ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

DOTTORATO DI RICERCA IN

SCIENZE E TECNOLOGIE AGRARIE, AMBIENTALI E ALIMENTARI

Ciclo 37

Settore Concorsuale: 07/F1 - SCIENZE E TECNOLOGIE ALIMENTARI

Settore Scientifico Disciplinare: AGR/15 - SCIENZE E TECNOLOGIE ALIMENTARI

TECHNOLOGICAL, SENSORY, AND NUTRITIONAL ASSESSMENT OF ECOFRIENDLY FOOD LIPIDS

Presentata da: Cesare Ravagli

Coordinatore Dottorato

Diana Di Gioia

Supervisore

Maria Caboni

Co-supervisore

Federica Pasini

Esame finale anno 2025

Supervisor

Prof. Maria Fiorenza Caboni

Department of Agricultural and Food Science

Alma Mater Studiorum, University of Bologna

P.zza Goidanich 60, 47521 Cesena (FC), Italy

Reviewers

1)Professor Vito Verardo

Food Science and Technology

University of Granada UGR · Department of Nutrition and Food Science

vitoverardo@ugr.es

2)Professor Emanuele Boselli

Food Science and Technology

Faculty of Science and Technology of the Free University of Bolzano

emanuele.boselli@unibz.it

3*) Professor Ana María Gómez-Caravaca

Analytical Chemistry

University of Granada Department of Analytical Chemistry

anagomez@ugr.es

4*) Professor Maria Cristina Messia

Food Science and Technology

University of Molise Department of Agricultural, Environmental and Food Sciences

messia@unimol.it

*** = Substitute reviewers**

Borsa di dottorato del Programma Operativo Nazionale Ricerca e Innovazione 2014-2020 (CCI 2014IT16M2OP005), risorse FSE REACT-EU, Azione IV.4 “Dottorati e contratti di ricerca su tematiche dell’innovazione” e Azione IV.5 “Dottorati su tematiche Green." CUP. J35F21003390006

Abstract

The food industry extensively utilizes oils and fats from both animal and vegetable origins for various products. In 2023, global consumption of fatty substances reached 416 million tons, with projections suggesting this will double over the next 30 years. The implementation of the European Regulation 2019/649, which modifies the previous 1925/2006 regulation, has necessitated the food industry's focus on avoiding *trans* fatty acids by reducing hydrogenated fat usage, thus increasing palm oil consumption. In Italy, palm oil imports for food use surged from 40,000 tons/year during 2005-2008 to 77,000 tons in 2011, nowadays Italy is the seventh largest importer of palm oil in the world, with 1,65 billion $ imported in 2022. Despite its high productivity and technological benefits, palm oil has faced nutritional and environmental criticism. Improved production techniques have mitigated the nutritional concerns, while the Roundtable on Sustainable Palm Oil (RSPO) has addressed environmental issues by certifying sustainable palm oil. Given the fixed quantities and higher costs of certified palm oil, and lingering consumer mistrust, the industry must seek alternatives to enhance sustainability and safety in the supply chain. This thesis explores utilizing by-products from other industries (wine, cereal, and tomato) to improve the recovery of fatty substances, considering their bioactive compounds (e.g., tocopherols and sterols). Developing mixtures of oils and fats from these by-products, with added fractions of other fats, can enhance the safety and shelf life of formulations, with a focus on preventing lipid oxidation. Particularly, by-products from the Italian cereal sector, such as rice germ and wheat germ, offer promising sources of valuable fats. In 2020, Italy produced about 4 million tons of durum wheat, with the germ containing approximately 15% extractable fat, rich in *cis*-linoleic acid and monounsaturated fatty acids (MUFAs). Similarly, rice germ, a by-product of rice husking, yields significant quantities of linoleic and palmitic acids. Additionally, grape seeds from the wine industry and tomato seeds from the canning industry provide unsaturated fatty acids and antioxidants, beneficial for thermal stability and frying. This research aims to develop industrially viable oil and fat mixtures from these by-products, enhancing supply chain sustainability and reducing waste. It involves comprehensive chemical, sensory, and industrial analyses of raw materials, extraction, co-extraction, and refining methods. Furthermore, the development of rapid, non-destructive screening methods for fat quality assessment is crucial to minimize solvent and reagent usage, aligning with sustainable practices.

Keywords: palm oil, Italian agricultural supply-chain, chromatographic techniques, baked products, lipid oxidation, thermal stress, product formulation, non-destructive analyses.

Table of Contents

1.Introduction

a. Vegetable oils and fats, the general context

Vegetable oils and fats are one of the most important components of the dietary intake of worldwide population. According to the United States Department of Agriculture (USDA), 210.3 million metric tons of vegetable oils were produced globally during the 2022/2023 season. Furthermore, the global production of vegetable oils continues to rise, with a particular focus on palm oil, soybean oil, and sunflower oil. Studies on oils represent one of the main areas of research today, especially as consumers become increasingly careful and informed to nutritional and health aspects (Madhujith and Sivakanthan, 2019). Lifestyle-related diseases, including those associated with diet, such as coronary heart diseases, are the leading cause of death in developed and developing countries alike. In this context, oils play a crucial role in maintaining human well-being. Oils differ in their characteristics based on their composition, both in terms of fatty acids and minor compounds, and are utilized as cooking oils and ingredients in a wide variety of foods. Vegetable fats are mainly originated by the same matrices of the oils and tend to be more used as ingredients in product formulations such as sweets, ready-to-eat meals and vegetarian/vegan products, in substitution of animal fat sources such as butter and lard. In the following pages a brief description of the mostly used vegetable oils and fats in the food industry will be presented, in order to gain focus on the main advantages and disadvantages that these matrices present.

Palm oil

Palm oil is a semi-solid fat at room temperature, derived from the pulp of the oil palm fruit. This oil is predominantly produced in Indonesia and Malaysia, which together account for 87% of global production, estimated, for the 2023/2024 season at 66 million metric tonnes (USDA 2024). Worldwide, 80% of palm oil production is used in the food industry, 19% in cosmetics, soaps, pharmaceuticals, lubricants, paints, and varnishes industries, and 1% in biodiesel production. The palm oil is the most efficient crop in terms of land use for oil production. The plant yields its first harvest after 30 months and produces an average of 40 kg of oil annually. Palm oil is almost entirely composed of triglycerides, with 50% of its fatty acids being saturated, predominantly palmitic acid, and the remainder being unsaturated fatty acids, mainly oleic acid. Additionally, crude palm oil contains components like vitamin E (mainly as tocopherols up to 500 mg/kg of crude oil and alpha-tocopherols 150-200 mg/kg), carotenoids (500-2000 mg/kg), and phytosterols (40-90 mg/100 g). Some industrial refining methods can reduce the vitamin E content by up to 40% and most of the carotenoids (Gibon, De Greyt and Kellens, 2007). This fat is used as an ingredient in various food production and processing chains, such as biscuits, croissants, brioches,

snacks, rusks, crackers, as well as in spreads, soups, ice creams, glazes, baby foods, and chips (Traitler and Dieffenbacher, 1985; Aryana *et al.,* 2003). Moreover, palm oil can also be used as frying oil (Matthäus, 2007) and for producing margarine and shortenings (Imoisi *et al.,* 2015). Its widespread use is due to its vast technological versatility and unique properties that influence the structure, flavour, appearance, and often the shelf life of many products. The main characteristics justifying the extensive use of palm oil in food products are neutral taste and odour, high saturated fatty acid content, stability at high cooking temperatures, solid or semi-solid state, depending on the type of product needed is possible to obtain different states due to the high presence of non-hydrogenated saturated fatty acids (50%), crucial for the chemical and physical properties of some food products. Particularly smooth and creamy consistency, Cost-effectiveness and high yield per hectare (Aryana *et al.,* 2003). And the ability to obtain fractions with different physicochemical characteristics through physical fractionation. Indeed, palm oil can be easily fractionated based on the melting point into a so-called olein fraction (palm olein), which is liquid at room temperature due to a higher percentage of monounsaturated fatty acids compared to saturated ones, and a stearin fraction (palm stearin), which is solid at room temperature. Palm oil is the least expensive and most widely produced vegetable oil globally. Its versatility leads to its extensive use in food, cosmetics, and biofuel production. Between 2003 and 2013, palm oil production nearly doubled, and the increase in demand has resulted in a significant expansion of the land used for its cultivation (Figure 1). Since the cultivation of oil palm is limited to humid tropical environments, much of this expansion has occurred at the expense of carbon-rich and biodiversity-rich tropical forests. The destruction of tropical forests has led to a drastic increase in greenhouse gas emissions and a decline in biodiversity. Furthermore, the greenhouse gas emissions due to land-use change are higher than the greenhouse gas emissions from the production process itself.

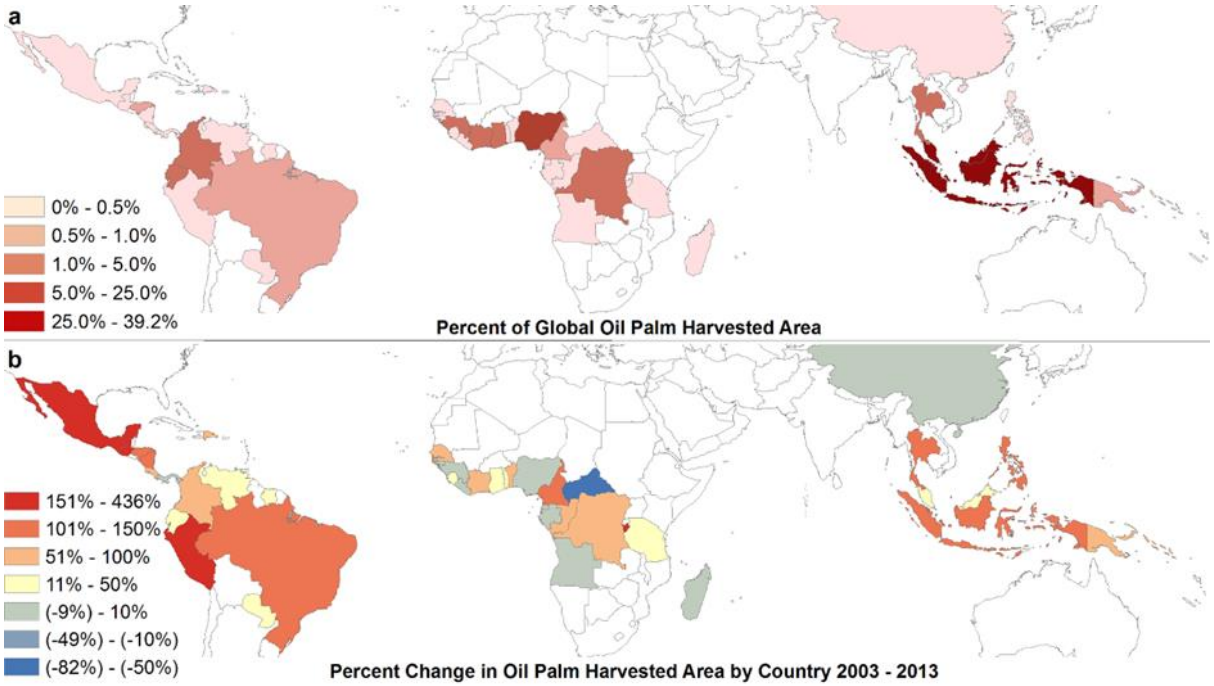

Figure 1.1: (a) Percentage of the total area of oil palm recorded by the FAO in 2013. (b) Percentage changes in oil palm harvested area by country from 2003 to 2013.

The increase in global palm oil production is largely due to the expansion of biofuel markets in the European Union. Biofuels derived from palm oil can be used as alternatives to fossil fuels, such as diesel. The use of biofuels is expected to drastically reduce emissions, unlike fossil fuels, which release large amounts of greenhouse gases when burned. However, deforestation, the drying of peat soils, and the use of fossil fuels for the cultivation and processing of the plantations themselves lead to greater greenhouse gas emissions, exacerbating climate change and accelerating the loss of biodiversity (Fitzherbert *et al.,* 2008; Hansen *et al.,* 2015). In recent years, consumers and non-governmental organizations (NGOs) have called on companies to purchase sustainable palm oil. The main organization responsible for certifying sustainable palm oil is the Roundtable on Sustainable Palm Oil (RSPO), and this certification system requires producers to follow various criteria, including management transparency, conservation of natural resources, and assessment of social and environmental impacts (Vijay *et al.,* 2016). In addition to having a balanced composition of fatty acids, palm oil is rich in nutrients such as carotenoids and tocopherols, which have antioxidant properties and play an important role in the stability and quality of the oil. Several scientific studies have significantly downplayed the negative role of saturated fatty acids in raising blood cholesterol levels. According to a study conducted by Kelly and collaborators (2002), diets enriched with palmitic acid, which is found in palm oil, do not lead to an increase in blood cholesterol; in fact, they have relatively neutral effects. In general, it is important to bear in mind that it is strongly recommended to limit the

total amount of fatty acids consumed in the diet, regardless of the source of these nutrients (Kadandale, Marten and Smith, 2019). In December 2014, Regulation (EU) No. 1169/2011 mandated the specification of each vegetable oil in the food product formulation. Consequently, palm oil is now identifiable on ingredient lists with the specific name *Elaeis Guineensis* Oil instead of the generic term vegetable oils. Additionally, since December 2014, GIFT (Great Italian Food Trade) has launched a petition to ban palm oil in food products. Thus, an increasing number of products on supermarket shelves are labelled "palm oil-free" or similar. The claim "palm oil-free" stems from two main issues: the environmental sustainability and the health risks of palm oil, especially for children, due to its high saturated fat content and the potential presence of processing contaminants. However, it should be noted that these contaminants have significantly decreased in recent years due to improved extraction and refining technologies. Eliminating palm oil necessitates a re-evaluation of formulations, considering both production costs and the product's shelf life and sensory characteristics.

Coconut oil

Coconut oil is extracted from the pulp of coconuts (*Cocos nucifera L.*) grown in tropical and subtropical regions, particularly in Asia and the Pacific islands. The Philippines is the world's leading producer of coconut oil, in 2023, the global production was estimated at 3.5 million tons, with the Philippines producing about 1.8 million tons, Indonesia 850,000 tons, and India 400,000 tons. Production trends for 2024 indicate a slight increase, with projections showing global production reaching 3.6 million tons, driven by expanding demand in the food and cosmetics industries (USDA 2024). The oil is obtained from the fruit's endosperm, which, when dried, is known as "cover" containing 65-68% oil and 4-7% moisture. The extraction process involves two stages: the first stage extracts the oil through pressing, while the second stage uses solvent extraction. Coconut oil is particularly rich in low molecular weight saturated fatty acids and lauric acid (about 50% of the fatty acids), classifying it as a "lauric fat" along with palm oil and palm kernel oil. These fats are notable for their sudden transition from solid to liquid across a wide range of temperatures. Coconut oil is solid at room temperature (21°C) but melts completely and abruptly just below body temperature, over 90% of its fatty acids are saturated, which gives it high oxidative stability. Among vegetable oils, coconut oil is the richest in medium-chain triglycerides (MCTs), such as C6, C8, and C10, who in the latest years had been studied for their relative activities in mental health pathologies prevention, and also nutritional merits (Peyronel, 2019; Ashton *et al.,* 2021) making it a key component in formulations for infants and medical foods for individuals who cannot absorb long-chain fatty acids.

The colour of crude coconut oil ranges from light yellow to brown, while deodorized oil appears pale yellow. The odour and taste are intricately linked to the presence of minor amounts of lactone (≤ 150 ppm), with crude oil having a rancid smell. Due to its low concentration of unsaturated fatty acids, coconut oil has high oxidative resistance but hydrolyses two to ten times faster than regular oils, leading to an unpleasant soapy taste. Coconut oil is extensively used in the confectionery, margarine, and ice cream industries, even after fractionation to obtain stearins with different melting points. Its unique properties, such as high oxidative stability and medium-chain fatty acid content, make it suitable for various applications, including baby foods and medical nutrition.

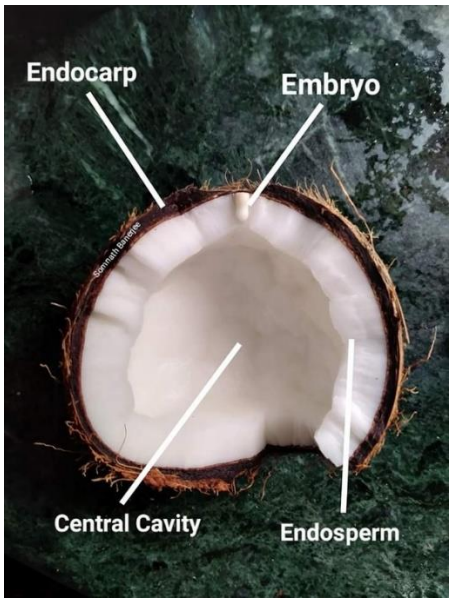

Figure 1.2. Coconut section, endosperm represents the source of the oil, the endocarp and the husk usually undergo thermal valorisation.

