auto- and photo-oxidation.

While total phenols content analysis provided a general overview, it could not underscore the specific phenolic compounds whose contents decreased or remained constant. The content of hydroxytyrosol and tyrosol derivatives, the ones contributing to the health claim, remained constant and above the legal limit for health claims.

Sensory profiles clearly demonstrated a loss of characteristic attributes over time, with some disappearing entirely by the end of study period.

In summary, these changes in various parameters lead to the conclusion that storage and consumption conditions have a direct and fast impact on the overall quality of olive oil. This information is crucial for both producers and consumers concerned with maintaining high quality olive oil. While evaluating and highlighting these quality changes is important, estimating the duration of grade retention using mathematical tools like predictive models is even more critical to avoid time and financial waste.

Kinetic models, which fitted well with the experimental data, predicted longer shelf life for oils stored in darkness compared to those exposed to light, emphasizing the negative impact of photo-oxidation. The predicted shelf life ranged from 15 to 41 days under light exposure and 61 to 63 days in darkness for PV quality parameters. These predictive models estimated olive oil shelf life under household consumption and different storage conditions, while the changing trends observed in the experimental data highlighted the importance of proper storage in preventing quality degradation during household use. A note on “preserve under dark and

consume within 60 days after opening" and "if preserved under light, consume within 2 weeks after opening" could represent additional labeling information to consumers, raising awareness on the need and means to preserve EVOO quality and safety.

This study provides valuable insights into olive oil conservation methods that can help maintain its quality grade, benefiting both producers and educating consumers about proper storage and consumption practices to maintain the quality of this essential component of the Mediterranean diet.

The findings from this study, highlighting the impact of domestic consumption and different storage conditions on olive oil quality, could lead to more informative labeling practices and inspire innovations in packaging design or storage solutions in the future. This perspective emphasizes how scientific research can directly influence industry practices, potentially leading to new standards in olive oil packaging and storage recommendations. This research also opens avenues for innovation in food science, demonstrating how interdisciplinary approaches combining physicochemical and sensory analyses with kinetic modelling can provide extensive knowledge. Such insights help to bridge the gap between laboratory results and real world applications, eventually serving producers, consumers and above all the food industry.

References

A

Asp, N. G., Bryngelsson, S., (2008). « Health Claims in Europe: New Legislation and PASSCLAIM for Substantiation ». The Journal of Nutrition 138 (6): 1210S-5S. Available at: https://doi.org/10.1093/jn/138.6.1210S.

Angerosa, F., (2002). « Influence of volatile compounds on virgin olive oil quality evaluated by analytical approaches and sensor panels. » European Journal of Lipid Science and Technology, 104, 639-660. Available at: https://doi.org/10.1002/1438-9312(200210)104:9/10%3C639::AID-EJLT639%3E3.0.CO;2-U.

Abbattista, R., Losito, I., Castellaneta, A., De Ceglie, C., Calvano, C.D., Cataldi, T.R.I., « Insight into the Storage-Related Oxidative/Hydrolytic Degradation of Olive Oil Secoiridoids by Liquid Chromatography and High-Resolution Fourier Transform Mass Spectrometry » (2020) Journal of Agricultural and Food Chemistry, 68 (44), pp. 12310 – 12325. Available at: https://doi.org/10.1021/acs.jafc.0c04925.

B

Balaky, H., Rasul, N., Khudher, H., Romel, S., Surchi, B., (2020). « Effect of heating on changes of chlorophyll content and oxidative stability in olive pomace oil ». Journal of Critical Reviews 7: 8282–87. Available at: https://doi.org/10.31838/jcr.07.19.935.

Baccouri, B., Sieren, T., Mohamed, S. N., Willenberg, I. «Fingerprinting of Tocopherol, Phenolic Compounds and Oxidative Properties of Unstudied Minor and Rare Tunisian Olive Oils». South African Journal of Botany 2023, 156, 54–64. Available at: https://doi.org/10.1016/J.SAJB.2023.03.003.

Becerra-Herrera, M., Sánchez-Astudillo, M., Beltrán, R., Sayago, A., (2014). « Determination of phenolic compounds in olive oil: New method based on liquid–liquid micro extraction and ultra high performance liquid chromatography-triple–quadrupole mass spectrometry ». Lebensmittel-Wissenchaft & Technologie - Food Science and Technology 57 (1): 49-57. Available at: https://doi.org/10.1016/j.lwt.2014.01.016.

Banco, A. P., Puertas, C. M., Trentacoste, E. R., Gariglio, N. F., Jofré, V. P. « Promising Olive Varieties for Extra Virgin Oil Production in Mendoza, Argentina ». J. Saudi Society of Agricultural Sciences. 2022. Available at: https://doi.org/10.1016/J.JSSAS.2022.06.003.

C

Caipo, L., Sandoval, A., Sepúlveda, B., Fuentes, E., Valenzuela, R., Metherel, A. H., Romero, N., (2021). « Effect of Storage Conditions on the Quality of Arbequina Extra Virgin Olive Oil and the Impact on the Composition of Flavor-Related Compounds (Phenols and Volatiles) ». Foods 10 (9): 2161. Available at: https://doi.org/10.3390/foods10092161.

Cairone, F., Petralito, S., Scipione, L., Cesa, S., (2021). « Study on Extra Virgin Olive Oil: Quality Evaluation by Anti-Radical Activity, Color Analysis, and Polyphenolic HPLC-DAD Analysis ». 2022. « Application of accelerated shelf-life test (ASLT) procedure for the estimation of the shelf-life of extra virgin olive oils: A validation study ». Food Packaging and Shelf Life 34 : 100990. Available at: https://doi.org/10.1016/j.fpsl.2022.100990.

Calligaris, S., Lucci, P., Milani, A., Rovellini, P., Lagazio, C., Conte, L., Nicoli, M.C., (2022). « Application of accelerated shelf-life test (ASLT) procedure for the estimation of the shelf-life of extra virgin olive oils: A validation study ». Food Packaging and Shelf Life 34: 100990. Available at: https://doi.org/10.1016/j.fpsl.2022.100990.

Campestre, C., Angelini, G., Gasbarri, C., Angerosa, F., (2017). « The compounds responsible for the sensory profile in monovarietal virgin olive oils ». Molecules 22 (11): 1833. Available at: https://doi.org/10.3390/molecules22111833.

Caponio, F., Bilancia, M.T., Pasqualone, A., Sikorska, E., Gomes, T., (2005). « Influence of the exposure to light on extra virgin olive oil quality during storage ». European Food Research and Technology 221 (1): 92–98. Available at: https://doi.org/10.1007/s00217-004-1126-8.

Caporaso, N., Savarese, M., Paduano, A., Guidone, G., Marco, E. De., Sacchi, R.; (2015). « Nutritional quality assessment of extra virgin olive oil from the Italian retail market: Do natural antioxidants satisfy EFSA health claims? » Journal of Food Composition and Analysis 40 (juin): 154-62. Available at: https://doi.org/10.1016/j.jfca.2014.12.012.

Choe, E., & Min, D.B. (2006). « Mechanisms and factors for edible oil oxidation ». Comprehensive Reviews in Food Science and Food Safety, 5, 169–186, Available at: https://doi.org/10.1111/j.1541-4337.2006.00009.x.

Conte, L., Milani, A., Calligaris, S., Rovellini, P., Lucci, P., & Nicoli, M. C. (2020). «Temperature Dependence of Oxidation Kinetics of Extra Virgin Olive Oil (EVOO) and Shelf-Life Prediction ». Foods, 9, 295, Available at: https://doi.org/10.3390/foods9030295.

Cherif, M., Rodrigues, N., Veloso, A. C. A., Pereira, J. A., Peres, A. M., (2021). « Kinetic Study of the Microwave-Induced Thermal Degradation of Cv. Arbequina Olive Oils Flavored with Lemon Verbena Essential Oil». Journal of the American Oil Chemists' Society 98 (10): 1021-32. Available at: https://doi.org/10.1002/aocs.12519.

Cherif, M., Rodrigues, N., Veloso, A. C. A., Zaghdoudi, K., Pereira, J. A., Peres. A. M., (2021a). «Kinetic-thermodynamic study of the oxidative stability of Arbequina olive oils flavored with lemon verbena essential oil». Lebensmittel-Wissenchaft & Technologie - Food Science and Technology 140 (avril): 110711. Available at: https://doi.org/10.1016/j.lwt.2020.110711.

Cioffi, G., Pesca, M., Caprariis, P., Braca, A., Severino, L., Tommasi, N., (2010). « Phenolic compounds in olive oil and olive pomace from Cilento (Campania, Italy) and their antioxidant activity ». Food Chemistry - 121 (juillet): 105-11. Available at: https://doi.org/10.1016/j.foodchem.2009.12.013.

Commission Implementing Regulation (EU) 2022/2105. (2022). Following the method outlined in COI/T.20/Doc. No. 34/Rev. 1 (2017) on the characteristics of olive oil and the relevant methods of analysis. Official Journal of the European Union, L282, 28-31.