Sunflower oil

Sunflower (*Helianthus annuus L.*) is an annual plant native to America; from whose seeds a light-yellow oil is extracted. In 2023, global sunflower oil production was estimated at 21.5 million tons, with Ukraine and Russia being the largest producers, contributing around 10 million tons and 6 million tons respectively. The trend for 2024 indicates a slight increase in production, expected to reach 22 million tons, driven by the growing demand for healthier cooking oils and the increased cultivation of high oleic sunflower varieties (USDA 2024). This oil is typically subjected to chemical refining to remove unpleasant odours and flavours, and it is characterized by high percentages of unsaturated fatty acids, particularly linolenic acid, known for its cholesterol-lowering properties, which can reach percentages of 55-69%. However, due to this high concentration of polyunsaturated acids, sunflower oil is more susceptible to rancidity and lipid peroxidation during cooking, especially frying,

producing toxic radicals and oxidants, making it primarily suitable as a raw dressing for salads. This has led to the selection, cultivation, and techniques of spontaneous or induced mutation to obtain new varieties with much higher percentages of oleic acid, such as Sunflower Oil Mild Oleic (SOMO) and Sunflower Oil High Oleic (SOHO). The former is characterized by an oleic acid content of 60-75%, while the latter exceeds 75%, reaching up to 90%, compared to the 20% oleic acid present in traditional sunflower oil. These oils are more heat-resistant and are therefore suitable substitutes for saturated fats in the preparation of foods requiring a liquid fat with high oxidative stability (about 10 times higher than traditional sunflower oil). High oleic sunflower oil (and the one enriched with vitamin E and natural antioxidants) is therefore an excellent oil for frying, as it is stable at temperatures above 200°C, unlike traditional sunflower oil. Some applications of high oleic sunflower oil include spray oil, used as a protective coating for cereals, fruits, nuts, baked goods such as crackers, cookies, croutons, etc. Bottled oil, for general domestic uses such as cooking or pan-frying, salads, sauces, gravies, etc. Frying oil, for preparations where the characteristics of frying with liquid oil are preferred, and ingredient, used by food processors in preparations such as salad dressings, mayonnaise, margarine, sauces, baking mixes (pastry or bread), etc. Oxidative stability is the main goal in the production of high oleic sunflower oil and is linked both to the higher concentration of oleic acid and the reduction of linoleic acid content, the primary fatty acid in traditional sunflower oil. From a nutritional perspective, lower concentrations of linoleic acid compared to other seed oils confer reduced inflammatory potential, as linoleic acid promotes the production of prostaglandins in the body and an excess would unbalance the omega-6 to omega-3 ratio, leading to potential systemic inflammation. Another advantage of using an oil that provides high doses of oleic acid is the regulation of plasma lipoproteins. Indeed, oleic acid tends to increase the production of HDL (also known as “good cholesterol”), improving the lipid profile and preventing cardiovascular diseases. Sunflower oil is also characterized by a high content of alpha-tocopherol, which partially preserves it from oxidative degradation, although the refining process can reduce this content by up to 40%.

Soybean oil

Soybean oil is derived from the seeds of the soybean plant (*Glycine max*) and is one of the most widely produced and consumed vegetable oils globally, mainly to the double product obtained from its extraction, food oil and protein feed in the form of flour residue. As of 2023, global soybean oil production reached approximately 59 million tons, with major producers including the United States, Brazil, and Argentina, which together account for about 80% of the world's supply (USDA 2024). United States

accounted for around 11 million tons, while Brazil and Argentina contributed 9.5 million and 8.5 million tons, respectively. Projections for 2024 indicate a slight increase in production, driven by expanding agricultural areas and improved yield efficiencies. Soybean oil is characterized by a fatty acid profile that includes around 51% polyunsaturated fats (linoleic acid, 44-62%, and linolenic acid, 4-11%), 24% monounsaturated fats (oleic acid), and 15% saturated fats (palmitic and stearic acids) (RIF). This high content of polyunsaturated fats (PUFA), particularly linoleic and linolenic acids, makes soybean oil prone to oxidation, which can affect its shelf life and stability. To address this, the oil often undergoes partial hydrogenation to improve its oxidative stability, though this process can lead to the formation of *trans* fatty acids, which have been associated with adverse health effects. To mitigate this, interest in high-oleic soybean varieties has increased, as these contain a higher proportion of monounsaturated fats and lower levels of polyunsaturated fats, enhancing stability without hydrogenation. Soybean oil also contains significant levels of natural antioxidants, such as tocopherols (vitamin E), which play a crucial role in protecting the oil from oxidative degradation. Alpha-tocopherol is present in concentrations of about 100-190 mg/kg, contributing to both the nutritional value and oxidative stability of the oil. In the food baking industry, soybean oil is valued for its neutral flavour, which does not interfere with the sensorial profile of the products, providing also desirable textural properties, such as tenderness and moisture retention. Its high smoke point (around 230°C) makes it suitable for frying applications as well.

Figure 1.3. Soybeans, the extraction of their vegetable oil represents a global scale commodity nowadays.

b. Vegetable fat sources in the Italian supply-chain

Baking industry in general tends to heavily depend on the afore-mentioned vegetable fats; since in the Italian case, most of these are imported from outside the reliance poses

significant challenges, including supply chain vulnerabilities and exposure to global market fluctuations. Furthermore, the importation of these oils substantially increases the carbon footprint of the industry. The transportation of large quantities of oil from distant countries like Indonesia, Malaysia, the United States, and Brazil contributes to greenhouse gas emissions, exacerbating environmental concerns. This dependency not only undermines the sustainability efforts within the Italian baking industry, but also highlights the need for more locally sourced and environmentally friendly alternatives to reduce the industry's overall ecological impact. Another goal is also to also give technological alternatives to at least decrease the fragility of the supplying activities. In the following pages are reported some valid alternatives that could prove useful in the sense of this project. In the following pages, vegetable oils, derived from Italian by-products will be exposed.

Rice oil

Italy is one of the leading rice producers in Europe, with a robust and well-established supply-chain. The cultivations include varieties such as Arborio, Carnaroli, and Vialone Nano, which are particularly prized for their use in risotto. The Italian rice industry is centred in the regions of Lombardy, Piedmont, and Veneto, where favourable climate conditions and fertile soils contribute to high yields. In 2023, Italy produced approximately 1.4 million tons of rice, keeping its position as a significant player in the European market. Rice oil, or more precisely rice bran oil, is the vegetable oil obtained from the processing of the outer layer of the rice grain; it is not very widespread globally, but it is increasingly in demand as an edible oil, especially among Asian populations. The oil extracted from rice germ (*Oryza Sativa L.*) holds about 40% oleic acid and 40% linoleic acid, followed by palmitic acid at 17%. An important characteristic of rice oil is its high concentration of vitamin E in a 1:1 ratio between tocopherols and tocotrienols and the presence of gamma-oryzanol, which consists of a mixture of ferulic acid esters with plant sterols and triterpene alcohols. Numerous studies attribute various beneficial effects to these substances, including the reduction of blood cholesterol and triglyceride levels through a combination of different mechanisms, including increased conversion of cholesterol to bile acids and inhibition of cholesterol absorption. The richness in tocopherols, monounsaturated fatty acids (oleic acid), and polyunsaturated fatty acids (linoleic acid) gives rice oil hypolipidemic properties and cardiovascular disease prevention functions, as well as antioxidant and anti-inflammatory functions. Additionally, rice oil has a delicate flavour and remarkable stability at elevated temperatures, making it suitable for cooking methods such as frying and sautéing. Furthermore, gamma-oryzanol, its characteristic

component, is resistant to typical frying temperatures (180 °C). To fully benefit from all the characteristics of this oil, it should be consumed raw.

Figure 1.4. Milled rice bran, rice bran represents an important by-product of the rice milling industry, and it is usually implemented as feed stock.

Grapeseed oil

The cultivation of grapes is considered one of the most important agro-economic activities globally, with over 75 million metric tons of grapes (*Vitis Vinifera L. ssp sativa*) produced worldwide in 2021, of which approximately 8.15 million metric tons were produced in Italy (FAOSTAT 2022). A significant amount of waste from the wine industry remains unused every year on an international scale. Grape seeds, byproducts of the wine industry, are often referred to as important agricultural and industrial waste with the potential to be utilized in both the pharmaceutical and food sectors. Grape seeds constitute 20-26% of the grape berry and contain 7.8-11% proteins and 10-20% oil, depending on the extraction conditions (Bail *et al.,* 2008). Grape seed oil is composed on average of 90% monounsaturated and polyunsaturated fatty acids, particularly linoleic acid (58-78%), followed by oleic acid and a smaller amount of saturated fatty acids. Unrefined oils contain some bioactive compounds with various biological effects, such as antioxidant and antimicrobial properties that explain also its stability and good resistance to oxidation reaction. Defatted seeds are a rich source of tannins, oligomeric proanthocyanins, mainly monomeric catechins and epicatechins, in concentrations much higher than other oils (Xagoraris *et al.,* 2021). The grape seed oil is also rich in tocopherols, which are among the most important natural antioxidants introduced through the diet, and its unsaponifiable fraction contains high levels of phytosterols (Kiralan *et al.,* 2019). The volatile profile includes many compounds, including monoterpenes, alcohols, esters, and carbonyls. The concentration of these compounds depends on the grape variety, cultivation practices, and climatic and biological factors (Yalcin *et al.,* 2017). Numerous modern researches focus on the

characterization and application of unrefined grape seed oils, mainly because of the plethora of compounds of interest, however, unrefined grape seed oil can develop quick oxidation reactions and flavour alteration (Nyam and Ken, 2014); it is also to be noted that the percentage of oil inside a seed is usually around the 14-16% of the total seed weight, making the extraction by pressing not economically sustainable (Kiralan *et al.,* 2019). Recent studies had led to an increase in the extraction yield by the implementation of super-critical fluids, like carbon dioxide (CO_2).

Figure 1.5. Grape seeds tend to represent less than 5% of the total weight of the grapefruit, but the high volumes of grapes produced for hectare make it so that the mass accumulated is estimated around 800 kg/hectare.

c. Baking industry, the Italian landscape

The Italian baking industry represents a cornerstone of the country's culinary tradition, and a significant segment of its food sector. Characterized by a wide variety of products, from artisanal breads to industrially produced pastries, the industry encompasses both small-scale local bakeries and large multinational corporations, with notable example found in Barilla, with the "Mulino Bianco" brand, Ferrero and other smaller realities such as Balocco, Galbusera and Saiwa. Bread remains the most produced and consumed baked good, with regional varieties playing a key role in defining local culinary identities, some notable examples are "Pane di Altamura", the "Coppia Ferrarese", the "Michetta" and many others. Alongside bread, pastries, cakes, biscuits form a substantial part of the industry, catering to both domestic consumption and export markets. In terms of market volumes, the Italian baking sector is robust, with an estimated annual production value exceeding €20 billion (FAOSTAT 2022). This industry is highly fragmented, with thousands of small and medium enterprises (SMEs) contributing to the diverse array of baked goods available in the market. Despite the dominance of traditional bread and pastry products, the industry has seen

a growing demand for healthier and more convenient options, leading to an increase in the production of gluten-free, organic, and ready-to-eat products. Italy is also a significant player in the global baking industry, with a strong presence in both import and export markets. The country imports raw materials such as wheat, sugar, and dairy, while exporting a variety of finished products, particularly in the premium segment. Italian baked goods, especially those with Protected Designation of Origin (PDO) status, are highly valued in international markets for their quality and authenticity. Among the wide array of baked goods, “tarallini” and “frollini” biscuits were chosen as subject of study mainly for: their widespread consume all over Italy, the relatively modernized production industries, and their relative differences, both on a sensorial and technological point of view.

Taralli and “Tarallini”

Taralli are common savoury snacks typical of the Italian cuisine, often flavoured with spices, onion, or salt. In recent years, taralli have gained popularity worldwide as a snack and as bread substitute. The main difference found in their counterpart, known as “Tarallini” is held in the smaller dimensions of the latter, also, their production tends to be located in the artisanal shops of the Puglia region, meanwhile “Taralli” are more typical of the Campania region. From a commercial standpoint, they belong to the “baked goods” category, which is, in fact, a highly heterogeneous category of products, classified both by technological and commercial criteria. What unites these products are the basic ingredients: flour, water, and a leavening agent (Cabras and Martelli, 2004). Taralli are a typical product from Southern Italy, specifically originating from the Puglia region. The history of this product dates to approximately the 15th century, during a period when the southern regions faced a famine. According to legend, the first taralli were made by a mother who, with the few ingredients she had at home—olive oil, flour, salt, and wine—had to improvise a recipe to feed her children. After kneading the dough, she shaped it into strips, formed rings, let them rest, and then baked them, thus creating what we now call "Taralli." The origin of their name is still unclear; some believe it derives from the Latin term “torrère,” which means to toast, referring to their crunchy and slightly brown appearance. Others suggest it may come from the Italic term “tar,” meaning to wrap, or from the French term “danal,” which refers to round bread, due to its rounded shape (Caponio *et al.,* 2009). Italian taralli are bread substitutes characterized by a ring shape and a crumbly, crunchy texture, with a diameter ranging from 2-3 cm to about 9-10 cm, and a thickness ranging from 7-8 mm to 1.5 cm (Caponio *et al.,* 2009). These products are made from a dough of flour, water, salt, vegetable oil (or another fat), and sometimes with the addition of wine. The processing technology initially involves a brief boiling, which prevents leavening

before baking and gives the surface a glossy appearance. Nowadays, on an industrial scale, taralli production technology may involve the use of a specific machine called a "tarallatrice" (Barbieri *et al.,* 2018), a machine that cuts and rolls strips of dough, passing them through moving tubes that shape the dough into a round form with a hole in the center, approximately 3 cm in diameter and 8 mm in thickness.

Figure 1.6 and 1.7. "Tarallini" and taralli, the shape and the flavours tend to vary by means of regional recipe, consumers preferences and seasonal availability of ingredients (wild fennel as an example).

General recipe

Generally speaking, the recipe for taralli and "tarallini" takes in account for 0.4 L of water, 0.2 L of extra virgin olive oil (EVOO), and 0.02 Kg of salt for every 1 Kg of flour, with the possible addition of spices (0.005 Kg) (Caponio *et al.,* 2011). In

industrial production, the ingredients are mixed for about 25 minutes using a spiral mixer. The energy exerted during the mixing of water and flour promotes the formation of the gluten structure; the presence of gluten is crucial for the dough's performance as it provides elasticity and plasticity. Once the dough is prepared, it is left to rest for about 45 minutes (Caponio *et al.,* 2009). It is then sectioned and placed into an automatic tarallo-forming machine, which uses different types of extruders to create smooth, uniform, and thin strips of dough that are subsequently cut, rolled, and inserted into moving tubes that shape the dough into the typical circular form of taralli (approximately 3 cm in diameter and 8 mm in thickness). After exiting the "tarallatrice", the taralli are boiled for about one minute, then placed on a rack and left at room temperature for 30 minutes to facilitate surface drying. Next, they are baked in air tunnel ovens at 230°C for 20 minutes (Caponio *et al.,* 2009). Once baking is complete, the taralli are left to cool at room temperature for a short period before being packaged using packaging machines.

Consume trends

Among Italians' preferences for potential snacks, fruits and vegetables rank first, followed by bread substitutes, such as taralli, and finally, yogurt. According to research conducted during the COVID-19 pandemic, which forced the population to stay at home and adapt to remote working, the needs of workers have changed, with a growing demand for simpler, quicker, and longer-lasting solutions. This situation led consumers to use bread substitutes instead of fresh bread during main meals. Regarding the sale of taralli, there was an increase in revenue of 64.8 million euros. This new trend has prompted modern retailers (Conad, Coop, Esselunga, Lidl, etc.) to expand the shelf space dedicated to the bread substitutes sector. Additionally, 49% of consumers appreciate the fact that these products are packaged in single portions, making them very convenient for on-the-go consumption. The research also shows that 26% of the sample consists of women who consume snacks daily outside of meals, compared to 21% of men (ANSA).

Biscuits, Italian panorama

The term "biscuit" identifies a small, dry, and crumbly baked confectionery product, made from soft wheat flour, sugar, and minor components such as milk, salt, and flavouring agents. It is characterized by a low moisture content (1-5%) and long shelf life (Chevallier *et al.,* 2000). The word "biscuit" was coined in the Middle Ages and derives from the Latin term "*panis biscoctus*", meaning bread baked twice, referring to dried rusks made from flour, salt, and water, prepared as a survival ration for sailors. The first producers discovered that by adding fats and sugar to small pieces of dough

and baking them in a typical hot oven until they reached a light golden colour, they could produce biscuits that were less dry and crunchy. Furthermore, if left to dry further at lower temperatures, their texture would improve, and their shelf life would be extended (Cauvain and Young, 2007). With the advent of industrialization, biscuit production was the first industrial process to be mechanized, leading to the creation of numerous varieties over time, which can be classified according to ingredients, shape, and texture into:

Hard Dough Biscuits: also known as dry biscuits, these are the direct descendants of the original sailor's hardtack. They are made from a hard and elastic dough, achieved through prolonged processing, with low fat (6-10%) and sugar (10-15%) content, and the primary ingredients are flour and water.

Semi-Hard Dough Biscuits: these products are based on the use of shortcrust pastries, which are fragile, less plastic, and moderately fatty. Although considered lean, shortcrust biscuits contain higher fat (12-19%) and sugar (14-20%) content than dry biscuits.

Soft Dough Biscuits: commonly known as shortcrust pastry biscuits, these also originate from shortcrust doughs, which are richer in fats and sugars; therefore, they have a slightly soft texture.

Very Soft or Liquid Dough Biscuits: these have a very soft, sometimes almost liquid dough (batter) and are produced from highly heterogeneous recipes, all based on the use of little or no flour, eggs, and a high content of fats (15-28%) and sugars (20-60%) (Lanza, 2006).

According to Mediterranean dietary habits, the consumption of baked goods represents about 22-24% of daily energy intake, with around 10% of daily requirements coming primarily from the raw material, flour, which is not only a source of energy nutrients (carbohydrates, fats, and proteins) but also of minerals (potassium, magnesium, phosphorus, copper, and zinc) and vitamins (thiamine, riboflavin, vitamin B6, and folic acid). Currently, biscuits hold a privileged position in the market in terms of production and consumption compared to other baked goods. According to recent research by Global Market Insights, the bakery products industry accounts for 10% of the food market. It is estimated that the global bakery products market will reach $339.3 billion by 2026, with an annual growth rate of 3.54%. With the spread of the COVID-19 pandemic, biscuit sales increased by 44.3%, this growing consumption rate of long-shelf-life processed products provides convenience for consumers, and the ongoing shift toward convenient food products is aiding market growth. Moreover, the sector's

development is currently also linked to increased awareness of health issues associated with food. More and more consumers, in fact, are seeking foods that are high in fiber, healthy, and low in fat. According to a survey by Innova Market Insights, one of the main future trends will involve an increase in bakery products that are increasingly vegan-friendly, to reduce the use of animal fats.

"Frollini" biscuits

"Frollini" biscuits are characterized by their rich, buttery flavour and crumbly texture. The traditional recipe for "frollini" includes a high proportion of fat, typically butter, combined with flour, sugar, and eggs. This composition results in a dough that is less elastic and more fragile compared to other biscuit types, giving "frollini" their distinctively tender and melt-in-the-mouth quality. Sensorially, "frollini" are appreciated for their delicate sweetness, smooth mouthfeel, and rich, buttery aroma. From a rheological point of view, they exhibit low elasticity and high plasticity, allowing for easy shaping but making them more prone to breaking. "Frollini" differ from other biscuits in their higher fat content and crumbly texture, distinguishing them from harder biscuits like "*biscotti secchi*" and softer, moister options like sponge biscuits. In Italy, frollini hold a significant place in the baked goods market, reflecting the country's preference for high-quality, indulgent treats. The Italian production of "frollini" emphasizes artisanal methods and the use of premium ingredients, combined with the implementation of regional and typical ingredients that contribute to a development of unique profiles related to the site of production. Some examples of widespread "frollini" biscuits in the Italian market are represented by "Macine", "Galletti" and "Baiocchi".

Figure 1.8 and 1.9. "Frollini" biscuits, both in traditional recipe, and in variant with chocolate chips adding. The simplicity of the recipe tends to let producers get creative with the final product.

d. Lipids and oxidation

Lipids represent a vital fraction in most of the foods consumed, not only they tend to cover a majority of the calory index of most products, but they also contribute to the development of flavours, aromas and textures when interacting with both protein and starchy structures. In the products presented these phenomena are of outmost importance, and to have a good product, feasible to the customer, an opportune study and understanding of the characteristics and kinetics that rule this category of molecules is necessary. In the following pages an overview of the general structure of food lipids, their differences and chemical transformations that take place will be presented, specifically looking at the impact that these reactions have on the product, and the favourable conditions in which they take place.

Lipids and baked products

Lipids are a diverse group of hydrophobic molecules that play a critical role in food systems, both as structural components and as sources of energy. Generally speaking, the chemical characteristics of lipids are largely defined by their fatty acid composition. Saturated fatty acids, which lack double bonds, tend to be solid at room temperature and exhibit a higher melting point due to strong intermolecular forces (Asokapandian, Sreelakshmi and Rajamanickam, 2021). In contrast, unsaturated fatty acids contain one or more double bonds, introducing kinks into the fatty acid chains that hinder close packing, resulting in lower melting points and a liquid state at room temperature. The degree of unsaturation also influences lipid reactivity, particularly their susceptibility to oxidation. *Cis* and *trans* configurations of unsaturated fatty acids further diversify lipid properties, with *trans* fats, often formed during industrial hydrogenation, being of particular concern due to their association with adverse health effects. From a nutritional perspective, lipids are indispensable, serving as a dense energy source, contributing to the absorption of fat-soluble vitamins (A, D, E, and K), and providing essential fatty acids that the human body cannot synthesize, such as linoleic and alpha-linolenic acids. However, the type and amount of dietary fat consumed are critical to health outcomes. Saturated and *trans* fats have been linked to increased risk of cardiovascular diseases, whereas unsaturated fats, particularly omega-3 fatty acids, have been associated with anti-inflammatory effects and cardiovascular benefits. Moreover, the balance between omega-6 and omega-3 fatty acids is crucial in maintaining homeostasis and preventing chronic diseases. A fundamental challenge in the stability of food lipids is their susceptibility to oxidation, a complex process that significantly impacts the shelf life, safety, and nutritional quality of lipid-containing

foods. Lipid oxidation involves the reaction of unsaturated fatty acids with oxygen, leading to the formation of hydroperoxides, which further decompose into secondary products such as aldehydes, ketones, and alcohols. These oxidation products can cause rancidity, off-flavours, and loss of nutritional value. The oxidation process is influenced by factors such as the degree of unsaturation of fatty acids, presence of pro-oxidants (e.g., metal ions), and environmental conditions (e.g., light, heat, and oxygen availability). In the context of food science, lipids include triglycerides, phospholipids, and sterols.

Triglycerides

Triglycerides are compounds formed by the esterification of glycerol with three molecules of fatty acids. These fatty acids consist of a hydrophobic linear chain of carbon atoms with a hydrophilic carboxyl group at one end. The fatty acids present in triglycerides can be saturated if the hydrocarbon chain lacks double bonds, or unsaturated if it contains one or more double bonds. Fats and oils are composed of mixtures of triglycerides with different melting points, which is why they exhibit a melting range that could be broad depending on the type of fat. The breadth of this melting range depends on various factors, such as the degree of hydrogenation, the composition of the fatty acids, and their distribution within the triglyceride molecule. Short-chain fatty acids, those with a high degree of unsaturation, and those with a *cis* configuration tend to lower the melting point. The melting profile of fats and oils in biscuits affects various rheological aspects such as aeration mechanisms, mouthfeel, shelf life, and other quality parameters (Ghotra, Dyal and Narine, 2002; Carrai, 2010). The triglycerides that make up fats can assume various polymorphic forms; each form varies in its crystalline structure, free energy, and other physical and chemical properties, though their chemical composition remains the same. Polymorphism is particularly important in baked goods because texture, plasticity, and other physical properties depend on the polymorphic forms that develop during food processing. The different crystalline forms that a fat can assume are known as α, β′, and β, which differ in melting point and crystallographic properties. The β form is the most stable; however, the β′ form is generally preferred because it consists of a fine crystalline network that imparts plasticity and excellent creaming properties, i.e., the ability to incorporate air when the fat is whipped with sugar. A well-plasticized fat exhibits excellent creaming properties, giving the product a lighter and airier texture. The β′ crystalline form is influenced by several factors, including palmitic acid content, as well as the distribution and position of palmitic and stearic acids within the triglyceride. It is known that palm oil tends to form β′ crystals, while coconut and palm kernel oils tend to form β crystals.

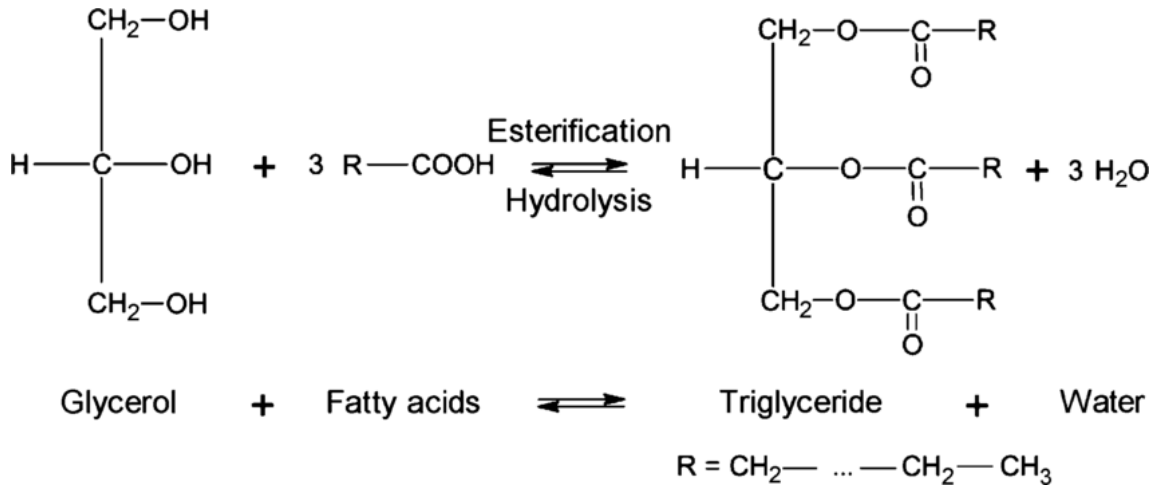

Figure 1.10. Triglycerides formation and structure.

Phospholipids

Phospholipids tend to have unique roles in food systems due to their amphiphilic structure, which comprises both hydrophilic and hydrophobic ends. This dual characteristic makes phospholipids essential as natural emulsifiers, facilitating the formation and stabilization of emulsions in food products such as mayonnaise, dressings, and margarine. In these products, phospholipids contribute to the texture, mouthfeel, and overall sensory experience of the food. Beyond their functional properties, phospholipids are also crucial in modulating the nutritional profile of food products. For instance, they are key components of cell membranes and are involved in various biological processes, including lipid metabolism and cell signalling. Additionally, phospholipids contribute to the bioavailability of fat-soluble nutrients and are recognized for their potential health benefits, such as improving liver function and supporting cognitive health. In the context of food processing, phospholipids can also impact product stability, influencing factors such as oxidation and shelf life. Their role in preventing lipid oxidation is particularly important, as it helps maintain the quality and safety of lipid-rich foods by minimizing rancidity and the development of off-flavours (Asokapandian, Sreelakshmi and Rajamanickam, 2021; Habeeb *et al.,* 2024).

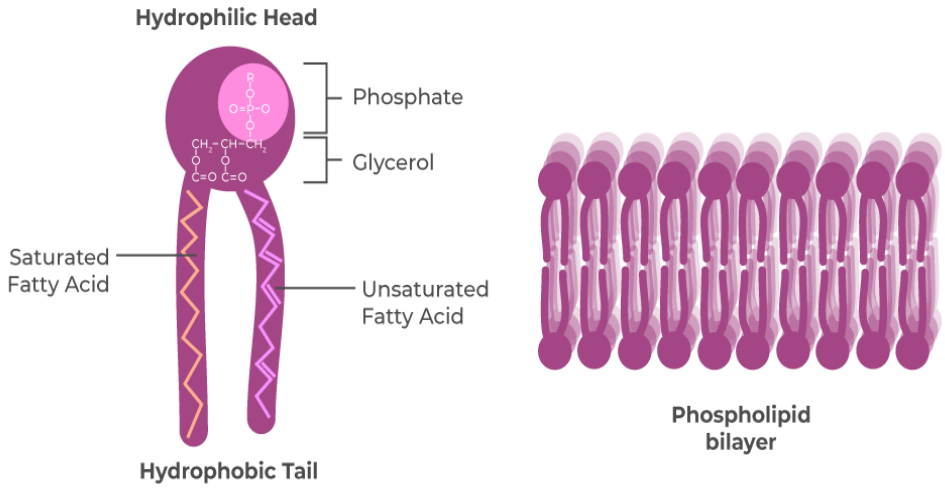

Figure 1.11. Phospholipid structure and arrangement, the latter gives form to the cytoplasmatic membrane common to most of the living organisms.

Sterols

Sterols, particularly cholesterol and phytosterols, are integral components of food systems, primarily due to their structural and functional roles in biological membranes, in general their structure is characterized by a complex ring structure derived from squalene, consisting in a tetracyclic cyclopenta[α]-phenanthrene structure with a hydroxyl group at C-3 and a flexible side chain with 8–10 carbons at C-17. In food, sterols contribute to the stability and integrity of cell membranes, impacting the texture and physical properties of various products, especially those derived from animal sources, such as dairy and meat. Cholesterol, the most well-known sterol in animal-based foods, is crucial for maintaining membrane fluidity and permeability, which can influence the texture and mouthfeel of food products (Li, Liu and Guo, 2024). Phytosterols, the plant-based counterparts to cholesterol, are increasingly recognized for their health benefits, particularly their ability to lower Low-density lipoprotein (LDL) cholesterol levels when incorporated into the diet (Aguchem *et al.,* 2024). This has led to the fortification of various food products, such as margarine and dairy alternatives, with phytosterols to enhance their nutritional profile and provide cardiovascular benefits. In food systems, sterols also play a role in the emulsification process, contributing to the formation and stabilization of emulsions, similar to phospholipids. Furthermore, sterols have antioxidant properties that can protect lipid-containing foods from oxidation, thus extending shelf life and preserving quality. The interaction of sterols with other lipids and their influence on the crystallization process can also affect the consistency and sensory attributes of food products.

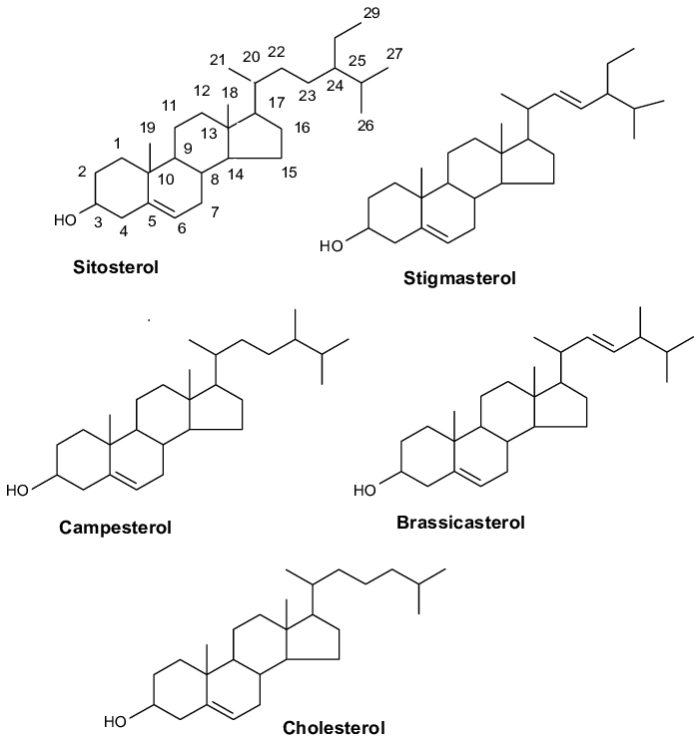

Figure 1.12. Different kinds of phytosterols, the multiple ring structure, coupled with the presence of multiple π-bonds and a hydroxylic tail leads to molecules with good antioxidant activity and emulsifying properties.

The reactions of the lipid fraction

Lipids can undergo various reactions depending on the conditions of humidity, temperature, presence of ion metals and so on. Their chemical structure leads to molecules that have high reaction rates, leading to a plethora of molecules obtainable by a single lipid. This characteristic is why lipids are a crucial parameters and protagonists of the aroma of food products, the wide variety of molecules that originate from their reactions are normally low molecular weight molecules, aldehydes, ketons, terpenes, alkanes, alkenes and organic acids, characterized by a low threshold of sensitivity by the human senses. These molecules are somewhat appreciated in certain foods, because are signals of fermentation processes and/or of a successful aging process (aged cheeses, fish fermented products, dried stockfish etc…). However, in the specific case of food lipids in fresh products, the molecules produced tend to develop off-flavours and specific defects, not compatible with the nature and sensorial profile of the food: in vegetable oils the aldehydes and ketons produced lead to “greasy”, “rancid” and “pungent” aromas, and the mouthfeel of said products tends to be ruined by the same molecules; in baked products, in which a strong thermal stress is applied to the lipids in the formulation, the same problem can concur, and moreover the

structure and texture of the goods could be hindered by the morphism and reactions of the lipids. By all these means is necessary to have a deep understanding on the reactions that the lipid fraction can undergo, giving a particular focus on the oxidation processes, which are one of the key factors of baked goods shelf-life.

Hydrolytic and Oxidative rancidity

Lipids undergo changes in their chemical composition over time. Rancidity can be categorized into two types: hydrolytic and oxidative.