Commission Implementing Regulation (EU) 2022/2105. (2022). According to COI/T.20/Doc. No. 35 on the characteristics of olive oil and the relevant methods of analysis. Official Journal of the European Union, L282, 28-31.

Commission Implementing Regulation (EU) 2022/2105. (2022). According to COI/T.20/Doc. No. 19/Rev. 5/2019 method on the characteristics of olive oil and the relevant methods of analysis. Official Journal of the European Union, L282, 28-31.

Commission Delegated Regulation (EU) 2022/2104. (2022). On marketing standards for olive oil, and repealing Commission Regulation (EEC) No 2568/91 and Commission

Implementing Regulation (EU) No 29/2012. Official Journal of the European Union, L275, 1-74.

D

Douzane, M., Daas, M. S., Meribai, A., Guezil, A., Abdi, A., Tamendjari, A., (2021). « Physico-Chemical and Sensory Evaluation of Virgin Olive Oils from Several Algerian Olive-Growing Regions ». Oilseeds & safety Crops and Lipids 28: 55. Available at: https://doi.org/10.1051/ocl/2021044.

De la Torre, R., Fitó, M., Covas, M. I. « The Bioavailability of Olive Oil Phenolic Compounds and Their Bioactive Effects in Humans ». Olives and Olive Oil in Health and Disease Prevention. 2021, 193–203.

Available at: https://doi.org/10.1016/B978-0-12-819528-4.00022-5.

E

EFSA European Commission Regulation EC. Establishing a List of Permitted Health Claims Made on Foods, Other than Those Referring to the Reduction of Disease Risk and to Children's Development and Health. Official Journal of the European Union 2012, No. 432/2012 (L136), 1–40.

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2011). Scientific Opinion on the substantiation of health claims related to polyphenols in olive oil and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. European Food Safety Authority Journal, 9(4), 2033. Available at: https://doi.org/10.2903/j.efsa.2011.2033.

Escudero, A., Ramos, N., La Rubia, M. D., Pacheco, R., (2016). « Influence of Extreme Storage Conditions on Extra Virgin Olive Oil Parameters: Traceability Study ». Journal of Analytical Methods in Chemistry 2016: 7506807. Available at: https://doi.org/10.1155/2016/7506807.

Esposto, S., Taticchi, A., Urbani, S., Selvaggini, R., Veneziani, G., Di Maio, I., Sordini, B., Servili, M., (2017). « Effect of light exposure on the quality of extra virgin olive oils

according to their chemical composition ». Food Chemistry 229: 726–33. Available at: https://doi.org/10.1016/j.foodchem.2017.02.151.

European Commission Regulation (EU) No 432/2012 of 16 May 2012 Establishing a List of Permitted Health Claims Made on Foods, Other than Those Referring to the Reduction of Disease Risk and to Children's Development and Health. Official Journal of the European Union 2012, L136, 1–40.

European Parliament and the Council of the European Union. Regulation (EC) No. 1924/2006 of the European Parliament and of the Council of 20 December 2006 on Nutrition and Health Claims Made on Foods. Official Journal of the European Union 2006, L404, 9–25.

European Commission. (2015). Commission Implementing Regulation (EU) 2015/1830 of 7 October 2015 amending Implementing Regulation (EU) No 29/2012 on marketing standards for olive oil. Official Journal of the European Union, L266, 9–10. Retrieved from https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32015R1830.

F

Fernandes, G. D., Ellis, A. C., Gámbaro, A., Barrera-Arellano, D., (2018). « Sensory evaluation of high-quality virgin olive oil: panel analysis versus consumer perception ». Current Opinion in Food Science, Sensory Science and Consumer Perception Food Physics & Materials Science, 21 (juin): 66-71. Available at: https://doi.org/10.1016/j.cofs.2018.06.001.

Ferro, M.D., Lopes, E., Afonso, M., Peixe, A., Rodrigues, F.M., Duarte, M.F., (2020). « Phenolic profile characterization of 'Galega Vulgar' and 'Cobrançosa' Portuguese olive cultivars along the ripening stages ». Applied Sciences 10 (11): 3930. Available at: https://doi.org/10.3390/app10113930.

Figueiredo-González, M., Olmo-García, L., Reboredo-Rodríguez, P., Serrano-García, I., Leuyacc-del Carpio, G., Cancho-Grande, B., Carrasco-Pancorbo, A., González-Barreiro, C. « Singular Olive Oils from a Recently Discovered Spanish North-Western Cultivar: An Exhaustive 3-Year Study of Their Chemical Composition and In-Vitro Antidiabetic Potential». Antioxidants (2022), 11 (7), 1233. Available at: https://doi.org/10.3390/antiox11071233.

G

García-González, A., Quintero-Flórez, A., Ruiz-Méndez, M. V., Perona, J. S., (2023). « Virgin Olive Oil Ranks First in a New Nutritional Quality Score Due to Its Compositional Profile ». Nutrients 15 (9): 2127. Available at: https://doi.org/10.3390/nu15092127.

Genovese, A., N., Yang, R., Linforth, R., Sacchi, I., Fisk. (2018). « The role of phenolic compounds on olive oil aroma release ». Food Research International 112 (octobre): 319-27. Available at: https://doi.org/10.1016/j.foodres.2018.06.054.

Gagour, J., Oubannin, S., Bouzid, H. A., Bijla, L., Moudden, H. E., Sakar, E. H., Koubachi, J., Laknifli, A., & Gharby, S. (2022). « Physicochemical characterization, kinetic parameters, shelf-life and its prediction models of virgin olive oil from two cultivars ("Arbequina" and "Moroccan Picholine") grown in Morocco ». Oilseeds & fats, Crops and Lipids, 29, 39, Available at: https://doi.org/10.1051/ocl/2022033.

Garcia-Oliveira, P., Jimenez-Lopez, C., Lourenço-Lopes, C., Chamorro, F., Pereira, A.G., Carrera-Casais, A., Fraga-Corral, M., Carpena, M., Simal-Gandara, J., & Prieto, M.A. (2021). « Evolution of Flavors in Extra Virgin Olive Oil Shelf-Life ». Antioxidants, 10, 368, Available at: https://doi.org/10.3390/antiox10030368.

Guillaume, C., & Ravetti, L. (2016). « Shelf-Life Prediction of Extra Virgin Olive Oils Using an Empirical Model Based on Standard Quality Tests ». Journal of Chemistry, 1, 6, Available at: https://doi.org/10.1155/2016/6393962.

Garcia-Oliveira, P., Jimenez-Lopez, C., Lourenço-Lopes, C., Chamorro, F., Pereira, A.G., Carrera-Casais, A., Fraga-Corral, M., Carpena, M., Simal-Gandara, J., Prieto, M.A. «Evolution of Flavors in Extra Virgin Olive Oil Shelf-Life ». Antioxidants (2021), 10, 368. Available at: https://doi.org/10.3390/antiox10030368.

Guasch-Ferré, M., Liu, G., Li, Y., Sampson, L., Manson, J. E., Salas-Salvadó, J., Martínez-González, M. A., Stampfer, M. J., Willett, W. C., Sun, Q., Hu, F. B. « Olive Oil Consumption and Cardiovascular Risk in U.S. Adults » . Journal of the American College of Cardiology. (2020), 75 (15), 1729–1739. Available at: https://doi.org/10.1016/j.jacc.2020.02.036.

H

Hijawi, T. « Characterizing of Oil Quality and Fatty Acid Profiles of Old Olive Trees in Palestine ». Journal of Oleo Science 2021, 70 (11), 1585–1606. Available at: https://doi.org/10.5650/JOS.ESS21066.

I

International Olive Council. (2017). Method COI/T.20/Doc. No. 35/Rev. 1 on the determination of olive oil quality parameters. International Olive Council, 1-9. Available at: https://www.internationaloliveoil.org/wp-content/uploads/2019/11/Method-COI-T.20-Doc.-No-35-Rev.-1-2017.pdf.

International Olive Council. (2017). Method COI/T.20/Doc. No. 34/Rev. 1 on the determination of olive oil quality parameters. International Olive Council, 1-14. Available at: https://www.internationaloliveoil.org/wp-content/uploads/2019/11/COI-T.20-Doc.-No-34-Rev.-1-2017.pdf.

International Olive Council. (2019). Method COI/T.20/Doc. No. 19/Rev. 5 on the determination of olive oil quality parameters. International Olive Council, 1-9. Available at: https://www.internationaloliveoil.org/wp-content/uploads/2019/11/Method-COI-T.20-Doc.-No-19-Rev.-5-2019-2.pdf.

International Olive Council (IOC). (2011). "Method for the Organoleptic Assessment of Virgin Olive Oil." COI/T.20/Doc. No 15/Rev. 2. Available online at: https://www.internationaloliveoil.org/wp-content/uploads/2019/11/COI-OT-MO.-1-Rev.2-2011-Eng.pdf.

IOC. (2015). « INTERNATIONAL OLIVE COUNCIL », novembre 2015.

IOC. (2022). 'Trade Standard Applying to Olive Oils and Olive Pomace Oils'.