- **Hydrolytic Rancidity**: This alteration occurs in the absence of O_2. The reaction leads to the hydrolysis of triglycerides and the subsequent release of glycerol and short-chain fatty acids. The release of volatile fatty acids (e.g., butyric, caproic, and caprylic acids) with a low number of carbon atoms results in the development of unpleasant odours and flavours, thereby reducing the food's shelf life (Figure 13).

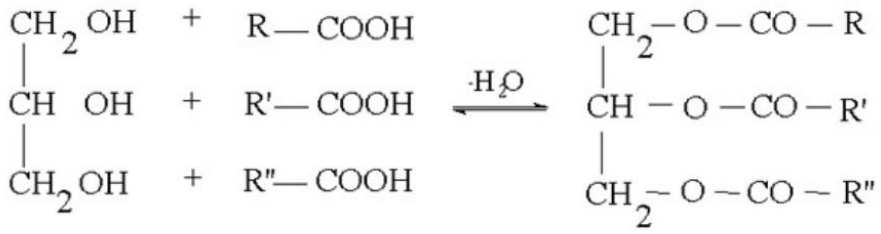

Figure 1.13: Hydrolytic rancidity reaction.

This alteration is promoted by the presence of moisture, light, and heat, and is caused by the action of endogenous enzymes known as lipases. These enzymes catalyse the oxidation of unsaturated fats, producing peroxides and thermolabile compounds that survive the cooking process (Smith *et al.,* 2004).

- **Oxidative Rancidity**: This type of reaction is caused by the exposure of fats to oxygen in the air and is an autocatalytic radical reaction where oxygen is the primary agent responsible for the chemical oxidation of fats. The reaction is facilitated by the presence of pro-oxidant agents such as light, metal ions (iron, copper, and manganese), high temperatures, and the degree of unsaturation of fatty acids. The matrix on which oxygen acts determines the reactivity of the oxidation. Fats with a high degree of unsaturation, rich in oleic, linoleic, and linolenic acids, degrade more easily compared to hydrogenated fats. Reducing the degree of unsaturation of fats significantly increases the food's shelf life at the expense of its nutritional properties (Capella, 1997).

The general mechanism of lipid oxidation involves three phases (Figure 14):

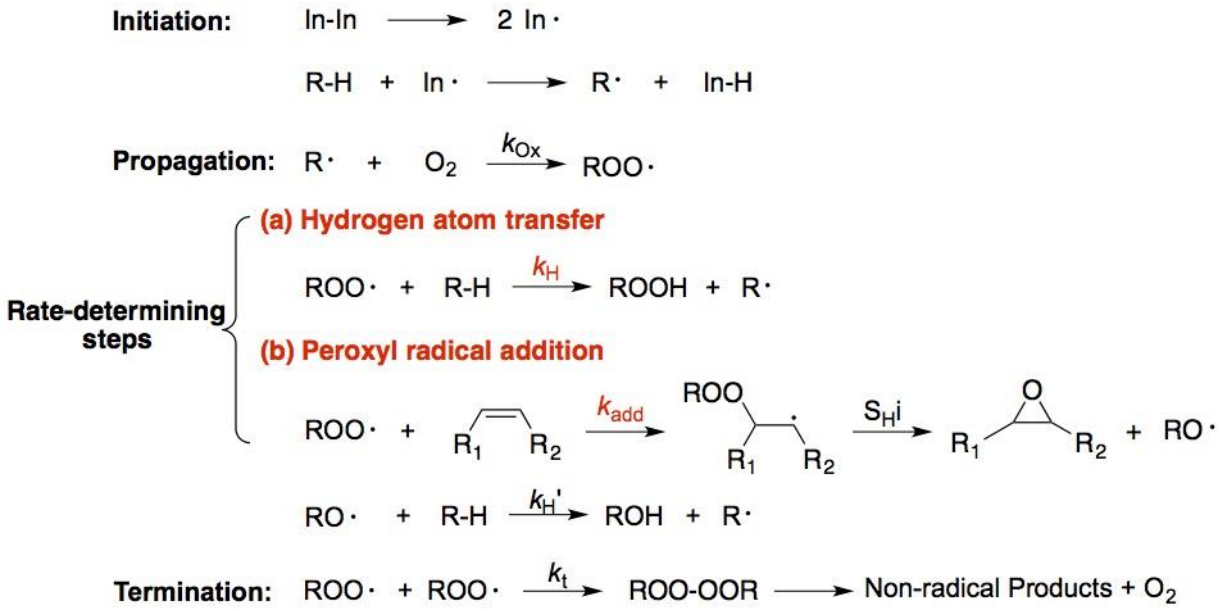

Figure 1.14: Initiation, propagation, and termination of lipid autoxidation.

In the initiation phase, a free radical (R•) is formed following the detachment of a hydrogen atom from a fatty acid. The formation of free radicals increases with the degree of unsaturation of fatty acids. Light is the primary factor triggering autoxidation. The propagation phase follows, which is divided into two sub-phases: in the unimolecular propagation phase, atmospheric oxygen reacts with free radicals to form peroxides (ROO•), the primary compounds of the oxidative process. These highly unstable compounds react with a new unsaturated fatty acid (RH) in the vicinity, leading to the formation of a hydroperoxide (ROOH) and a new free radical (R•). In this phase, the reaction rate is slow, and to preserve the food's shelf life, it is crucial to prolong this phase as much as possible. In the bimolecular propagation phase, the reaction rate increases exponentially, triggered when the concentration of hydroperoxides is sufficiently high for them to react with each other, forming peroxyl radicals (ROO•), alkoxyl radicals (RO•), and water. As the reaction progresses and hydroperoxides accumulate, they transform into secondary products such as aldehydes, ketones, and alcohols. Oxidation tends to terminate when polymerization reactions occur between different radicals, by which point the food product has already deteriorated beyond acceptable limits. The oxidation reaction evolves differently depending on the degree of unsaturation of the fatty acid. In monounsaturated fats, the detachment of a hydrogen atom does not occur at the double bond but at the two adjacent carbons, namely the Cα (e.g., either C8 or C11) (Figure 15).

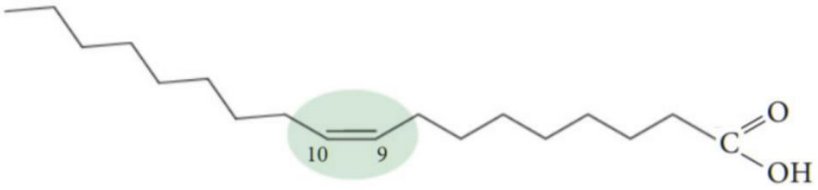

Figure 1.15: Chemical structure of oleic acid.

Following radicalization, the Cα carbon undergoes sp^2 hybridization, creating a resonance system that allows the unpaired electron's charge to be distributed. Oxygen can attack both radical ends, leading to the formation of four hydroperoxides with cis and trans configurations. As the number of double bonds in polyunsaturated fats increases, the reaction tends to be faster because of the greater ability to distribute the electron across multiple resonance forms, thereby stabilizing the resulting molecule and making the reaction more energetically favourable (Chaiyasit *et al.,* 2007; Barden and Decker, 2016) (Figure 16).

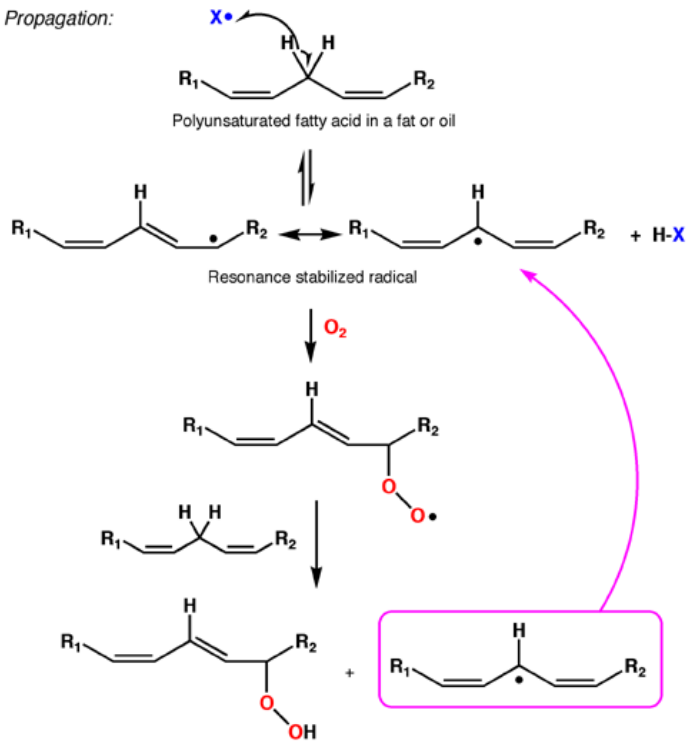

Figure 1.16. Visual representation of the resonance stability that polyunsaturated fatty acids have in case of oxidation reaction. It is estimated that the reaction rate of these fatty acids is 10 times higher than monounsaturated fatty acids. (Picture taken from the "Organic chemistry course 320N" of Iverson University of Texas at Austin, Spring 2025).

Products of lipid rancidity and oxidation

All chemical changes related to oils and fats subjected to high temperatures promote phenomena such as oxidation, hydrolysis, polymerization, isomerization, or cyclization. These reactions, in turn, influence the sensory, nutritional, and safety properties of oils. Moreover, these mechanisms can be triggered by various factors, including the presence of oxygen, moisture, trace metals, and free radicals, as well as other factors such as air contact, oil unsaturation levels, presence of oxidants and antioxidants, in addition to temperature and the duration of thermal treatment. Oxidation is the most significant cause of deterioration in oils and fats (Butinar, 2008). In the following paragraph a list of the principal oxidation products will be presented, giving a particular focus on the sensorial and quality influences that these molecules have on the food product.

Peroxides

Peroxides are compounds in which oxygen uses one of its two electrons to bond with another oxygen atom. They are the primary products of lipid oxidation, formed by the action of oxygen and specific enzymes present in the fruit, such as lipoxygenases, which, upon contact with oil due to cellular damage, oxidize fatty acids. Additionally, peroxides can further degrade into secondary oxidative products, such as alcohols, aldehydes, and ketones (Manzocco *et al.,* 2020). The production and decomposition of peroxides are influenced by various factors, such as oxygen, the composition of fatty acids, and temperature. A large amount of oxygen can be dissolved in the oil when the partial pressure of oxygen in the headspace of the packaging is high, and the oxidation of the oil increases with the concentration of soluble oxygen. Furthermore, metallic catalysts can accelerate their decomposition (Zhang *et al.,* 2021). Peroxides are odourless and tasteless, making them imperceptible at the organoleptic level, but being very unstable, they decompose easily, leading to the formation of aldehydes and ketones, which are responsible for rancid off-flavours. A high peroxide value indicates an already initiated, irreversible oxidation process. The peroxide content, expressed as the number of milliequivalents of oxygen per kilogram of oil (meq O_2/kg of oil), indicates the degree of primary oxidation of the oil, i.e., its tendency to become rancid (Manzocco *et al.,* 2020). In fact, in many studies, this index is evaluated to monitor the chemical and sensory quality of lipid-rich products, especially during food storage (Calligaris *et al.,* 2008). In the finished product, the concentration of peroxides can also depend by the level of native peroxides already present in the oil or fat used as an ingredient. The evolution of these products is driven by the interactions of phenomena occurring during the dough's cooking and processing, such as the availability of oxygen during leavening and the cooking temperature. For this reason, it is important to use oil with a low degree of oxidation. However, when the lipid ingredient has a high level

of oxidation, a considerable number of primary and secondary products accumulate in the matrix (Manzocco *et al.,* 2020).

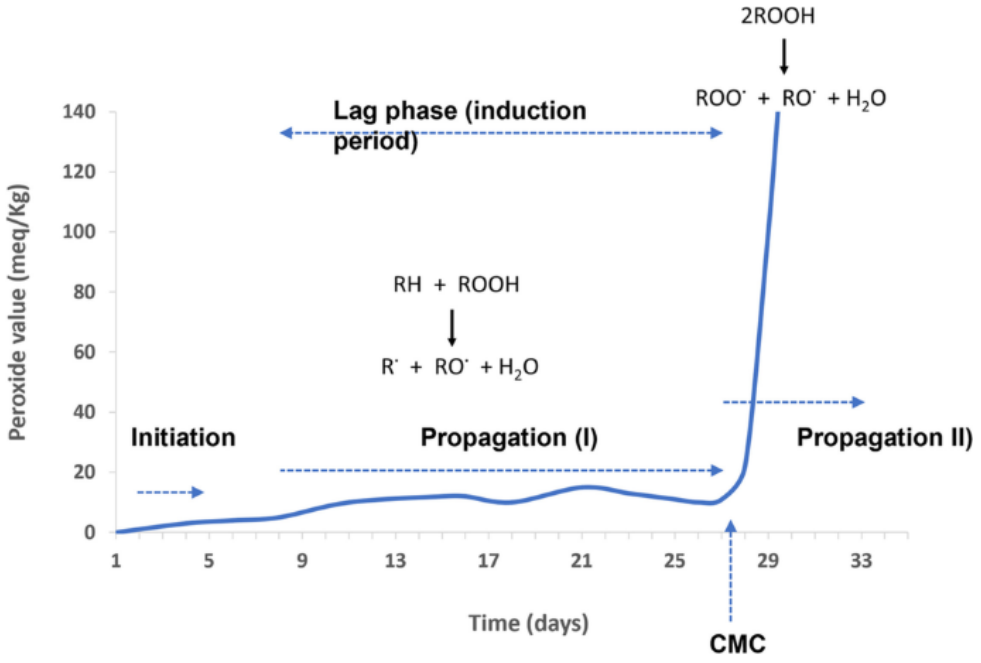

Figure 1.17. An example of how the concentration of peroxides increases exponentially after the first phases of initiation and propagation (Musakhanian, Rodier and Dave, 2022).

Aldehydes

Aldehydes are among the most important volatile compounds that form during the oxidative degradation of lipids, significantly influencing the sensory quality of food products. Chemically, aldehydes are characterized by the presence of a carbonyl group (C=O) attached to a hydrogen atom, making them highly reactive. This reactivity is due to the polarity of the carbonyl group, which can easily undergo further reactions, such as polymerization or interaction with other food components, thus influencing the overall stability and quality of the food product. In the context of lipid oxidation, the aldehydes formed are often aliphatic, with chain lengths typically ranging from C3 to C9. The degree of unsaturation and the position of the double bonds in the original fatty acids influence the structure and type of aldehyde formed. Their formation is primarily driven by the oxidative processes involving unsaturated fatty acids, particularly polyunsaturated fatty acids (PUFAs). The mechanism of aldehyde formation is linked to the kinetics of hydroperoxide demolition, in which under the influence of heat, light, or catalytic metals, these hydroperoxides decompose into secondary oxidation products, including a variety of aldehydes (Frankel, 2012). Lipid hydroperoxides (ROOH) are relatively unstable, especially under conditions of heat, light, or in the presence of transition metal ions such as iron (Fe^{2+}) or copper (Cu^{+}). These conditions

can catalyse the homolytic cleavage of the O-O bond in the hydroperoxide, leading to the formation of alkoxyl radicals (RO•) and hydroxyl radicals (HO•). The decomposition of hydroperoxides can proceed via two primary pathways:

Homolytic Cleavage (Type I Cleavage):

In this pathway, the O-O bond in the hydroperoxide breaks homolytically, producing an alkoxyl radical (RO•) and a hydroxyl radical (HO•). The alkoxyl radical is highly reactive and can undergo β-scission, a process where the bond adjacent to the carbon bearing the oxygen radical is cleaved. This cleavage leads to the formation of smaller, more volatile molecules, including aldehydes (Frankel, 2012).

Heterolytic Cleavage (Type II Cleavage):

In this pathway, the hydroperoxide can undergo heterolytic cleavage, typically catalyzed by acids or certain metal ions, leading to the formation of an alcohol and an alkoxyl radical. This alkoxyl radical can also undergo β-scission, forming aldehydes or ketones depending on the position of the cleavage (Frankel, 2012).

Aldehydes play a crucial role in determining the sensory profile of food products. Hexanal, for example, is a six-carbon aldehyde that is commonly formed from the oxidation of linoleic acid, a predominant fatty acid in many vegetable oils. Hexanal is associated with a characteristic "green" or "grassy" odour, which, in moderate concentrations, can contribute to the fresh and pleasant aroma of products such as freshly cut grass or unprocessed vegetables. However, as lipid oxidation progresses, the accumulation of hexanal and other aldehydes can lead to the development of off-flavours described as "greasy" or "rancid," particularly in vegetable oils (Zhang *et al.,* 2021). These off-flavours are detrimental to the sensory quality of food products, reducing consumer acceptability and indicating a decline in product freshness and stability. The impact of aldehydes on the sensory properties of food is not limited to hexanal. Other aldehydes such as pentanal, heptanal, nonanal, and 2,4-decadienal also contribute to the complex flavour profiles of oxidized lipids (Orsavova *et al.,* 2015). Pentanal, for instance, imparts a sweet and nutty aroma, while nonanal is known for its citrus-like scent. 2,4-Decadienal, on the other hand, is associated with deep-fried flavours, commonly found in oxidized oils and fried foods. In food systems, the most relevant aldehydes include hexanal, as previously mentioned, along with propanal, butanal, pentanal, heptanal, octanal, nonanal, 2-hexenal, and 2,4-decadienal. These aldehydes are frequently monitored in food analysis as indicators of lipid oxidation and shelf-life stability, and their formation is influenced by factors such as the degree of

unsaturation in the lipid content, the presence of pro-oxidants like metal ions, and environmental conditions such as temperature and exposure to light.

Ketones

Ketones represent another class of secondary oxidation products that arise from the degradation of unsaturated fatty acids, playing a pivotal role in the sensory deterioration and, to a lesser extent, the nutritional quality of food lipids. Chemically, ketones are characterized by the presence of a carbonyl group (C=O) attached to two alkyl or aryl groups, which differentiates them from aldehydes that have at least one hydrogen atom bonded to the carbonyl carbon. This structural configuration grants ketones relatively greater stability in comparison to aldehydes and other oxidation products. The formation of ketones is primarily driven by the autoxidation of polyunsaturated fatty acids, with linoleic acid (C18:2n-6) being one of the most prominent precursors due to its abundance in many vegetable oils and processed foods (Kiralan *et al.,* 2019). The autoxidation process is initiated by the abstraction of a hydrogen atom from a bis-allylic methylene group in linoleic acid, leading to the formation of a lipid radical. This radical then reacts with molecular oxygen to form a lipid peroxyl radical, which, in turn, generates lipid hydroperoxides (LOOH) through propagation reactions, the hydroperoxides then undergo homolytic cleavage to form alkoxy radicals (RO•), which subsequently break down into smaller volatile compounds, such as methyl ketones and cyclic ketones. In the context of linoleic acid oxidation, the most representative ketones formed include 2-pentanone, 2-heptanone, and 3-octen-2-one. These molecules are notable for their contributions to undesirable sensory characteristics in foods. For example, 2-pentanone and 2-heptanone are associated with fruity, green, or solvent-like odours, while 3-octen-2-one contributes metallic and mushroom-like notes (ben Hammouda *et al.,* 2017). The formation of such volatile ketones is a critical factor in the overall quality degradation of foods, particularly in terms of flavours and aroma profiles. From a nutritional standpoint, ketones are generally considered inert and do not have a direct impact on the essential nutritional properties of lipids, such as their role in energy provision or essential fatty acid content. However, the formation of ketones and other oxidation products is often indicative of the broader degradation of lipid structures, which may result in a reduction of the overall nutritional value of the food product due to the destruction of essential fatty acids and fat-soluble vitamins (e.g., vitamins A and E).The most commonly encountered ketones in food lipids are methyl ketones, particularly those with an odd number of carbon atoms, such as 2-nonanone and 2-decanone, which are known for their distinctive soapy, waxy, or fruity odours. These ketones are especially prevalent in the oxidation of dairy fats, such as butter and cream, where they contribute to the

off-flavours characteristic of spoiled or oxidized fat. Additionally, ketones like 3-octen-2-one are found in oxidized vegetable oils and fried foods, further influencing their flavour deterioration.

Alcohols

Alcohols are oxidation products formed through the breakdown of polyunsaturated fatty acids (PUFAs), chemically, they are characterized by the presence of a hydroxyl group (-OH) attached to a saturated carbon atom, which makes them relatively more polar and less volatile than other oxidation products such as aldehydes and ketones. Alcohols formed during lipid oxidation can be primary, secondary, or tertiary, depending on the position of the hydroxyl group within the carbon chain. The formation of alcohols typically follows the initial stages of autoxidation, where fatty acids are subjected to oxidative stress, leading to the generation of lipid hydroperoxides. These lipid hydroperoxides can undergo a range of decomposition reactions, including reduction, which leads to the formation of alcohols. Specifically, peroxyl radicals (ROO•) formed during the oxidation of unsaturated fatty acids can be reduced to hydroperoxides (ROOH), which are then reduced further by antioxidants or enzymatic activity into alcohols (Abdelhedi *et al.*, 2016). The type and structure of alcohols formed depend on the nature of the fatty acid substrate, with common examples being 1-pentanol, 1-hexanol, and 2-butanol derived from the oxidation of fatty acids such as linoleic, linolenic, and arachidonic acids. From a nutritional perspective, alcohols are generally inert and do not directly affect the essential nutritional properties of food lipids. Sensory changes associated with alcohols are particularly notable, as these compounds contribute to both pleasant and unpleasant aroma profiles, depending on their concentration and type. Low-molecular-weight alcohols, such as 1-pentanol and 1-hexanol, are commonly found in oxidized oils and fats, imparting “grassy”, “green”, or “fruity” aromas. In small amounts, these compounds may contribute positively to the overall flavour complexity of certain foods, but in higher concentrations, they are often associated with off-flavours that signal the onset of rancidity. Secondary alcohols, such as 2-butanol, formed from the reduction of secondary hydroperoxides, can add musty or earthy notes to the sensory profile of oxidized fats. Their presence is often linked with advanced lipid degradation, particularly in fried foods, processed meats, and dairy products. Among the most representative alcohols in lipid oxidation are 1-octen-3-ol and 2-heptanol. 1-octen-3-ol, often referred to as the "mushroom alcohol," is derived from the oxidation of linoleic acid and is known for its strong, mushroom-like aroma, which is frequently associated with rancid fats (Zhang *et al.*, 2025). It is a key marker in the detection of oxidation in oils, especially in products with high PUFA content. Similarly, 2-heptanol,

formed during the oxidation of heptanal, contributes sharp, pungent notes that further degrade the sensory qualities of oxidized fats and oils.

Organic acids

Organic acids, such as caprylic (C8), butyric (C4), and hexanoic (C6) acids are short- to medium-chain fatty acids that are commonly formed during the degradation of lipids in food matrices. Chemically, these acids are carboxylic acids with varying aliphatic chain lengths, where the carboxyl group (-COOH) is attached to a hydrocarbon chain. Their formation in foods primarily occurs through lipolysis, where triglycerides are hydrolysed into free fatty acids and glycerol by the action of lipases, these processes can be enzymatically driven or initiated by heat, oxygen, and light, which can induce oxidation and breakdown of the lipids. Lipid peroxidation also contributes to the formation of these organic acids, particularly through β-oxidation and autoxidation pathways that fragment longer-chain fatty acids into volatile, shorter-chain acids. The kinetics of their formation is influenced by several factors, including the availability of unsaturated fats, oxygen, water activity, and temperature (Frankel, 2012). Enzymatic activity, such as that of microbial lipases in fermented or aged foods, can also result in significant accumulation of organic acids. From a sensory perspective, these organic acids are critical contributors to the aroma and flavour profile of foods, especially baked goods and vegetable oils. For instance, butyric acid imparts a strong, rancid, and cheesy aroma, which can be desirable in certain fermented or aged products but is generally considered an off-flavour in baked products and fresh vegetable oils. Hexanoic and caprylic acids, while less perceptible than butyric acid, also exhibit distinct fatty, sour, and sometimes “goaty” odours. In baking, the controlled formation of these acids can contribute to the complexity of the aroma, but excessive production typically results in undesirable rancidity.

Minor compounds

Given the high instability of the oxidation products, and also the high amount of possible paths in which oxidation processes can undergo and proceed into there are several minor volatile organic compounds (VOCs) such as terpenes, alkanes, and alkenes often released during the last phases of the reactions, which contribute significantly to the overall aroma and flavour profile of foods. These compounds tend to originate from the oxidative degradation of unsaturated fatty acids, particularly polyunsaturated fatty acids (PUFAs). Terpenes, a class of hydrocarbons derived from isoprene units, are typically present in trace amounts in vegetable oils and certain baked goods due to their presence in plant materials. Their volatility and ability to interact with lipid matrices mean that they are often produced during heat processing or

oxidative stress. Chemically, terpenes consist of repeating isoprene units, which can undergo isomerization and oxidation, generating volatile fragments that impart citrus, floral, or pine-like aromas depending on their structure. Alkanes, which are saturated hydrocarbons with the general formula CnH2n+2, and alkenes, their unsaturated counterparts with one or more double bonds (CnH2n), are produced as a result of lipid peroxidation and the cleavage of fatty acids. These compounds are particularly formed during thermal degradation, where high temperatures facilitate the breakdown of long-chain fatty acids through scission reactions, releasing smaller, volatile hydrocarbon fragments. For example, the oxidative breakdown of linoleic or linolenic acids can yield various alkanes and alkenes through β-scission or homolytic cleavage mechanisms. Alkanes, being chemically inert and relatively odourless, contribute less directly to the aroma of foods. However, in baked products and oils, their presence can indicate advanced stages of lipid oxidation, where substantial degradation has occurred. Alkenes, due to their unsaturated nature, are more reactive and can undergo further oxidation, potentially forming secondary volatile compounds like aldehydes and ketones that impart a more pronounced flavour impact. In terms of aroma contribution, terpenes are known for their distinct and often desirable sensory qualities in foods, contributing to fresh, floral, or herbal notes, particularly in products like olive oil, breads, cheeses and others. On the other hand, the presence of alkanes and alkenes generally signals the onset of rancidity and off-flavours, with the latter exhibiting slightly more reactivity and a potential to develop undesirable oxidative by-products. In vegetable oils and baked products, these VOCs often signal the decline in product quality, as their formation is closely associated with oxidative spoilage and loss of freshness.

Conventional analyses for the evaluation of lipid oxidation

Given the high heterogeneity of the molecules obtainable from the different oxidation pathways, it's easy to understand how the analysis of the oxidation products tend to be diverse. Normally most of the molecules researched are identifiable by chromatographic systems, in which the separation and identification of said products are normally imputable to different weights and polarity. Examples of these analyses are found in the identification of the mono and di-glycerides (MAG and DAG), these compounds are normally obtained by the enzymatic demolition of triglycerides, and tend to give to the food product various off-flavours; over the years the methods used ranged from thin-layer chromatography (Privett and Blank, 1961), passing through standard gas-chromatographic systems (Firestone, 1994). Other parameters identifiable through chromatography are the volatile compounds (VOCs), such as aldehydes and ketones, that are normally identified and quantified through the usage of mass

spectrometry, focusing on the headspace of the matrices (Noshad, Behbahani and Karabagias, 2023; Cai *et al.,* 2025; Chang *et al.,* 2025). Certain parameters and molecules are not easily identifiable in chromatographic systems, given their high thermal instability. That is the case for compounds such as peroxides and hydroperoxides, for these molecules were in fact constituted methods that evaluated general parameters imputable to the presence of said molecules, such as the Peroxide Value (PV), in which the peroxides react with specific reagents, such as iodine solution and atomic Fe^{2+} to create coloured chemical complexes that can be identified by bare eye (Shantha and Decker, 1994; *ISO 6885:2016(en), Animal and vegetable fats and oils — Determination of anisidine value*)

e. Non-destructive analyses

The necessity of non-destructive analyses nowadays

With the advent of more sustainable approaches on the food productive chain comes also the necessity for the development of more sustainable analytic procedures. Chemical analyses and extractions of analytic compounds, such as the lipid fraction, have always used methods based on the usage of strong, volatile solvents: hexane, chloroform, dichloromethane, pyridine and diazomethane are a few of the molecules that, during the period ranging from the 1960s to the 1980s represented most of the reagents used chemical labs. However, the situation, with the advent of studies on the effects that such substances have on the organism, highlighted how dangerous can they be. Hexane is neurotoxic, with prolonged exposure leading to peripheral neuropathy; it is also highly flammable, posing fire risks in laboratory settings (Issever *et al.,* 2002). Chloroform is a suspected carcinogen and hepatotoxin, with inhalation potentially causing central nervous system depression and cardiac arrhythmias (‘Public Health Goals for Trihalomethanes in Drinking Water’, 2020). Dichloromethane, or methylene chloride, is metabolized to carbon monoxide, which can lead to carbon monoxide poisoning; it is also a potential carcinogen and may cause optic neuropathy and hepatitis upon acute exposure (*CDC - NIOSH Pocket Guide to Chemical Hazards - Methylene chloride*, no date). Pyridine is toxic and flammable, with exposure resulting in symptoms such as dizziness, headache, and nausea; chronic exposure may lead to liver, heart, and kidney damage, and it is classified as possibly carcinogenic to humans. With these cases, representing some of the many, found in literature, extensive research has been conducted during the years ranging from the 1990s to the recent years in order

to develop less dangerous methods, and to also avoid cases of chronic pollution, derived from the high impact that these substances have on the environment and their difficulty in the disposal. These necessities went also hand in hand with the need for quicker and simpler procedures, being the chemical analyses, often, the quality control instrument for most of the food, pharmaceutical and steelwork industries. In this Phd thesis the focus will be specifically put on the Near-Infrared Spectroscopy (NIR) and its application in the food industry.

Near-Infrared (NIR) spectroscopy

Near-Infrared Spectroscopy (NIR) is an analytical technique that measures the absorption of near-infrared light by molecular bonds, particularly those involving hydrogen, such as O-H, N-H, and C-H groups (Jr, Workman and Weyer, 2007). This absorption results from overtones and combinations of fundamental vibrations, providing information about molecular composition and structure. The origins of NIR trace back to the 1960s, when Karl Norris and colleagues at the U.S. Department of Agriculture pioneered its application for assessing the quality of agricultural products. Their work demonstrated the potential of NIRS for rapid, non-destructive analysis, laying the groundwork for its broader adoption (Williams and Norris, 2001). Historically, NIR has been deployed across various fields. In agriculture, it has been utilized for determining moisture, protein, and fat content in grains and other commodities. In the pharmaceutical industry, NIRS serves in quality control and process monitoring. Additionally, it has applications in medical diagnostics, such as monitoring tissue oxygenation and cerebral hemodynamic. Typically, in NIR system wavelengths between 780 nm and 2500 nm are emitted to the samples, that can be in liquid, powder or solid form, then, the molecular bonds, primarily those involving hydrogen atoms such as C-H, O-H, and N-H tend to absorb some of the radiation given. This absorption arises from overtone and combination vibrations of these bonds. NIR instruments typically consist of a light source, Typically, a tungsten-halogen lamp, providing a stable and broad spectrum of NIR radiation.; a sample holder; a wavelength selection system (such as a monochromator or interferometer), and a detector, like InGaAs (indium gallium arsenide) or PbS (lead sulfide) detectors, which are sensitive to NIR wavelengths and convert the absorbed light into an electrical signal; a wavelength selector, either a monochromator (grating-based) or an interferometer (as in Fourier-transform NIR systems) to isolate specific NIR wavelengths; and then a computer, equipped with specific software used for data acquisition, processing, and interpretation through chemometric algorithms. The choice of measurement mode transmission, reflectance, or diffuse reflectance depends on the sample's physical

characteristics and the specific analytical requirements. It is possible to divide the workflow of these kind of instruments in four distinct phases:

Illumination:

The NIR light source directs radiation onto the sample.

Interaction:

Depending on the mode (transmission, reflectance, or diffuse reflectance), the light interacts with the sample, with some wavelengths being absorbed while others are transmitted or reflected.

Detection:

The detector captures the light that emerges from or is reflected by the sample, converting it into an electronic signal.

Data Processing:

The signal is analysed to create a spectrum, which represents the sample's molecular composition and structure. Advanced chemometric methods enhance the interpretation of complex spectra. Modern Near-Infrared Spectroscopy instruments are designed to accommodate various measurement techniques, including transmission, reflectance, and diffuse reflectance, to analyse diverse sample types effectively.

Figure 1.18. Example of NIR system, specifically, a NIR-Flex Solids N-500 (BUCHI, Switzerland). The instruments always include a package of installation to be implemented on PC, to register and elaborate the data acquired through reading of samples.

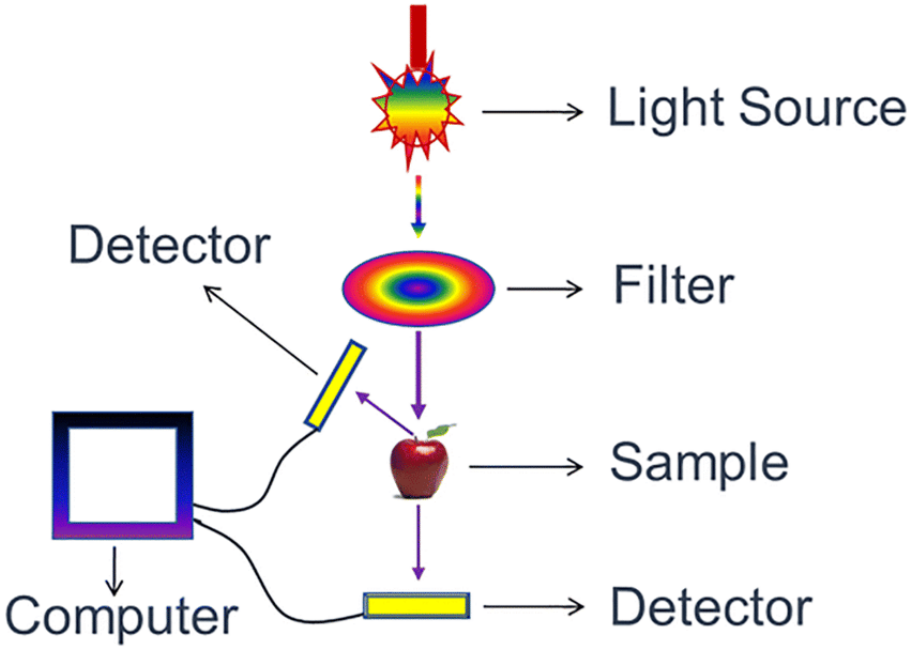

Figure 1.19. Schematic workflow of NIR instrument (Chandrasekaran et al., 2019).