International Olive Council. (2022). Trade standard applying to olive oils and olive pomace oils (COI/T.15/NC No. 3/Rev. 19). International Olive Council. Available at: https://www.internationaloliveoil.org/wp-content/uploads/2022/12/Norme-comerciale-REV-19_ENK.pdf.

J

Jimenez-Lopez, C., Carpena, M., Lourenço-Lopes, C., Gallardo-Gomez, M., Lorenzo, J. M., Barba, F. J., Prieto, M. A., Simal-Gandara, J. « Bioactive Compounds and Quality of Extra Virgin Olive Oil ». Foods 2020, 9 (8), 1014. Available at: https://doi.org/10.3390/foods9081014.

K

Kalua C. M., Allen M. S., Bedgood D. R., Bishop A. G., Prenzler P. D., & Robards K. (2005). « Olive oil volatile compounds, flavour development and quality: A critical review ». Food Chemistry, 100, 273-286, Available at: https://doi.org/10.1016/j.foodchem.2005.09.059.

Kanavouras, A., Munoz, P. H., & Coutelieris, F. A. (2006). « Packaging of Olive Oil: Quality Issues and Shelf-life Predictions ». Foods Reviews International, 22, 1-24. https://doi.org/10.1080/87559120600865149.

Klisović, D., Novoselić, A., Lukić, I., Brkić Bubola, K., (2022). « Extra virgin olive oil under simulated consumption conditions: Evaluation of quality, health, and flavour properties». Journal of Food Composition and Analysis 110: 104570. https://doi.org/10.1016/j.jfca.2022.104570.

Kaya, A., Tekin, A. R., & Oner, M. D. (1993). « Oxidative stability of sunflower and olive oils: comparison between a modified active oxygen method and long term storage ». Food Science and Technology, 26, 464-468. Available at: https://doi.org/10.1006/fstl.1993.1091.

L

Li, X., & Wang, C. (2018). « Shelf-life of Extra Virgin Olive Oil and Its Prediction Models ». Journal of Food Quality, 2018, 1639260. Available at: https://doi.org/10.1155/2018/1639260.

Li, X., Zhu, H., Shoemaker, C.F., & Wang, S.C. (2014). « The effect of different cold storage conditions on the compositions of extra virgin olive oil ». Journal of the American Oil Chemists' Society, 91, 1559–1570. Available at: https://doi.org/10.1007/s11746-014-2496-0.

Lolis, A., Badeka, A.V., Kontominas, M.G., (2019). « Effect of bag-in-box packaging material on quality characteristics of extra virgin olive oil stored under household and abuse temperature conditions ». Food Packaging and Shelf Life 21: 100368. Available at: https://doi.org/10.1016/j.fpsl.2019.100368.

M

Mancebo-Campos, V., Salvador, M. D., Cioffi, G., (2023). « EFSA Health Claims-Based Virgin Olive Oil Shelf-Life ». Antioxidants 12 (8): 1563. Available at: https://doi.org/10.3390/antiox12081563.

Mancebo-Campos, V., Salvador, M. D., Fregapane, G., (2022). « Modelling Virgin Olive Oil Potential Shelf-Life from Antioxidants and Lipid Oxidation Progress ». Antioxidants 11 (3): 539. Available at: https://doi.org/10.3390/antiox11030539.

Martin-Torres, S., Tello-Jiménez, J. A., López-Blanco, R., González-Casado, A., & Cuadros-Rodríguez, L. (2023). « Multivariate stability monitoring and shelf-life models of deterioration of vegetable oils under real time ageing conditions – Extra virgin olive oil as a main case of study ». Food Packaging and Shelf Life, 37, 101070, Available at: https://doi.org/10.1016/j.fpsl.2023.101070.

Martínez-González, M. A., Sayón-Orea, C., Bullón-Vela, V., Bes-Rastrollo, M., Rodríguez-Artalejo, F., Yusta-Boyo, M. J., García-Solano, M. « Effect of Olive Oil Consumption on Cardiovascular Disease, Cancer, Type 2 Diabetes, and All-Cause Mortality: A Systematic Review and Meta-Analysis ». Clinical Nutrition 2022, 41 (12), 2659–2682. Available at: https://doi.org/10.1016/j.clnu.2022.10.001.

Marx, Í.M.G., Casal, S., Rodrigues, N., Pinho, T., Veloso, A.C.A., Pereira, J.A., & Peres, A. M., (2021). « Impact of the malaxation temperature on the phenolic profile of cv. Cobrançosa olive oils and assessment of the related health claim ». Food Chemistry 337: 127726. Available at: https://doi.org/10.1016/j.foodchem.2020.127726.

Marx, Í.M.G., Casal, S., Rodrigues, N., Cruz, R., Veloso, A.C.A., Pereira, J.A., Peres, A.M., (2022). « Does water addition during the industrial milling phase affect the chemical-sensory quality of olive oils? The case of cv. Arbequina oils ». Food Chemistry 395: 133570. Available at: https://doi.org/10.1016/j.foodchem.2022.133570.

Marx, Í.M.G., Casal, S., Rodrigues, N., Veloso, A.C.A., Pereira, J.A., & Peres, A.M., (2021). « Estimating hydroxytyrosol-tyrosol derivatives amounts in cv. Cobrançosa olive oils based on the electronic tongue analysis of olive paste extracts ». Food Science & Technology 147: 111542. Available at: https://doi.org/10.1016/j.lwt.2021.111542.

Martín-Tornero, E., Barea-Ramos, J.D., Lozano, J., Durán-Merás, I., Martín-Vertedor, D. « E-Nose Quality Evaluation of Extra Virgin Olive Oil Stored in Different Containers ». Chemosensors (2023), 11, 85. Available at: https://doi.org/10.3390/chemosensors11020085.

Macaluso M., Mercanti N., Pieracci Y., Mangia R., Verdini P.G., Zinnai A. «Unconventional Extraction and Storage Strategies in Order to Enhance the Shelf Life of Virgin Olive Oil » (2024) Foods, 13 (13), 2088. Available at: https://doi.org/10.3390/foods13132088.

Mastralexi, A., Nenadis, N., & Tsimidou, M.Z. (2014). « Addressing Analytical Requirements To Support Health Claims on "Olive Oil Polyphenols" (EC Regulation 432/2012) ». Journal of Agricultural and Food Chemistry, 63(45), 11315–11321. Available at: https://doi.org/10.1021/jf5005918.

Mele, M., Islam, M., Kang, H. M., Giuffrè, A., (2018). « Pre-and post-harvest factors and their impact on oil composition and quality of olive fruit ». Emirates Journal of Food and Agriculture 30 (juillet): 592-603. Available at: https://doi.org/10.9755/ejfa.2018.v30.i7.1742.

Moschopoulou, E., Moatsou, G., Syrokou, M. K., Paramithiotis, S., Drosinos, E. H., (2019). « 1 - Food quality changes during shelf life ». In Food Quality and Shelf Life, édité par Charis M. Galanakis, 1-31. Academic Press. Available at: https://doi.org/10.1016/B978-0-12-817190-5.00001-X.

O

Olmo-Cunillera, A., Casadei, E., Valli, E., Lozano-Castellón, J., Miliarakis, E., Domínguez-López, I., Ninot, A., Romero-Aroca, A., Lamuela-Raventós, R. M., Pérez, M., Vallverdú-Queralt, A., Bendini, A. « Aromatic, Sensory, and Fatty Acid Profiles of Arbequina Extra Virgin Olive Oils Produced Using Different Malaxation Conditions ». Foods 2022, 11 (21). Available at: https://doi.org/10.3390/foods11213446.

P

Pagliarini, E., Zanoni, B., & Giovanelli, G. (2000). « Predictive study on Tuscan extra virgin olive oil stability under several commercial conditions ». Journal of Agricultural and Food Chemistry, 48, 1345–1351. Available at: https://doi.org/10.9755/ejfa.2018.v30.i7.1742.

Passeri, V., Sammut, C., Mifsud, D., Domesi, A., Stanzione, V., Baldoni, L., Mousavi, S., Mariotti, R., Pandolfi, S., Cinosi, N., Famiani, F., Bufacchi, M. « The Ancient Olive Trees (Olea Europaea L.) Of the Maltese Islands: A Rich and Unexplored Patrimony to Enhance Oliviculture ». Plants 2023, 12 (10), 1988. Available at: https://doi.org/10.3390/plants12101988.

Pastor, R., Bouzas, C., Tur, J. A. « Beneficial Effects of Dietary Supplementation with Olive Oil, Oleic Acid, or Hydroxytyrosol in Metabolic Syndrome: Systematic Review and Meta-Analysis ». Free Radical Biology Medicine 2021, 172, 372–385. Available at: https://doi.org/10.1016/J.FREERADBIOMED.2021.06.017.

Paradiso, V.M., Gomes, T., Nasti, R., Caponio, F., Summo, C., (2010). « Effects of free fatty acids on the oxidative processes in purified olive oil ». Food Research International 43 (5): 1389–94. Available at: https://doi.org/10.1016/j.foodres.2010.04.015.

Psomiadou, E., & Tsimidou, M. (2002a). « Stability of virgin olive oil. 1. Autoxidation studies ». Journal of Agricultural and Food Chemistry, 50, 716–721. Available at: https://doi.org/10.1021/jf0108462.