Diverse types of instruments have been developed over the years, this was relative to not only increase the range and number of scans that could be possibly administered to the samples, but also in relation to the possibility of using the NIR systems to a plethora of diverse samples, different in each kind of chemical, physical and structural differences. Most of these machines were first developed to simply measure the quantity of radiation that a sample absorbed at a specific range, but, over time, different kind of alternatives were created. In the following pages some examples of differences between various NIR systems are reported.

Transmission:

In this configuration, the most classical one, NIR light passes through a sample, and the transmitted light is measured. It is the classical application of NIR instruments and tends to be the most adopted one in clear or liquid samples or thin films where the light can traverse the material with minimal scattering (Jr, Workman and Weyer, 2007).

Transflectance:

Reflectance measurements involve directing NIR light onto a sample's surface and detecting the light reflected back. This technique is advantageous for analysing opaque or solid samples where transmission is not feasible. The reflectance data can reveal surface properties and, to some extent, subsurface information, depending on the material's optical characteristics.

Diffuse Reflectance:

Diffuse reflectance spectroscopy is employed for samples with rough or particulate surfaces, such as powders or granular materials. In this mode, incident NIR light penetrates the sample, undergoing multiple scattering events before being diffusely reflected. The collected diffusely reflected light contains information about both surface and bulk properties of the sample. This technique is particularly useful for analysing heterogeneous materials without extensive sample preparation.

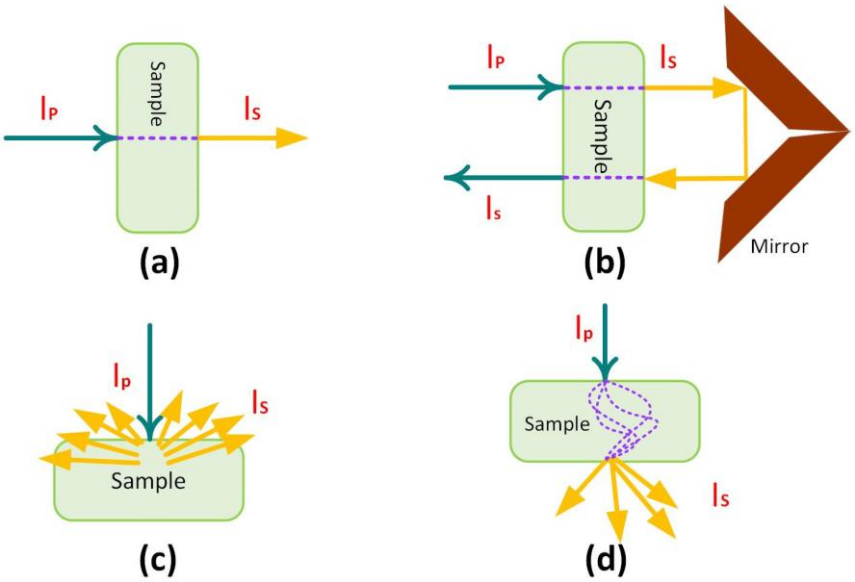

Figure 1.20. (a) Transmittance measurement mode, which is used with gases and semi-solids placed in a cuvette; (b) transflectance measurement mode, which is used with semi-solids without a cuvette; (c) diffuse reflectance measurement mode, which is used with solids where the measurement is taken from the NIR incidence; (d) transmittance through a scattering medium (*Zhang et al., 2022*).

Limits of the Near-Infrared Spectroscopy

By configuring different kinds of radiance emitted, number of scans made for each wavelength, and by modelling the type of detection, relative to the characteristics of the sample is possible to analyse a wide kind of samples. However, there are some limits to the NIR instruments and analyses, NIR absorption bands are typically

overtones and combinations of fundamental vibrations, resulting in lower molar absorptivity compared to mid-infrared regions (Ferrari, Mottola and Quaresima, 2004). This leads to weaker absorption signals, making NIRS less sensitive and potentially less accurate for detecting low-concentration analytes. Also, The NIR regions often exhibits broad and overlapping absorption bands, complicating the interpretation of spectra. This complexity necessitates advanced chemometric techniques and robust calibration models to accurately analyse and quantify components. Variations in sample temperature, particle size, and physical state can significantly impact NIR spectra, leading to inaccuracies. For instance, moisture content and sample heterogeneity can alter scattering properties, affecting the reliability of NIR measurements. NIR light has limited penetration depth in highly absorbing or scattering materials, restricting its effectiveness for analysing thick or dense samples. This limitation is particularly relevant in biological tissues and certain solid materials. Moreover, developing accurate calibration models requires extensive and representative sample sets. Additionally, NIR instruments may suffer from inter-instrument variability, making it challenging to transfer calibration models between different devices without significant adjustments. And finally, NIR measurements can be affected by environmental factors such as ambient light, humidity, and temperature fluctuations. These factors can introduce noise and variability into the spectra, necessitating controlled measurement conditions (Ferrari, Mottola and Quaresima, 2004). It is important to note that NIR analysi are certainly an appreciated novelty in the food analysis panorama, but they are not specific, NIR analysis are useful and precise as much as the reference analysis are, they work on prediction systems, and as such need to have consolidate data on which it’s possible to build good predictive systems.

NIR applications in food industry

Near-Infrared Spectroscopy has become an indispensable analytical tool in the food industry, offering rapid, non-destructive, and cost-effective analysis of various food products. Its applications span from quality control and process monitoring to authentication and adulteration detection, significantly enhancing food safety and quality assurance. NIR is extensively utilized for determining the compositional attributes of food products, including moisture, protein, fat, and carbohydrate content. For instance, in the dairy industry, NIRS facilitates the analysis of milk and cheese, enabling the assessment of fat and protein concentrations, which are critical for product standardization and quality assurance (Fodor *et al.,* 2024). Similarly, in meat processing, NIRS aids in evaluating parameters such as fat content and tenderness, ensuring consistency and adherence to quality standards (Squeo and Amigo, 2023).

The integration of NIR into production lines has allowed real-time monitoring of manufacturing processes. This capability is particularly beneficial in large-scale operations, such as cheese production, where NIRS provides rapid and precise assessments of product quality, facilitating timely adjustments to maintain desired specifications (Nadimi and Paliwal, 2024). In the baking industry, Near-infrared spectroscopy is also employed to monitor dough properties and baking conditions, in order to optimize product quality and consistency. Ensuring the authenticity of food products is paramount in the food industry, given the huge interest on both economic and social aspects of regional specialties and matrices characterized by national origins. In this field NIR systems have proven effective in detecting adulteration and verifying the origin of various food items. For example, it has been applied to authenticate honey by determining its botanical origin and detecting potential adulterants (Fodor *et al.,* 2024). In the case of meat products, NIRS assists in identifying species-specific characteristics, thereby preventing fraudulent practices. Beyond chemical composition, NIR systems are also employed to evaluate the physical properties of food products. In the fruit and vegetable sector, it is used to assess parameters such as ripeness, firmness, and sugar content, aiding in harvest timing and quality grading. For instance, NIR has been utilized to visualize sugar content distribution in white strawberries, facilitating quality assessment (Squeo and Amigo, 2023). NIR offers a non-invasive approach to detecting contaminants and spoilage in food products. In the seafood industry, it has been applied to classify the freshness of mackerels by predicting chemical indicators of spoilage, such as total volatile basic nitrogen and acid values (Squeo and Amigo, 2023). This capability enhances food safety by enabling the early detection of spoilage and contamination. The technique is also valuable for nutritional profiling, allowing for the rapid determination of nutrient content in various food products. This application is particularly relevant in the development of health-oriented food products and in ensuring compliance with nutritional labelling regulations. The widespread adoption of NIR in the food industry is attributed to its numerous advantages, including rapid analysis, minimal sample preparation, and the ability to analyse multiple components simultaneously. However, successful implementation requires careful calibration and validation of NIRS models, considering factors such as sample heterogeneity and environmental conditions that may influence spectral data. In conclusion, Near-Infrared Spectroscopy has established itself as a versatile and efficient analytical method in the food industry. Its applications in quality control, process monitoring, authentication, and safety assurance contribute significantly to the production of high-quality, safe, and authentic food products. Ongoing advancements in NIR technology and chemometric analysis are expected to further enhance its capabilities and expand its applications in the food sector.

2.Objectives

Given the context presented in the previous chapter, the objective of this PhD thesis is to reach 3 goals, all useful to the criticalities nowadays registered in the food industry: in first place to study, characterize and understand mechanics of oxidation, conservation, and physical stress on different kinds of vegetable oils native of the Italian landscape and food industry. This in order to comprehend how they behave in conditions similar to the abuse presented in industrial implants, such as storage in non-refrigerated tanks, transport in outdated and often dirty pipes, or long periods of waiting inside open-air vats. Vegetable oils are of outmost importance when it comes to the food industry, and specifically for the baking industry, where the qualities of the products are strictly related to the correct management of temperatures, dough mixing and fat addition in order to obtain fragrant, soft and chewy products. In fact, as previously presented, Italy is subject to a great interest in the market of baked products, mostly because of national and regional specialties that have unique flavours and shapes. More and more, these specialties tend to see an increase in the offer of competitors on the market, often formulated with poorer ingredients or more "specialized" ones, such as the fractions of coconut, palm and soy oil. The products made with these oils and fats are, as a result, more polluting than those made with local ingredients, by means of import and usage of non-sustainable alternatives. Moreover, to be competitive on the market, most of the local realities have also adopted the usage of import oils and fats, to at least be competitive in terms of price. So, as the second objective of this thesis, the formulation and sensorial-chemical characterization of baked products was conducted; looking specifically at different kinds of savoury and sweet products, such as "frollini" biscuits and "tarallini" formulated with mixes fats and oils of Italian origin that emulate the technological properties of standard lipids used in the industry, such as coconut and palm oil. The second objective will serve both as an assessment to the quality of the oils implemented in the formulations and also as potential new products on the market. Finally, the third objective of this PhD thesis is the development of novelty analyses on vegetable oils, to assess the general chemical quality of them without the usage of dangerous substances and with quicker and more sustainable methods, specifically the implementation of Near-Infrared spectroscopic analyses.

3.Materials and Methods

a. First goal: chemical characterization of 4 different kind of vegetable oils stressed with UV light and mild temperatures

Samples

The oil samples analysed in this research work were acquired partly through direct purchase from local supermarkets (CONAD CITY, Viale Gaspare Finali, 28, 47521 Cesena FC) and include:

- Extra Virgin Olive Oil "Apruntino Pescarese DOP" under the brand "Sapori & Dintorni" by Conad, 0.75 liters (EVOO),
- Grape Seed Oil "Olitalia," 1 liter (GRO),
- Sunflower Seed Oil by Conad, 1 liter (SO),

and partly through direct supply from private sources, specifically:

- High-Oleic Sunflower Oil (HOSO).

After sampling, approximately 10 g of each oil were placed in 100 ml Sovirel bottles with caps, and then heated in an oven at 100°C for various time intervals, namely 0, 30, 60, 90, 120, 180, and 300 minutes.

The same oil samples were also stressed using a UV-light chamber, specifically, a photo-oxidation chamber was designed, consisting of two UV lamps, a reflective structure, and a current transformer.

The two UV lamps, purchased from Stanley Electric Co. (Tokyo, Japan) and belonging to the UV-CCL WH Ass'y TYPE series (model UC/4E165/3), were mounted on the lid of an insulated polystyrene container, as shown in Figures 22 and 23. The lid, along with all internal walls of the container, was lined with aluminium foil to enhance the diffusion of UV light. The lamps were then connected to a current transformer to regulate the power output from the electrical socket (220 V) to the voltage required by the lamp ballasts (24 V). During the setup of the photo-oxidation system, a significant temperature increase was observed due to the radiation emitted by the lamps. Specifically, in the initial tests, the analysed oils reached temperatures exceeding 40°C within the first 20 minutes of treatment. To mitigate this effect, and to prevent oxidative phenomena attributable to thermal stress, refrigerated eutectic plates were placed inside the system. The temperature was monitored using two thermocouples: one in contact with a test oil placed in a test tube and the other in the system's headspace. These thermocouples, connected to an SCC-TC02 multimeter (National Instruments, Assago

(MI), Italy), tracked temperature variations in the product and air for seven hours via the LabVIEW 8.2 software. For each oil sample, 10 ml were placed in transparent 20 ml Pyrex glass test tubes sealed with Sovirel caps. These tubes were loaded into the system in groups of four, with one sample representing each oil type. To prevent direct contact with the eutectic plates, polystyrene supports were inserted to serve as separators between the plates and the test tubes. The samples, enclosed within the oxidation chamber, were then subjected to illumination for varying treatment times, from 0 to 480 minutes, specifically 0, 5, 10, 20, 30, 60, 240, and 480 minutes. During these periods, the air temperature never exceeded 30°C, and the temperatures of the analyzed oils remained below 20°C.

A total of 4 different types of vegetable oils were studied in this work, for each oil 15 different treatment were conducted (7 heat stress ranges and 8 UV light stress ranges), the samples were also made in two replicates in order to mitigate eventual statistic variability. A total of 120 samples were analysed and tagged, as shown in Table 3.1.

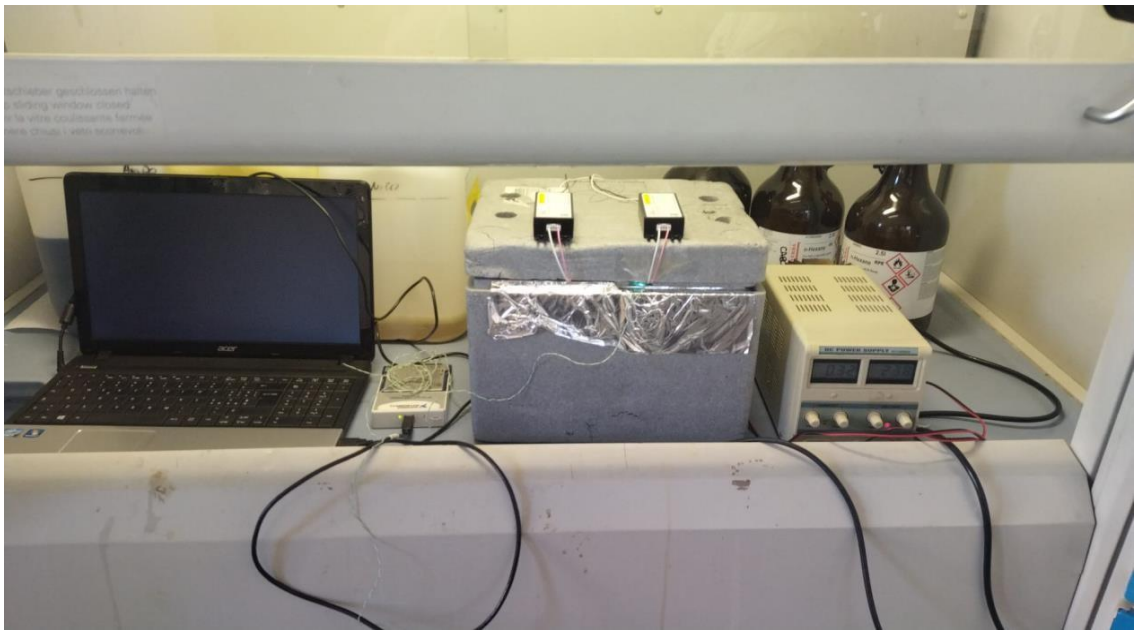

Figure 3.1. Photo-oxidation system. From left to right, the setup includes: a computer equipped with software for temperature monitoring via thermocouples, the thermocouple system, the oxidation chamber with UV lamps installed above it, and the current transformer.

Figure 3.2. External view of the lid, the ballasts were mounted on the external part of the system.

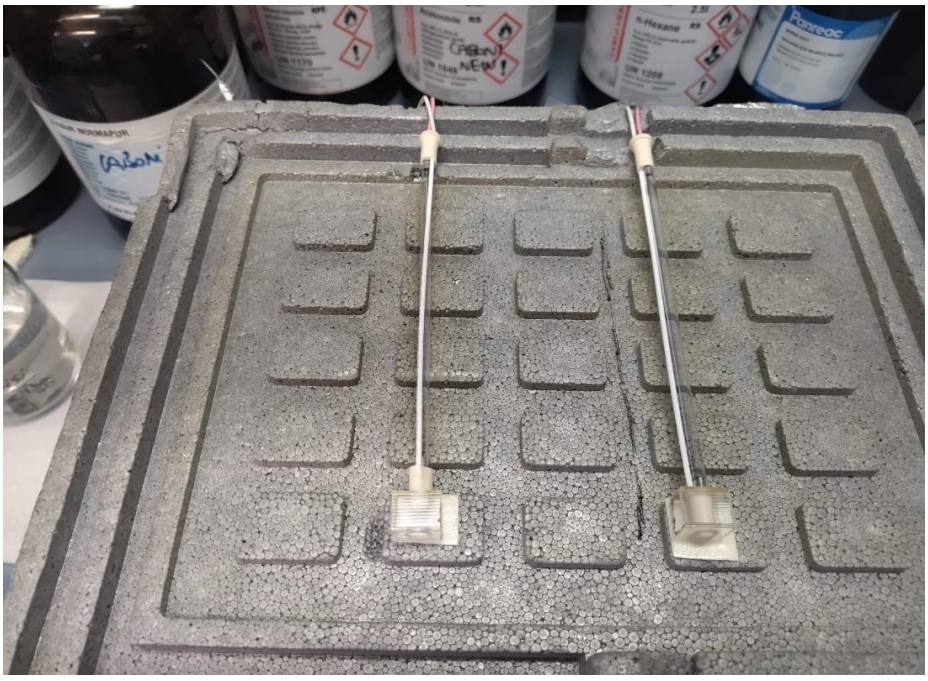

Figure 3.3. Internal view of the lid, the lamps were mounted inside the chamber with as little external contamination as possible.

SAMPLE NAME	TYPE OF OIL	TREATMENT (hh:mm:ss)	WAVELENGHT	TREATMENT (hh:mm:ss)	TEMPERATURE (°C)
GRO 0	Grapeseed oil	00:00:00	150 nm	00:00:00	100°C
GRO 1		00:05:00		00:30:00	
GRO 2		00:10:00		01:00:00	
GRO 3		00:20:00		01:30:00	
GRO 4		00:30:00		02:00:00	
GRO 5		01:00:00		03:00:00	
GRO 6		04:00:00		05:00:00	
GRO 7		08:00:00			
HOSO 0	High oleic sunflower oil	00:00:00	150 nm	00:00:00	100°C
HOSO 1		00:05:00		00:30:00	
HOSO 2		00:10:00		01:00:00	
HOSO 3		00:20:00		01:30:00	
HOSO 4		00:30:00		02:00:00	
HOSO 5		01:00:00		03:00:00	
HOSO 6		04:00:00		05:00:00	
HOSO 7		08:00:00			
SO 0	Sunflower oil	00:00:00	150 nm	00:00:00	100°C
SO 1		00:05:00		00:30:00	
SO 2		00:10:00		01:00:00	
SO 3		00:20:00		01:30:00	
SO 4		00:30:00		02:00:00	
SO 5		01:00:00		03:00:00	
SO 6		04:00:00		05:00:00	
SO 7		08:00:00			
EVO 0	Extra-virgin olive oil	00:00:00	150 nm	00:00:00	100°C
EVOO 1		00:05:00		00:30:00	
EVOO 2		00:10:00		01:00:00	
EVOO 3		00:20:00		01:30:00	
EVOO 4		00:30:00		02:00:00	
EVOO 5		01:00:00		03:00:00	
EVOO 6		04:00:00		05:00:00	
EVOO 7		08:00:00			

Table 3.1. Resume of the samples treated and analyzed for both the thermal and UV-stress.

Chemicals and Reagents

The following solvents and reagents used were purchased from Sigma Aldrich (St. Louis, MO, USA): methanol, chloroform, n-hexane, iso-octane potassium hydroxide, methyl tridecanoate, glacial acetic acid, potassium iodide, starch solution, sodium thiosulfate, *p*-anisidine. The standard mix GLC-463 was purchased from Nu-Check

(Elysian, MN, USA). The technical nitrogen used (95%≥ pure) was purchased from Sapio industries (Monza, Italy).

Fatty acids analysis by FAST-GC-FID

The determination of the fatty acid composition was carried out by gas chromatographic analysis of the relative methyl esters (FAME) obtained by cold basic transesterification of 20 mg of oil, according to the method of Christopherson and Glass, 1995 with some modifications. Methyl tridecanoate (C13:0; 1 mg/mL) was used as internal standard (IS). After a suitable dilution step with n-hexane, 0.30 µl of the upper phase was injected into a GC-2010 Plus gas chromatograph (Shimadzu, Tokyo, Japan) equipped with a flame ionization detector (FID) with a split injection port (1:100) set at 250 °C. The chromatographic separation was carried out using a BPX 70 column (SGE, Analytical Science. Ringwood, VIC, Australia, 10 m x 0.1 mm of internal diameter and 0.2 µm of thickness of the film with cyanopropyl phase, polarity equivalent to 70% cyanopropyl-siloxane) and the chromatographic conditions described by Marzocchi *et al*. (2018). The fatty acids were identified based on the known retention times of a standard blend of fatty acids (GLC-463 NuCheck. Elysian, MN, USA) and were quantified by the internal standard method, expressing them as weight percentage of total FAME (mg/100 mg of FAME). The chromatograms were recorded and processed using the Perkin-Elmer TotalChrom Navigator program (version 6.2.1) and each determination was carried out in triplicate (n=3).

Determination of the peroxide value (PV) by iodometric titration

The method used in this research is in accordance with the provisions of EC Reg. N. 2568/91 and subsequent amendments 2g of sample was weighed into a 250 ml flask. Subsequently, 25 ml of an acetic acid / chloroform mixture (3:2, v/v) and 1 ml of a saturated potassium iodide solution were added, which was freshly prepared to avoid the presence of iodine and iodates. After mixing and five minutes in the dark, 75 ml of distilled water were added to extinguish the reaction and 2 ml of starch solution, as a specific indicator to reveal the presence of iodine. Finally, the solution was titrated with 0.01 N sodium thiosulfate until the equivalence point was reached.

Determination of the p-anisidine value (p-AV)

In accordance with the official ISO 6885: 2006 (*ISO 6885:2016(en), Animal and vegetable fats and oils — Determination of anisidine value*) procedure, 0.3 g of the oil sample was weighed into a 10 ml flask and dissolved in iso-octane. 2.5 ml of this solution were placed inside a glass cuvette and the absorbance was then measured at 350 nm, against a blank as a pure iso-octane. Then 0.5 ml of *p*-anisidine solution (0.25g

of *p*-anisidine reagent in 100ml of acetic acid) were added to the cuvettes of both iso-octane (blank) and sample solution, placed in the dark for 10 minutes and measured their absorbance at 350 nm. The *p*-anisidine value was then calculated as indicated by the official procedure.

$$p - AV = \frac{v * \{1.2 * [(Absc2 - Absb2) - (Absc1 - Absb1)]\}}{w}$$

p-AV = *p*-anisidine value

v = volume of iso-octane used

1.2 = correction factor

Absc1 = absorbance of the sample before adding the p-anisidine solution

Absc2 = absorbance of the sample after adding the p-anisidine solution and waiting for 10 minutes in a dark place

Absb1 = absorbance of the pure iso-octane before adding the p-anisidine solution

Absb2 = absorbance of the iso-octane after adding the p-anisidine solution and waiting for 10 minutes in a dark place

w= exact weight of the sample used

Volatile compounds determination by SPME-GC-MS

The volatile compounds of the oil samples were analysed by headspace solid phase microextraction-gas chromatography/mass spectrometry (HS-SPME-GC/MS), using a GC-MSeQP2010 Plus (Shimadzu, Tokyo, Japan) equipped with an AOC 5000 autosampler (Shimadzu, Tokyo, Japan). with an aluminium cap equipped with a silicone septum. The SPME was carried out using a fibre of 2 cm long x 0.11 µm in diameter, coated with divinylbenzene / carboxen / polydimethylsiloxane (DVB / CAR / PDMS) 50/30 µm thick (Supelco, Bellofonte, PA, USA). Samples, weighted in a 10 ml amber vial with an aluminium cap and silicon septum, were equilibrated for 10 minutes at 40 °C. Fiber was exposed in the head space of the vials for 40 minutes at the same temperature and desorbed at 250 °C for 10 min in the split mode afterwards. The chromatographic separation was obtained with a Zebron-Wax fused-silica capillary column (30 m x 0.25 mm i.d. x 1.0 mm f.t.) (Phenomenex, Torrance, CA, USA) and the following GC conditions: carrier gas: helium; column flow: 1 ml / min; split ratio: 1:10 v / v; injector temperature: 230 °C; programmed oven temperature: from 50 °C (kept for 10 min) to 200 °C at 3 °C / min and kept for 3 min; from 200 °C to 240 °C at 10 °C / min and kept the final temperature for 5 min; running time: 72

minutes. MS conditions: temperature of the ion source of the mass spectrum: 200 ° C; scanning mass range: 30-250 m / z; acquisition mode: Total Ion Current (TIC) mode. The GCMS solution software, version 2.50 SU1 (Shimadzu, Tokyo, Japan) was used and the volatile compounds identification was performed by comparing their mass spectra with those reported in literature and the NIST Mass Spectral Database (NIST 08, National Institute of Standards and Technology, Gaithersburg, MD, USA). The quantification of the results was carried out via external standard (hexanal), injected at various concentrations in n-hexane, using the same conditions previously described.

Determination by gas chromatographic analysis of oxidized fatty acids (OFA)

The analysis of the oxidized fatty acids was carried out by a trans-methylation of the fatty acids and subsequent injection into a gas chromatograph GC20-25 Shimadzu (Tokyo, Japan) with ionization detector of flame (GC-FID). Chromatographic separation was carried out using a Restek RXi5ms FAST column (low polarity phase; 5% diphenyl, 95% dimethyl-polysiloxane Crossbond, length 10 m, internal diameter 0.1 mm, stationary phase with 0.10 μm film thickness). GC-FID conditions: carrier gas: hydrogen; injection volume: 0.30 μl; injector temperature: 325 ° C; column flow: 1.80 ml / min; split ratio 1:50. The oven temperature program was: 130 °C for 0 minutes, then it was raised to 5 °C / min up to 250 °C and then at 20 °C / min up to 325 °C; total running time: 27.75 minutes. The oxidized fatty acids were identified as a zone of the chromatogram reuniting the fatty acids of interest, compromised for the most part of C18 fatty acids, which account to circa 88% of the total, this zone was elaborated not taking into account single peaks in virtue of the high similarities and possibility of signal interlaps between the single molecules. The chromatograms were recorded and processed using the Perkin-Elmer TotalChrom Navigator program (version 6.2.1). The results were expressed in mg OFA /g of fat, using dilaurin as internal standard.

Statistical analysis

The samples were analysed in three replicates and the results were expressed as mean value ± standard deviation (s.d.). One-way analysis of variance, ANOVA (Tukey's honest significant difference multiple comparison) were evaluated using Statistica 8 software (2006, StatSoft, Tulsa, OK, USA). *p*-values lower than 0.05 were considered statistically significant.

b. Second goal: chemical and sensorial characterization of different kinds of product formulations, "tarallini" and "frollini" biscuits, over shelf-life

Chemical and Reagents

Wheat flour, extra virgin olive oil (EVOO), high oleic sunflower oil (HOSO), sunflower oil (SO), rice oil (RO), coconut oil (CO), palm oil (PO), butter (B), white wine, sodium chloride, sugar, eggs, lemon zest and chemical yeast for the preparation of "tarallini" and biscuits were purchased at a local supermarket. All solvents and chemicals used were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO; USA).

Samples – "tarallini"

The "tarallini" samples were formulated with the following ingredients: 59% wheat flour, 24% white wine, 16% fat and 1% salt. About the lipid fraction, five different oils and blend oils were used, as shown in Table 1. In order to identify the best oils for a blend formulation (with or without extra virgin olive oil) suitable for the preparation of "tarallini", a previous screening of different vegetable oils was carried out. On the basis of the chemical-analytical results (i.e., free acidity and peroxide value, oxidative stability, fatty acid composition and minor lipid compounds) and costs, specific oils were selected and blended for "tarallini" formulation. The ingredients were weighed and mixed with a professional kneader equipped with a hook mixing tool until the dough reached a suitable consistency (approximately 10 min). Successively, the dough was left relaxing for 20 min and then shaped manually in small rings (typical "tarallini" shape). The cooking phase was performed in two stages: (i) boiling in water until the product rose to the surface and (ii) approximately 35 min in a rotational oven at 180 °C. After cooling, "tarallini" were stored at room temperature in closed bags and analysed within 1 day from baking.

Samples	Oil/blend oils
Tctrl	100% EVOO (extra virgin olive oil)
TA	100% HOSO (high oleic sunflower oil)
TB	87.5% EVOO + 12.5% SO (sunflower oil)
TC	75% EVOO + 25% RO (rice oil)
TD	87.5% HOSO + 12.5% CO (coconut oil)

Table 3.2. Sample coding of "tarallini" samples and the oils used for their lipid fraction.

Lipid extraction

According to the AOAC Official Method 960.39 (Latimer and Latimer, 2023), the lipid fraction of ground tarallini (10 g) was extracted with *n*-hexane by using a Soxhlet apparatus (Behr Labor-Technik, Fischer Scientific, Milano, Italy). Each extraction was performed twice (n = 2).

Fatty acids analysis by FAST-GC-FID

The fatty acid composition of tarallini and relative raw lipid matrices were determined as fatty acid methyl esters (FAMEs) by capillary gas chromatography analysis after alkaline treatment according to (Marzocchi *et al.,* 2018). FAME composition was measured in two replicates for each lipid extract (n = 2).

Tocol analysis

For the tocol determination, approximately 0.05 g of oil was dissolved in 0.5 mL of n-hexane. The solutions were filtered through a 0.45-μm nylon filter. The tocols were determined by HPLC (Agilent 1200 series, Palo Alto, CA, USA) equipped with a fluorimeter detector (Agilent) according to (Lajnef *et al.,* 2017). To identify the tocotrienols, their retention times in samples were compared with their retention times in a barley extract obtained by hot saponification (Panfili *et al.,* 2003); tocopherols were identified by co-elution with the respective standards. The calibration curve used for quantification was constructed with α-tocopherol standard solutions. Analysis was achieved in two replicates for each extract (n = 4).

Sterol analysis

To determine the phytosterol content, 0.5 mL of dihydrocholesterol (c = 2 mg/mL) was added to 250 mg of oil, and saponification was conducted at room temperature according to (Sander *et al.,* 1989). Before injection, samples were silylated (Sweeley *et al.,* 1963), and sterol separation was performed by GC/MS (GCMS-QP2010 Plus; Shimadzu, Tokyo, Japan) under the same chromatographic conditions reported by (Cardenia *et al.,* 2012). Phytosterol identification was achieved by comparing peak mass spectra with peaks of a standard mixture and by comparing them to the GC/MS data reported in the literature (Pelillo *et al.,* 2003). Analysis was conducted in two replicates for each lipid extract (n = 4).

Oxidative stability with OXITEST

Ten grams of ground tarallini was placed in the appropriate oxidation reactors in the OXITEST instrument (Velp Scientific, Usmate Velate, MB, Italy) at 90 °C and 6 bar of oxygen pressure, as reported by Riciputi and Caboni (2017). The analysis was repeated twice for each replicate (n = 2).

Hardness measurement

The fracturability and hardness of tarallini were determined with a texture analyser TA-XT2i (Stable Micro Systems, Godalming, UK) using a cell load of 25 kg and a P/2 probe for the penetration test (Barbieri *et al.,* 2018). The test settings were as follows: pre-speed 2 mm/s; test speed 2 mm/s; post-speed 2 mm/s; distance 50%; trigger value 0.010 kg. The test allowed us to determine the sample height (mm), the fracturability, as force for the first rupture (g), registered on the sample after penetration and the hardness as the maximum force (g). Each result was expressed as the mean of at least 10 repetitions standard deviation.

Sensory analysis

The descriptive sensory aspects of tarallini were evaluated according to Meilgaard, Carr, and Civille 2006 by a panel of ten trained assessors recruited from the staff of the Department of Agricultural, Environmental and Food Sciences of the University of Molise for their experience and familiarity with the product. The different samples were randomly coded during the sensory test. A total of nine descriptors were considered: four for the description of aroma and flavour (overall aroma, wine aroma, overall flavour, cereal flavour) and five for the tactile/textural sensations (crispiness as the crushing at first bite, consistency as resistance to chewing, friability, fat perception on the palate and palatability, i.e., the ease of swallowing the product after chewing). The panellists rated the intensity of each attribute using a grading scale from 1 to 9,

where 1 indicated the absence of sensations and 9 the maximum intensity of sensations. Scores for two replicates and averages were calculated. Furthermore, the assessors were asked to give an additional to express levels of satisfaction/appreciation for the following attributes: appearance, shape, overall aroma, overall flavour, crispiness, and palatability. All subjects involved in the sensory study were preliminary informed about the nature of the research and consented to their informed participation. No personal data were collected or used in any form.