Psomiadou, E., & Tsimidou, M. (2002b). « Stability of virgin olive oil. 2. Photo-oxidation studies ». Journal of Agricultural and Food Chemistry, 50, 722–727. Available at: https://doi.org/10.1021/jf010847u.

Perona, J., Botham, K., (2013). « Olive Oil as a Functional Food: Nutritional and Health Benefits ». In Handbook of Olive Oil: Analysis and Properties, 677-714. Available at: https://doi.org/10.1007/978-1-4614-7777-8_18.

Pristouri, G., Badeka, A., Kontominas, M.G. (2010). « Effect of packaging material headspace, oxygen and light transmission, temperature and storage time on quality characteristics of extra virgin olive oil ». Food Control 21 (4): 412–18. Available at: https://doi.org/10.1016/j.foodcont.2009.06.019.

Pizarro, M. L., Becerra, M., Sayago, A., Beltrán, M., & Beltrán, R. (2013). « Comparison of Different Extraction Methods to Determine Phenolic Compounds in Virgin Olive Oil ». Food Analytical Methods, 6(1), 123–132. Available at: https://doi.org/10.1007/s12161-012-9420-8.

R

Rifat, M., Naqvi, S. R., Khan, A. A., Mirani, A. A. (2023). « Optimization of olive oil extraction from olive pomace using solvent extraction and response surface methodology analysis of oil yield ». Fuel science 348 (septembre): 128633. Available at: https://doi.org/10.1016/j.fuel.2023.128633.

Reyes-Goya, C., Santana-Garrido, Á., Espinosa-Martín, P., Vázquez, C. M., Mate, A. «Wild and Cultivated Olive Trees: Nutraceutical Insights of Extra Virgin Olive Oils in Cardiovascular and Ocular Diseases ». Biochimica & Biophysica Acta (BBA) - Molecular Basis of Disease 2024, 1870 (1), 166904. Available at: https://doi.org/10.1016/j.bbadis.2023.166904.

Riolo, R., De Rosa, R., Simonetta, I., Tuttolomondo, A. « Olive Oil in the Mediterranean Diet and Its Biochemical and Molecular Effects on Cardiovascular Health through an Analysis of Genetics and Epigenetics ». International Journal of Molecular Sciences. 2022, 23 (24), 16002. Available at: https://doi.org/10.3390/ijms232416002.

Rodrigues, N., Casal, S., Peres, A.M., Baptista, P., Pereira, J.A. (2020). « Seeking for sensory differentiated olive oils? The urge to preserve old autochthonous olive cultivars. » Food Research International 128: 108759. Available at: https://doi.org/10.1016/j.foodres.2019.108759.

Rodrigues, N., Dias, L.G., Veloso, A.C.A., Pereira, J.A., Peres, A.M. (2016). « Monitoring olive oils quality and oxidative resistance during storage using an electronic tongue ». Food Science & Technology 73: 683–92. Available at: https://doi.org/10.1016/j.lwt.2016.07.002.

Rodrigues, N., Oliveira, L., Mendanha, L., Sebti, M., Dias, L., Oueslati, S., Veloso, A., Pereira, J.A., Peres, A.M. (2018). « Olive Oil Quality and Sensory Changes During House-Use

Simulation and Temporal Assessment Using an Electronic Tongue ». Journal of the American Oil Chemists' Society 95 (9): 1121-37. Available at: https://doi.org/10.1002/aocs.12093.

Rodrigues, N., Marx, Í.M.G., Casal, S., Dias, L.G., Veloso, A.C.A., Pereira, J.A., Peres, A.M. (2019). « Application of an electronic tongue as a single-run tool for olive oils' physicochemical and sensory simultaneous assessment ». Talanta 197: 363–73. Available at: https://doi.org/10.1016/j.talanta.2019.01.055.

Roselli, L., Cicia, G., Del Giudice, T., Cavallo, C., Vecchio, R., Carfora, V., Caso, D., Sardaro, R., Carlucci, D., De Gennaro, B. « Testing Consumers' Acceptance for an Extra-Virgin Olive Oil with a Naturally Increased Content in Polyphenols: The Case of Ultrasounds Extraction ». Journal of Functional Foods 2020, 69, 103940. Available at: https://doi.org/10.1016/j.jff.2020.103940.

T

Tarabanis, C., Long, C., Scolaro, B., Heffron, S. P. « Reviewing the Cardiovascular and Other Health Effects of Olive Oil: Limitations and Future Directions of Current Supplement Formulations ». Nutrition, Metabolism and Cardiovascular Diseases 2023. Available at: https://doi.org/10.1016/j.numecd.2023.08.014.

Tarapoulouzi, M., Agriopoulou, S., Koidis, A., Proestos, C., Enshasy, H. A. E., Varzakas, T., (2022). « Recent Advances in Analytical Methods for the Detection of Olive Oil Oxidation Status during Storage along with Chemometrics, Authenticity and Fraud Studies ». Biomolecules 12 (9): 1180. Available at: https://doi.org/10.3390/biom12091180.

Tsimidou, M. Z., Nenadis, N., Mastralexi, A., Servili, M., Butinar, B., Vichi, S., Winkelmann, O., García-González, D. L., Toschi, T. G., (2019). « Toward a harmonized and standardized protocol for the determination of total hydroxytyrosol and tyrosol content in virgin olive oil (VOO). The pros of a fit for the purpose ultra-high performance liquid chromatography (UHPLC) procedure ». Molecules 24 (13): 2429. Available at: https://doi.org/10.3390/molecules24132429.

V

Visioli, F.; Poli, A. « Fatty Acids and Cardiovascular Risk. Evidence, Lack of Evidence, and Diligence ». Nutrients 2020, 12 (12), 3782. Available at: https://doi.org/10.3390/nu12123782.

Appendix

Table A1. The relative amounts of change (in percentage) of quality parameters analyses for bottle exposed to light and kept in the dark.

	Light			Dark		
	PV	**K_{232}**	**K_{268}**	**PV**	**K_{232}**	**K_{268}**
Variation (%)	+81	+26	+28	+44	+30	+13

Table A2. Results of mean±SD of physicochemical quality parameters (FA, PV, extinction coefficients) during 63 days of opening bottles.

Quality parameters	Storage conditions	Storage time										
		0 day	7 days	14 days	21 days	28 days	35 days	42 days	49 days	56 days	63 days	P-value
FA (%)	Light	0.23 ± 0.00^{b}	0.23 ± 0.01^{abA}	0.23 ± 0.00^{bA}	0.23 ± 0.01^{abA}	0.23 ± 0.00^{bA}	0.23 ± 0.01^{abA}	0.25 ± 0.02^{aA}	0.23 ± 0.01^{abA}	0.24 ± 0.02^{abA}	0.23 ± 0.01^{abA}	0.0259
	Dark	0.23 ± 0.00^{a}	0.24 ± 0.02^{aA}	0.23 ± 0.01^{aA}	0.23 ± 0.00^{aA}	0.23 ± 0.00^{aA}	0.23 ± 0.01^{aA}	0.24 ± 0.02^{aA}	0.23 ± 0.00^{aA}	0.23 ± 0.00^{aA}	0.23 ± 0.01^{aA}	0.0269
	P-value	----	0.2400	0.3450	0.3870	0.2970	0.9980	0.3460	0.3480	0.1420	1	----
PV (mEq O_2 kg^{-1} of olive oil)	Light	10.64 ± 0.22^{h}	14.6 ± 0.4^{gA}	14.62 ± 0.13^{gA}	14.8 ± 0.2^{gA}	17.37 ± 0.18^{fA}	21.46 ± 0.36^{eA}	26.94 ± 0.34^{dA}	32.14 ± 2.24^{cA}	33.87 ± 0.31^{bA}	56.73 ± 0.95^{aA}	<0.0001
	Dark	10.64 ± 0.22^{d}	10.81 ± 0.01^{dB}	12.58 ± 0.27^{cB}	12.71 ± 0.24^{cB}	13.06 ± 0.38^{cB}	13.55 ± 0.22^{cB}	16.54 ± 0.19^{bB}	18.11 ± 1.45^{aB}	18.78 ± 0.34^{aB}	19.10 ± 0.41^{aB}	<0.0001
	P-value	----	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	----
K_{232}	Light	1.81 ± 0.02^{f}	1.93 ± 0.04^{efA}	2.01 ± 0.07^{deB}	2.06 ± 0.15^{deB}	2.08 ± 0.06^{cdeB}	2.11 ± 0.05^{cdB}	2.21 ± 0.03^{bcB}	2.29 ± 0.05^{bB}	2.36 ± 0.09^{abB}	2.46 ± 0.04^{aB}	<0.0001
	Dark	1.81 ± 0.02^{d}	2.02 ± 0.11^{dA}	2.25 ± 0.03^{cA}	2.28 ± 0.12^{cA}	2.35 ± 0.05^{cA}	2.39 ± 0.05^{cA}	2.43 ± 0.03^{bA}	2.49 ± 0.03^{aA}	2.54 ± 0.03^{abA}	2.59 ± 0.03^{aA}	<0.0001
	P-value	----	0.1450	<0.0001	0.0342	<0.0001	<0.0001	<0.0001	<0.0001	0.0025	0.0004	----
K_{268}	Light	0.16 ± 0.02^{e}	0.16 ± 0.01^{eA}	0.17 ± 0.01^{deA}	0.18 ± 0.01^{cdA}	0.19 ± 0.00^{cA}	0.19 ± 0.01^{cA}	0.20 ± 0.01^{bcA}	0.21 ± 0.00^{abA}	0.22 ± 0.00^{aA}	0.22 ± 0.00^{aA}	<0.0001
	Dark	0.16 ± 0.02^{b}	0.16 ± 0.01^{bA}	0.17 ± 0.00^{abA}	0.17 ± 0.01^{abB}	0.18 ± 0.00^{aB}	0.17 ± 0.01^{abB}	0.17 ± 0.00^{abB}	0.18 ± 0.00^{aB}	0.18 ± 0.01^{aB}	0.18 ± 0.01^{aB}	<0.0001
	P-value	----	0.6210	0.4290	0.0274	0.0002	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	----

$^{a, b, c, d, e, f, g, h}$ letters used to compare means of samples from different periods of time for the same analysis. Means that do not bear a common letter differ significantly.

$^{A, B}$ letters used to compare means of samples from different storage conditions (dark or light) at the same period of time. Means that do not bear a common letter differ significantly.

Table A3. Results of mean±SD of total phenols content and oxidative stability (TPC and OS) during 63 days of opening bottles.

Chemical parameters	Storage conditions	Storage time										
		0 day	**7 days**	**14 days**	**21 days**	**28 days**	**35 days**	**42 days**	**49 days**	**56 days**	**63 days**	**P-value**
OS (h)	**Light**	9.1±0.15^{a}	9.31±0.73^{aA}	9.25±0.16^{aA}	9.02±0.16^{abA}	9.09±0.19^{abA}	8.5±0.11^{bA}	7.87±0.19^{cB}	7.42±0.26^{cB}	6.33±0.11^{dB}	6.04±0.18^{dB}	<0.0001
	Dark	9.1±0.15^{abc}	9.3±0.28^{abA}	9.04±0.21^{abcdA}	8.92±0.16^{abcdA}	9.37±0.27^{aA}	8.68±0.64^{bcdA}	8.96±0.37^{abcdA}	8.62±0.17^{cdA}	7.86±0.21^{eA}	8.47±0.15^{deA}	<0.0001
	P-value	----	0.9650	0.1080	0.3650	0.0939	0.5440	0.0004	<0.0001	<0.0001	<0.0001	----
TPC (mg gallic acid Eq kg^{-1} oil)	**Light**	555.94±6.31^{a}	565.60±11.37^{aA}	562.19±26.28^{aA}	464.10±15.65^{bB}	455.47±10.79^{bB}	431.56±45.13^{bB}	424.23±28.53^{bA}	465.23±18.56^{bB}	463.90±101.11^{bA}	461.79±24.14^{bA}	<0.0001
	Dark	555.94±6.31^{a}	517.05±16.49^{aB}	530.87±47.27^{aA}	502.03±5.79^{aA}	501.43±19.80^{aA}	516.11±18.85^{aA}	513.03±117.57^{aA}	516.76±29.01^{aA}	500.93±17.73^{aA}	472.33±26.40^{aA}	0.297
	P-value	----	0.0006	0.2310	0.0001	0.0019	0.0048	0.1390	0.0101	0.4430	0.5290	----

$^{a, b, c, d}$ letters used to compare means of samples from different periods of time for the same analysis. Means that do not bear a common letter differ significantly.

$^{A, B}$ letters used to compare means of samples from different storage conditions (dark or light) at the same period of time. Means that do not bear a common letter differ significantly.

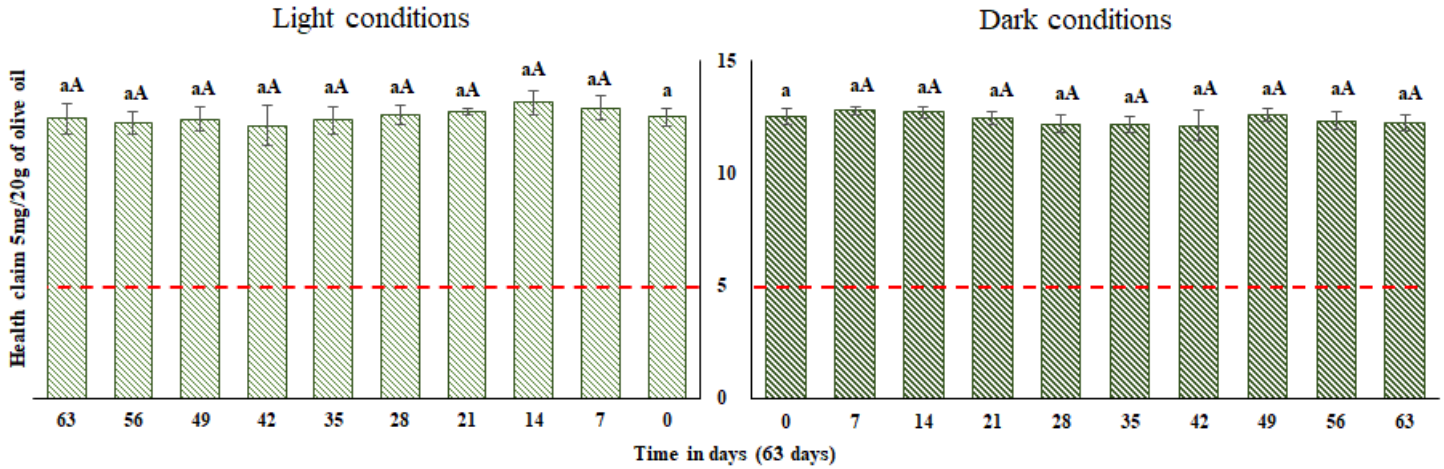

Figure A1. Time-evolution of olive oil health claim, supported on the measurement of hydroxytyrosol and tyrosol derivatives after acid hydrolysis, over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness. Red dashed line represents the EU limit for health claim labelling (European Commission regulation (EU) 432/2012).

1

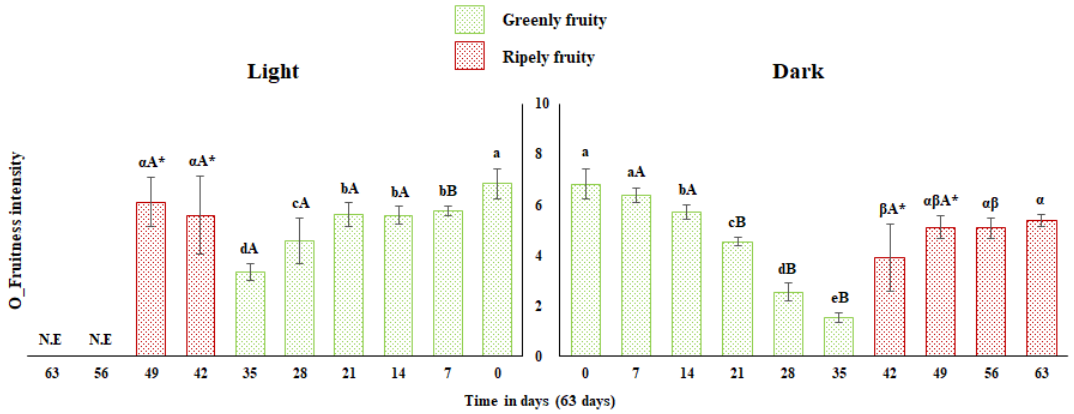

Figure A2. Time-evolution of olfactory fruitiness intensity (greenly fruity and ripely fruity notes) in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

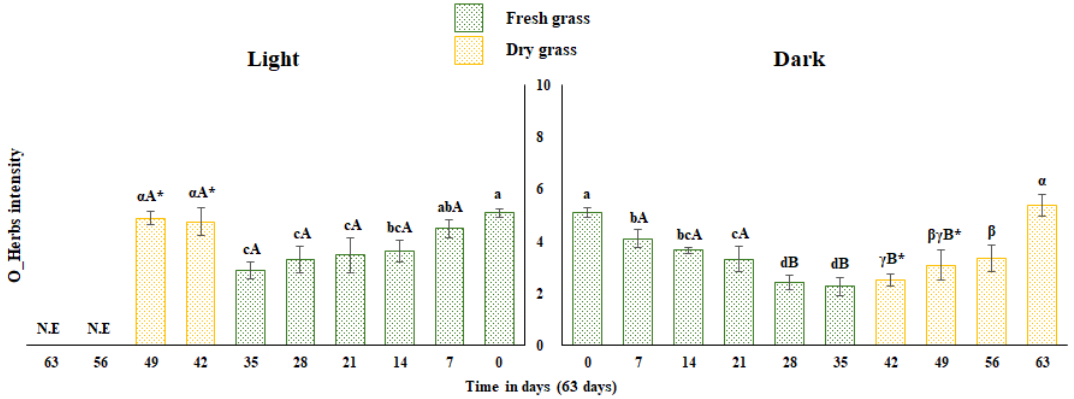

Figure A3. Time-evolution of olfactory herbs intensity (fresh grass and dry grass notes) in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

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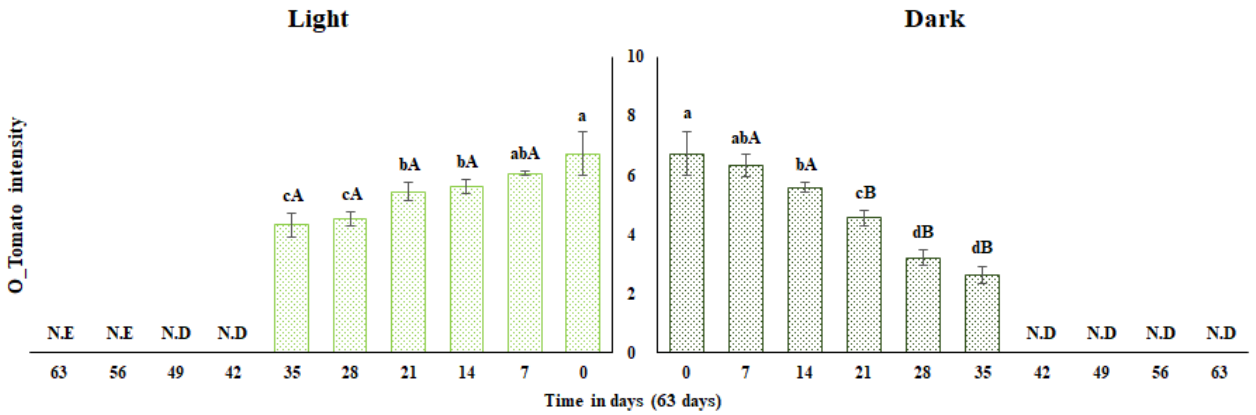

Figure A4. Time-evolution of olfactory tomato intensity in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

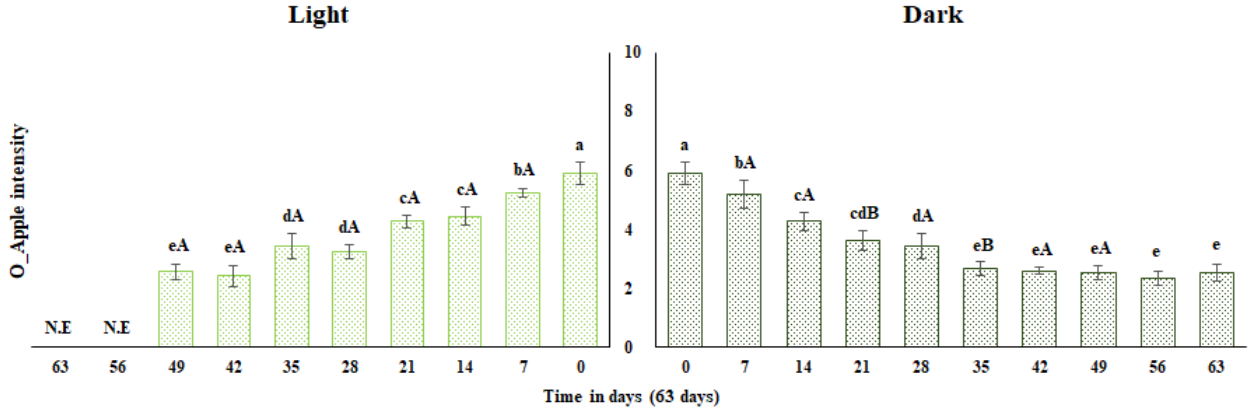

Figure A5. Time-evolution of olfactory apple intensity in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

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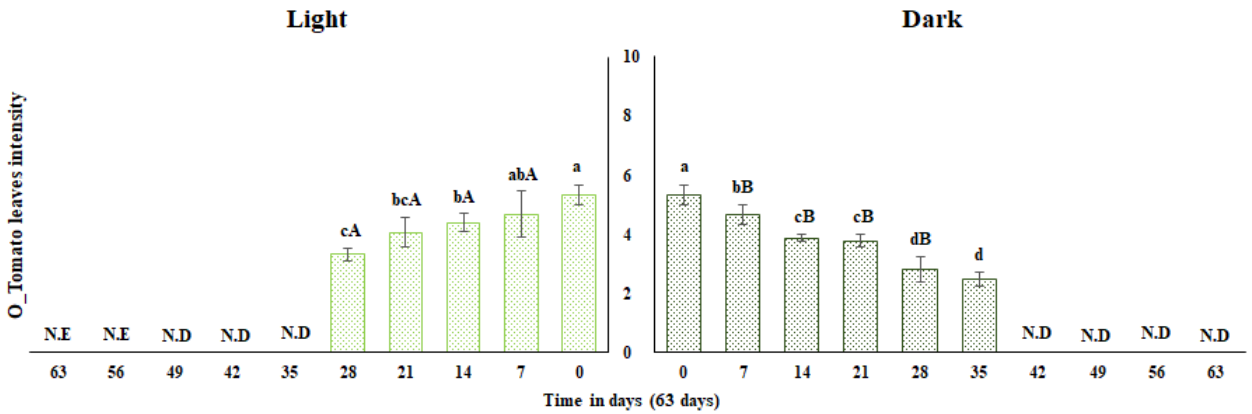

Figure A6. Time-evolution of olfactory tomato leaves intensity in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

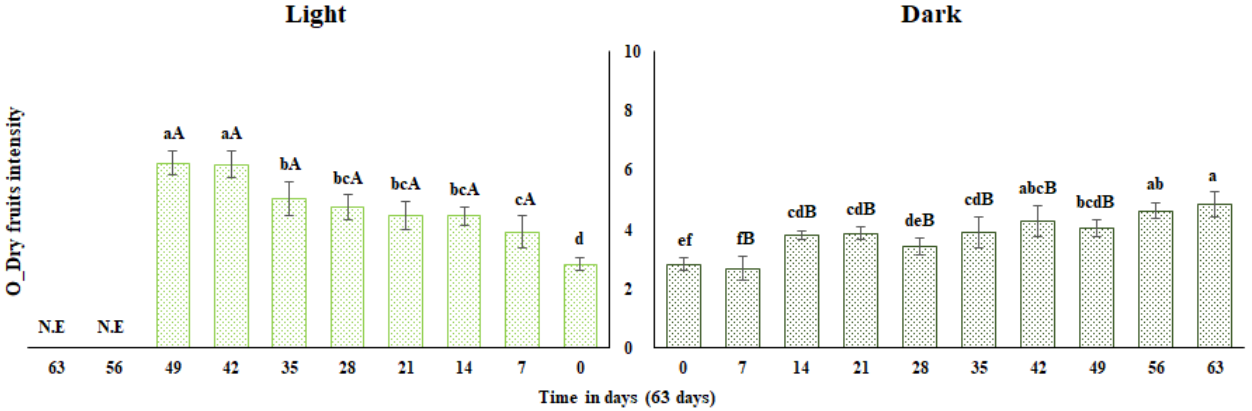

Figure A7. Time-evolution of olfactory dry fruits intensity in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

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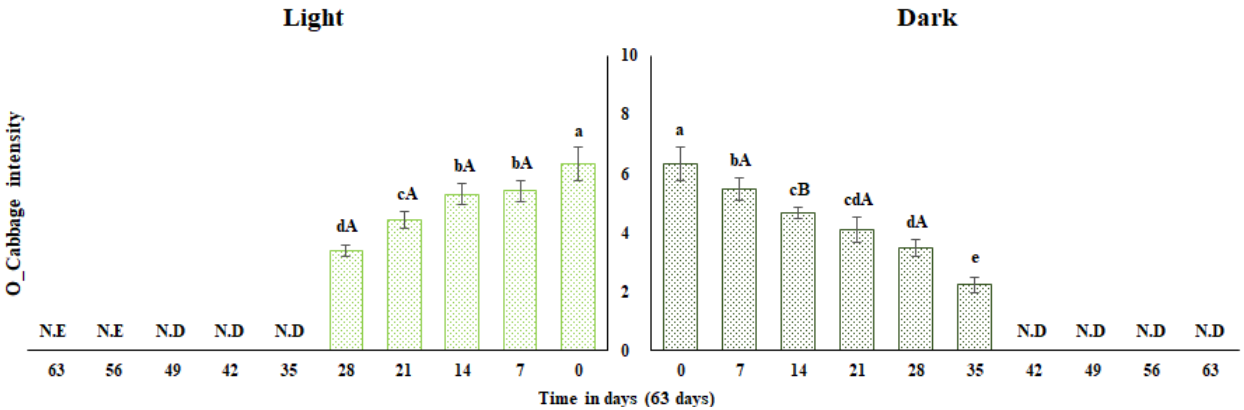

Figure A8. Time-evolution of olfactory cabbage intensity in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

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[1] a, b, c, d, e, f: letters used to compare means of samples from different periods of time for the same sensational attribute. Means that do not bear a common letter differ significantly.

A, B: letters used to compare means of samples from different storage conditions (dark or light) for the same sensational attribute. Means that do not bear a common letter differ significantly.

α, β, γ: Greek letters used to compare means of samples from different periods of time for the same sensational attributes as a second attribute in a graphic containing two attributes (Ripely fruity and Dry grass). Means that do not bear a common letter differ significantly.

A*, B*: letters with a symbol used to compare means of samples from different storage conditions (dark or light) time for the same sensational attributes as a second attribute in a graphic containing two attributes (Ripely fruity and Dry grass). Means that do not bear a common letter differ significantly.

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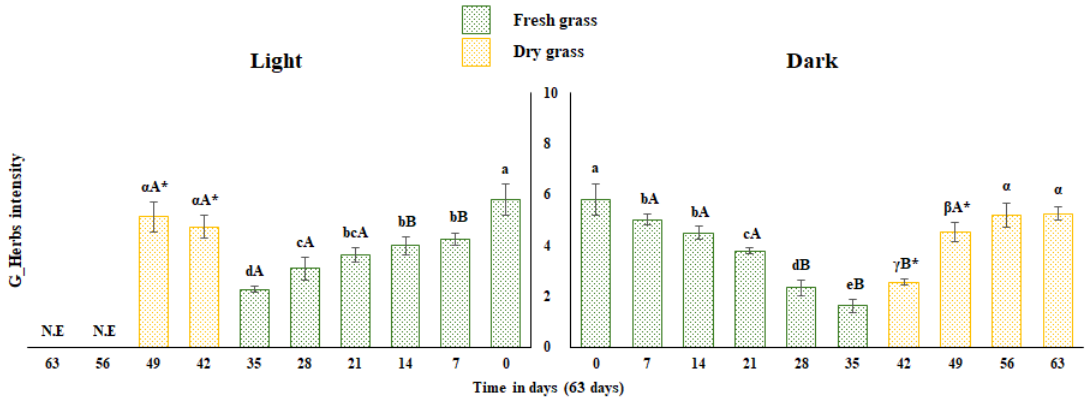

Figure A9. Time-evolution of gustatory herbs intensity (greenly fruity and ripely fruity notes) in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

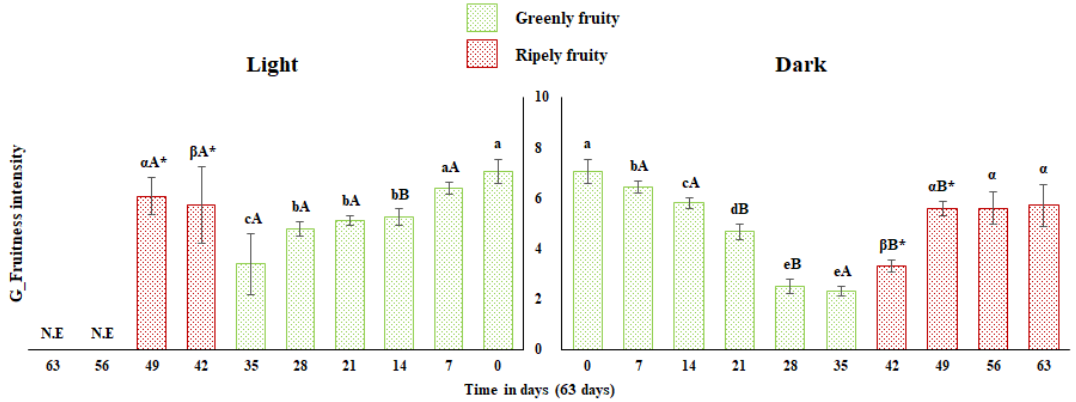

Figure A10. Time-evolution of gustatory fruitiness intensity (fresh grass and dry grass notes) in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

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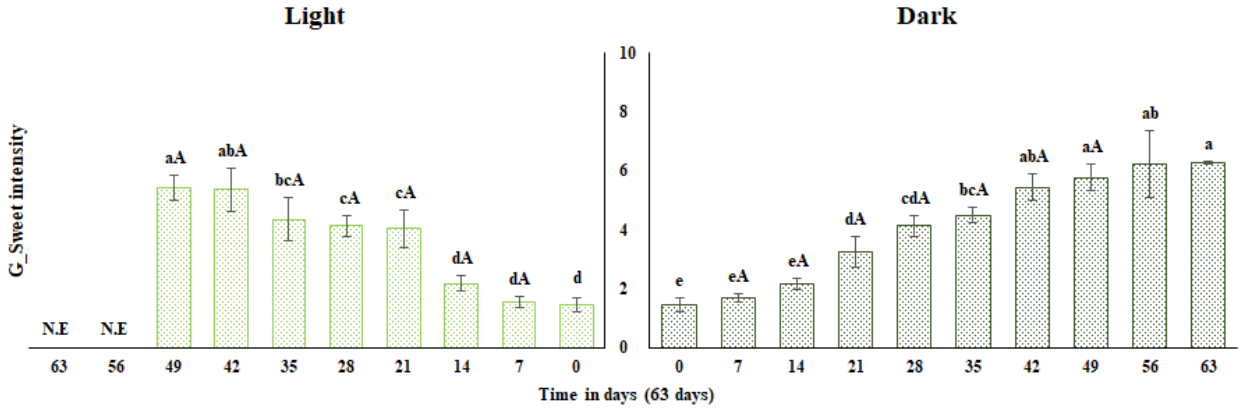

Figure A11. Time-evolution of gustatory sweet intensity in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

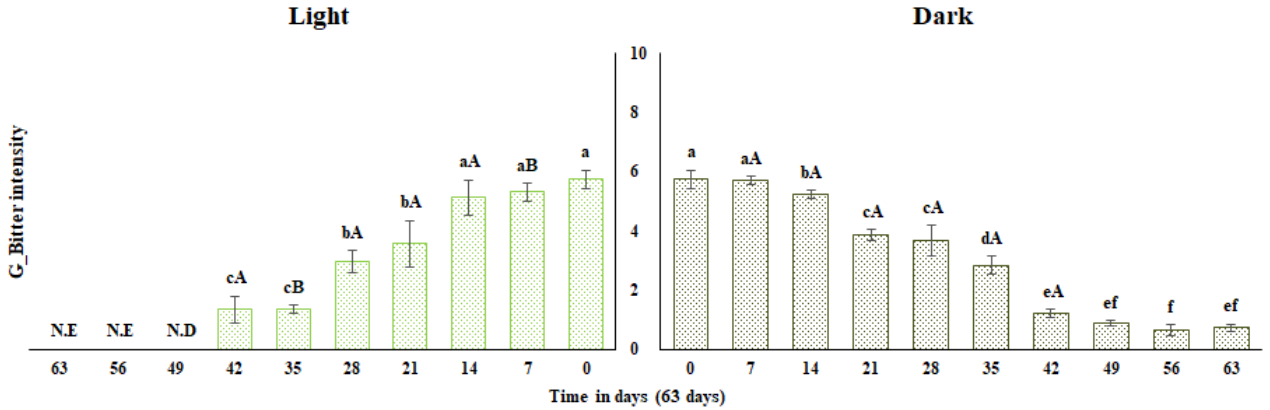

Figure A12. Time-evolution of gustatory bitter intensity in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

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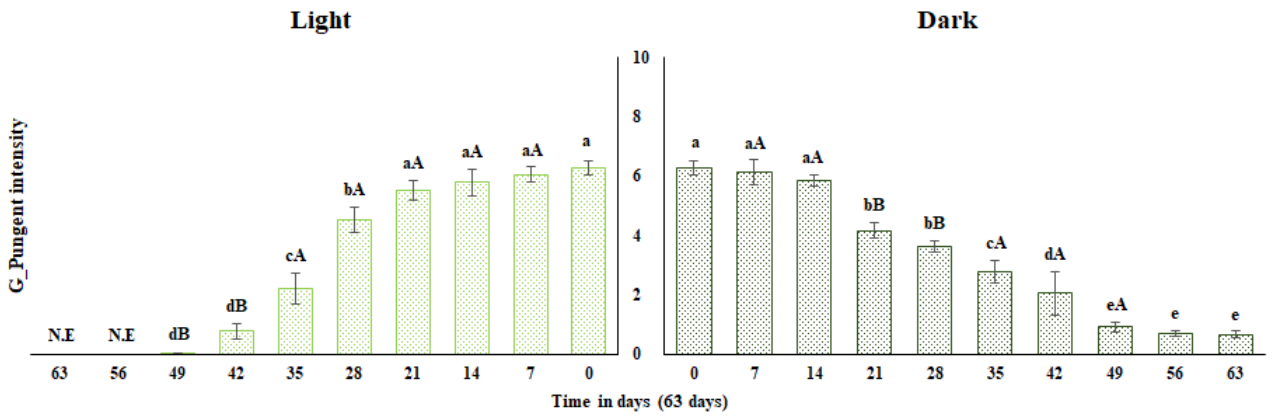

Figure A13. Time-evolution of gustatory pungent intensity in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

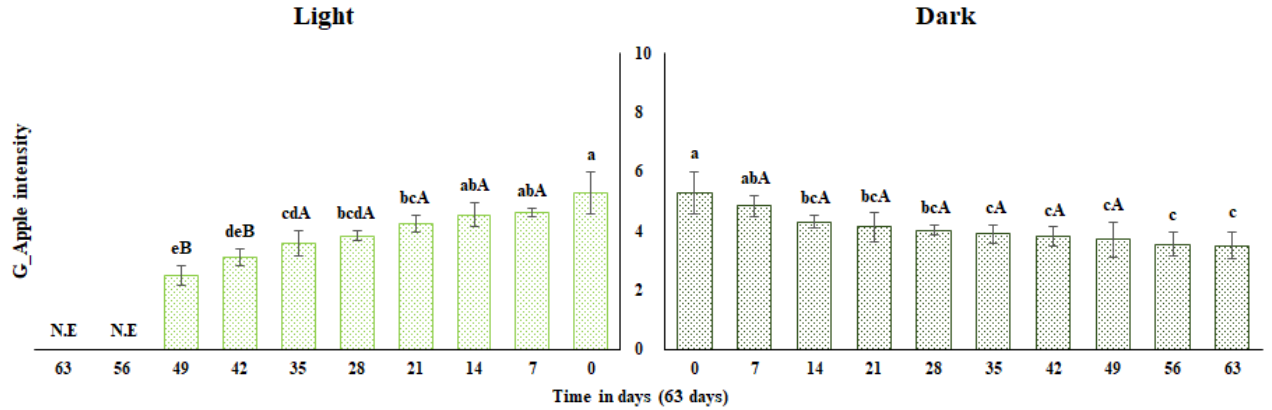

Figure A14. Time-evolution of gustatory apple intensity in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

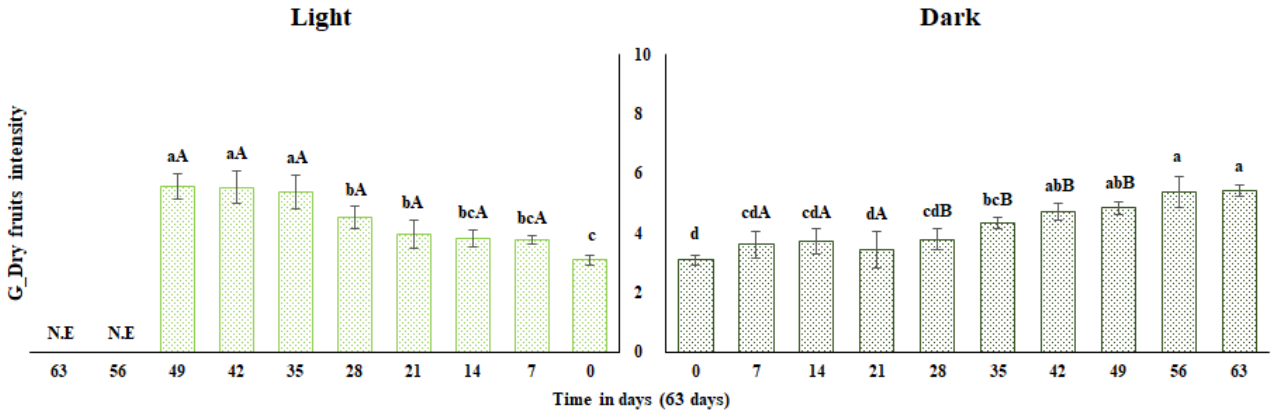

Figure A15. Time-evolution of gustatory dry fruits intensity in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

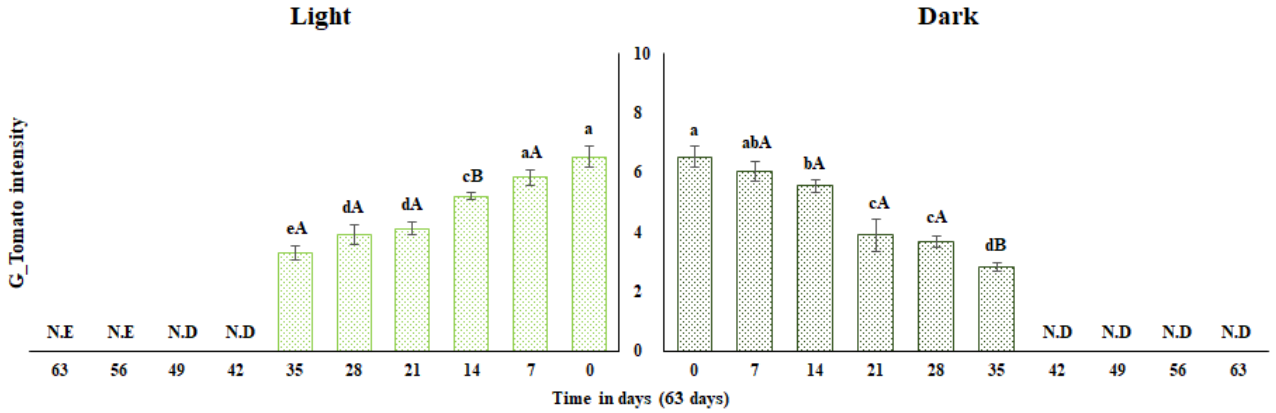

Figure A16. Time-evolution of gustatory tomato intensity in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

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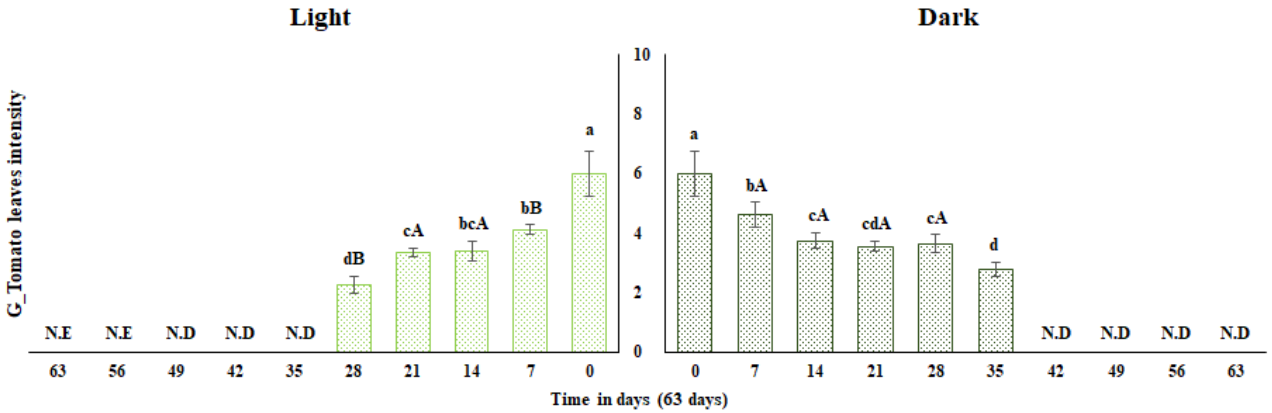

Figure A17. Time-evolution of gustatory tomato leaves intensity in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.

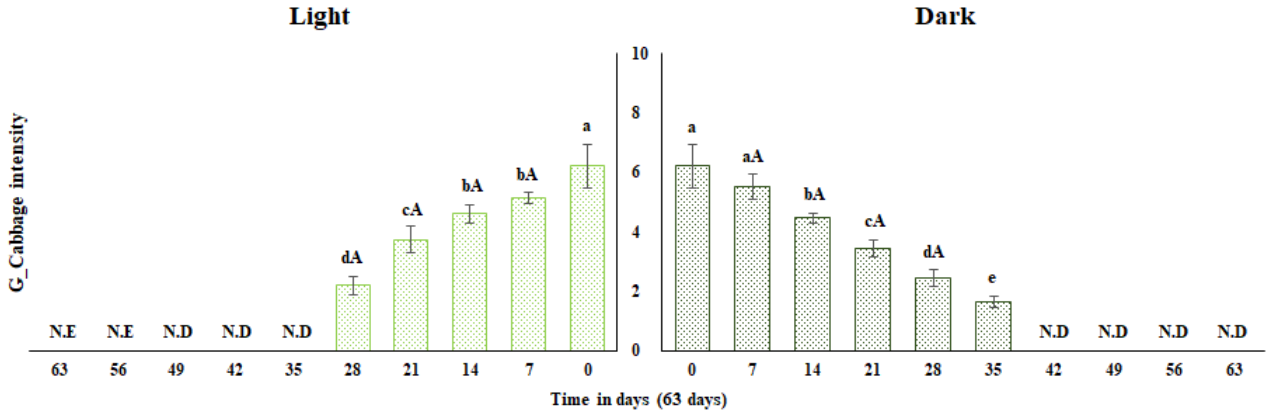

Figure A18. Time-evolution of gustatory cabbage intensity in olive oil over a 63-day period, simulating household consumption (with daily bottle opening and shaking, weekly oil removal), stored in glass bottles under conditions of either light exposure or darkness.