Determination of the Peroxide value (PV) by spectrophotometric detection

The method adopted was an application of the study of (Shantha and Decker, 1994)). The peroxide value was based on the measurement of Fe^{3+} ions formed by the oxidation of Fe^{2+} ions by hydroperoxides in the presence of ammonium thiocyanate (NH_4SCN). Circa 50 mg of the samples were collected and added with 9.9 ml of a Chloroform: Methanol solution (7:3, v/v); then 50 µl of a Fe^{2+} solution and 50 µl of an Ammonium Thiocyanate solution were added and the samples were kept in the dark for 5 minutes. Thiocyanate anions (SCN^-) reacted with Fe^{3+} ions to form a red-coloured complex that could be read spectrophotometrically at 500 nm (UV spectrophotometer 1601, Shimadzu, Kyoto, Japan), the results were then quantified via the following formula.

$$\frac{[ABS - ABS_{Blank}] - b}{m \times 55{,}84 \times w}$$

***ABS**: absorbance value of the solution at 500 nm.*

***ABS blank**: absorbance value of a blank sample.*

***m**: slope of the calibration curve.*

***b**: intercept of the calibration curve.*

***55.84**: Iron Elemental weight.*

***w**: sample weight.*

Volatile compounds determination by SPME-GC-MS

The analysis of volatile compounds was carried out using a Shimadzu Q 2010 GC-MS. The column used was a Restek RTX-WAX (30 m x 0.25 mm ID, 1 µm film thickness) (Restek Corporation, Bellefonte, USA). The method employed was adapted from that reported by Purcaro *et al.,* (2008). One gram of ground sample was weighed into a 10 mL amber vial, which was then sealed with a metal screw cap and silicone septum. The

determination of volatile compounds was performed using the SPME (Solid Phase Micro Extraction) technique with a Combi-Pal volatile autosampler, which fully automated the sample pre-conditioning, fiber exposure, and desorption steps, thereby minimizing operator-induced error. The analytical conditions adopted were as follows: GC conditions: carrier gas, Helium; column flow: 1 mL/min; split ratio: 1:10; injector temperature: 240 °C; total run time: 75.33 min. The temperature program started at 40 °C for 10 min, then increased to 200 °C at a rate of 3 °C/min, and further increased to 240 °C at a rate of 10 °C/min, holding this final temperature for 5 min. MS conditions: source temperature: 200 °C; interface temperature: 240 °C; mass range: 30–250 m/z: acquisition mode: scan from 3.5 min to 70 min. SPME conditions: the SPME fiber was 2 cm in length, 0.11 µm in diameter, and coated with a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) layer (Supelco, Bellefonte, PA, USA). Incubation time: 30 min at 40 °C, fiber exposure time: 10 min, desorption time: 7 min. The quantification of the results was carried out via external standard (hexanal), injected at various concentrations in *n*-hexane, using the same conditions previously described.

Statistical analysis

The relative standard deviation was obtained for all data collected. One-way analysis of variance (ANOVA) was evaluated using Statistica 8 software (2006; StatSoft, Tulsa, OK, USA). *p*-values lower than 0.05 were considered statistically significant using Tukey's honest significant difference (HSD) test. All chemical analyses were carried out in two replicates for each extract (n = 4 for each sample). Three-way ANOVA was used to evaluate the output of the sensory analysis, and the results were expressed according to Fisher's least significant difference (LSD) test.

Samples – "Frollini" biscuits

The dry ingredients included wheat flour (52%), sugar (16%), and chemical leavening agents. Liquid ingredients such as eggs (16%) and grated lemon zest were added to create a standard dough. For each formulation, fats were incorporated in specific ratios as shown in Table 3.3.

Samples	Oil/blend oils
B0	100% PO (palm oil)
B1	100% B (butter)
B2	50% B + 50% EVOO (extra virgin oilve oil)
B3	50% B + 50% HOSO (high oleic sunflower oil)
B4	100% HOSO
B5	87.5 CO (coconut oil) + 12.5% SO (sunflower oil)

Table 3.3. *Sample coding of "frollini" samples and the oils used for their lipid fraction.*

All ingredients were weighed and prepared individually before combining. The preparation process adhered to the following steps: wheat flour and sugar were sifted together to ensure uniform distribution. The chemical leavening agent was then incorporated. The fat(s) for each formulation were softened or melted as required. Solid fats like butter and coconut oil were creamed with sugar for formulations B1, B2, B3, and B5. Liquid fats (e.g., extra virgin olive oil and high-oleic sunflower oil) were emulsified with the eggs before mixing into the dry ingredients. The blended fat mixture was combined with the dry ingredients. The dough was kneaded gently to achieve homogeneity without overworking. Following, the dough was allowed to rest at a controlled temperature (10–15 °C) for 30 minutes, this step was critical for stabilizing the dough structure and ensuring uniform fat distribution. The rested dough was extruded using a pilot-scale extruder with a standard shortbread die. Shaped dough pieces were transferred onto baking trays lined with parchment paper and baked at 180°C for 20 minutes in a convection oven. After baking, the cookies were cooled at ambient temperature (20–22 °C) for 1 hour to stabilize their structure. Baked biscuits were then packed in certified LFGB-compliant polyamide-polyethylene (PA-PE) bags to ensure minimal exposure to oxygen and moisture. Packaging occurred under controlled humidity conditions to maintain the product's sensory and textural integrity during storage.

Lipid extraction

According to the AOAC Official Method 960.39, the lipid fraction of ground frollini (10 g) was extracted with *n*-hexane by using a Soxhlet apparatus (Behr Labor-Technik, Fischer Scientific, Milano, Italy). Each extraction was performed twice (n = 2).

Chemical analyses

The "frollini" biscuits were analysed using the same methods and reagents as illustrated for the previous chapter relative to the "tarallini", the analyses comprehended: Fatty acids methyl esters (FAME) identification, tocol analysis and peroxide value (PV) analysis. The operators that conducted the analyses were also the same. The content of Oxidated Fatty acids (OFA) was registered for the frollini, in virtue of their high shelf-life. The method is described in the chapter "*Determination by gas chromatographic analysis of oxidized fatty acids (OFA)*" of the First goal.

Oxidative stability with OXITEST

The "frollini" biscuits were analysed using the same methods reported for the "tarallini" analysis, the operators that conducted the analyses were also the same.

Hardness measurement

The "frollini" biscuits were analysed using the same methods used for the "tarallini" analysis, the operators that conducted the analyses were also the same.

Sensory analysis

The "frollini" biscuits were analysed using the same methods illustrated for the "tarallini" analysis, the operators that conducted the analyses were also the same.

Shelf-life trials

After preliminary analyses, 2 formulations of each "tarallini" and "frollini" samples, in addition to the control (T0 and B0), were stored to evaluate their shelf life. To simulate both commercial and home storage conditions, tarallini and frollini were packaged in certified LFGB-compliant polyamide-polyethylene (PA-PE) bags and were stored in thermostatic incubators at 20°C. As reported in Table 4, the sampling time was different for tarallini and frollini samples: from 0 to 75 days were selected for the tarallini, from 0 to 355 days for the frollini. At each shelf lifetime the samples were analysed with the methods reported above in order to evaluate their oxidative quality over time.

Tarallini sample code	Days of shelf-life (gg)	Frollini sample code	Days of shelf-life (gg)
Tn_0	0	**Bn_0**	0
Tn_7	7	**Bn_{28}**	28
Tn_{14}	14	**Bn_{50}**	50
Tn_{21}	21	**Bn_{90}**	90
Tn_{28}	28	**Bn_{120}**	120
Tn_{35}	35	**Bn_{175}**	175
Tn_{42}	42	**Bn_{240}**	240
Tn_{50}	50	**Bn_{300}**	300
Tn_{75}	75	**Bn_{355}**	355

Table 3.4. Sampling times for tarallini and frollini shelf-life evaluation. All the samples reported were stored for the same periods of time at 20°C. "n" is a generic indicator of the formulation, which are of different number for both the typologies of products.

c. Third goal: non-destructive analysis of refined vegetable oils, building and modelling of calibration curves for analytical parameters of quality in vegetable oils.

This specific segment of the analyses, and the work conducted during the PhD, was taken into account inside the headquarters of BUNGE Italy (Porto Corsini, Ravenna RA, Italy); a total of 6 months of activities were carried out during this time, and the data acquired were courtesy of the multiple professionals met during the period.

Samples

The analysed samples were fresh various vegetable oils, including sunflower oil, high oleic sunflower oil, corn oil, soy oil, peanut oil and commercial oil blends (frying mix, cooking mix etc…) stored immediately after the refining process, for a total amount of 160 samples analysed. The various vegetable oils were sampled all along the implant, after the refining process, in order to collect and then analyse the oils for internal evaluation of the refining implant.

NIR analysis

The samples were charged and analysed on a Vis-NIR PROXIMATE instrument (BUCHI, Switzerland), the instrument was equipped with a reading cell prototype, structured in a stainless-steel support, mounted with a sapphire glass and connect to a peristaltic pump, as shown in Figure 3.4. The prototype was implemented in the analysis of the spectra in order to, reduce the human error of charging and discharging the sample, by making it semi-automatic by means of pressure; reduce the quantity of solvents used to flush and wash the reading cells, by using the subsequent sample as a cleaning medium; and finally, to mitigate and control the temperature of the oil, during the analysis, by means of a series of serpentines connected by hoses to a chilling device, which, in case where it was needed, was able to provide higher or lower temperatures to the oils. All of the oils were analysed at temperatures between 20-25°C. The instrument worked in transflectance, from 900-1700 nm, 32 scans were made for each wavelength, scans were made with a resolution of 8 nm, optical length 1mm. The data were elaborated with the internal software of the PROXIMATE instrument (NIRCal). The raw data were first smoothed with the usage of the Multiplicative Scatter Correction (MSC) and then elaborated with first derivative. the elaboration consisted in the building of a simple regression curve, via Partial least squares (PLS), using real analytical values obtained from the analysis that will be presented in this paragraph.

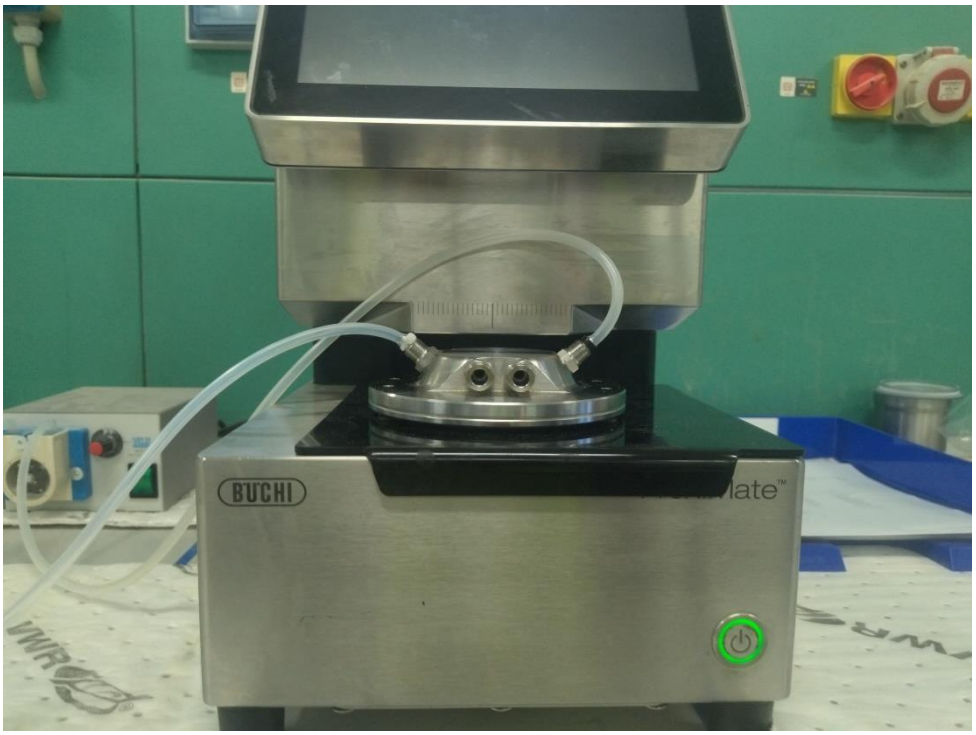

Figure 3.4. Flux camber prototype implemented in the NIR analysis of the oils, the samples were charged by a peristaltic pump with the hose on the left side of the image and discharged through the right one. The prototype also worked as the tranflectance medium.

Analytical analyses

Free Acidity

The free acidity of oil was determined following the ISO 660:2020 protocol. The procedure quantifies the free fatty acids present, expressed as a percentage of oleic acid. Reagents and chemicals comprehended Ethanol (≥98%), neutralized with a sodium hydroxide (NaOH) solution before use, sodium hydroxide (0.1 mol/L standardized solution), phenolphthalein solution (1% in ethanol) and distilled water. The instrumentation used were an analytical scale, Burettes (50 mL capacity, graduated to 0.1 mL), conical flasks (250 mL), a magnetic stirrer with heating plate, pipettes and micropipettes, volumetric flasks. Approximately 20 g of oil were accurately weighed into a 250 mL conical flask, and 50 mL of neutralized ethanol was added. The mixture was gently heated to ensure dissolution of the oil. After cooling, 2-3 drops of phenolphthalein indicator were added. The solution was titrated against the standardized 0.1 mol/L NaOH solution, with constant stirring, until the appearance of a persistent pale pink color lasting at least 30 seconds. The volume of NaOH required for neutralization was recorded, and the free acidity was calculated based on the following formula.

$$Free\ acidity\ (\%FFA\ in\ oleic\ acid) = \frac{V \times C \times M}{W} \times 100$$

V= Volume of sodium hydroxide solution used for titration (mL).

C= Concentration of the sodium hydroxide solution (mol/L).

M= Molar mass of oleic acid (282.47 g/mol)

W= Weight of the oil sample (G).

Assessment of Red and Yellow Colour

The colorimetric analysis of oil samples was conducted utilizing the Lovibond® PFXi-195 automatic colorimeter (Amesbury, United Kingdom), adhering to standardized protocols for precise measurement of red and yellow hues. Prior to analysis, the instrument was calibrated using certified conformance filters to ensure measurement accuracy. Oil samples were homogenized to ensure uniformity and then filtered to remove any particulate matter that could interfere with colour measurement, then a plastic sample cell was filled with the prepared oil sample, avoiding the introduction of air bubbles. A double reading for each oil sample was made, and colour parameters were registered in CIE scale.

Fatty acid analysis

The fatty acid composition of the oils was determined as fatty acid methyl esters (FAMEs) by capillary gas chromatography analysis after alkaline treatment according to BUNGE internal protocol. FAME composition was measured in two replicates for each lipid extract (n = 2).

4.Results and Discussions

a. First goal: chemical characterization of 4 different kind of vegetable oils stressed with UV light and mild temperatures

Oxidation is the most critical cause of deterioration in oils and fats. It is a mechanism characterized by a series of reactions that can be initiated by oxygen, humidity, trace metals, and free radicals, as well as other factors such as air exposure, temperature, treatment duration, packaging type, and the presence of prooxidants and antioxidants. Additionally, the degree of oil unsaturation plays a crucial role. For this reason, the fatty acid composition (FAME) of oils is initially assessed. The primary oxidation products are hydroperoxides, which are highly unstable and undergo further reactions to form secondary products such as hydrocarbons, alcohols, ketones, and aldehydes, which can be oxidized to carboxylic acids. Quantitative determination of oxidation is complex because classical methods that study oxidation reactions often focus on a single class of compounds within the complex mixture generated during the oxidation process (Butinar, 2008). For instance, measuring peroxide content is helpful for monitoring the initial phase of oxidation but does not provide a comprehensive evaluation of the oxidative state of a food product. This limitation arises because these compounds degrade as oxidation progresses, especially at high temperatures due to their significant instability. Another test useful for assessing advanced oxidative stages is the p-anisidine analysis (Butinar, 2008). Chemical changes in oils and fats subjected to high temperatures induce oxidation, hydrolysis, polymerization, isomerization, or cyclization reactions (Butinar, 2008). These reactions impact the sensory, nutritional, and safety properties of oils. For example, the production of unpleasant volatile compounds negatively affects the product's aroma profile, often imparting a rancid odour. For this reason, it is also useful to consider volatile analysis, particularly monitoring hexanal content, which increases during oxidation. However, as reported by Morales and collaborators (1997), this parameter is not always exhaustive. It is particularly unsuitable for extra virgin olive oil, where hexanal is already significantly present in the fresh product due to the lipoxygenase pathway. Additionally, evaluating the content of oxidized fatty acids (OFA) can be an effective method for monitoring the oxidative state of fatty matrices. OFAs can catalyse further reactions and/or degrade to form compounds that, in addition to imparting rancid flavour and aroma, may have antinutritional effects (Koutoulis, 2019). It is crucial to consider that the consumption of lipid oxidation products can partially influence the onset of certain degenerative diseases.

Fatty acids analysis by FAST-GC-FID

The characterization and quantification of total fatty acids in the samples under study, as described in the Materials and Methods chapter, was evaluated for all four oil types (extra virgin olive oil, grape seed oil, sunflower oil, and high-oleic sunflower oil) at time 0, in the fresh product, without thermal stress. This analysis aimed to assess the composition of the oils and determine whether it could influence their oxidative stability. Fatty acids, while not the sole factor to consider, can significantly affect oil stability during thermal treatment (Moulodi *et al.,* 2015). Certain fatty acids, due to their quantity and conformation, have a more pronounced influence on the oxidative stability of vegetable oils, particularly oleic, linoleic, and linolenic acids (Ali *et al.,* 2013). In all samples, various fatty acids were identified, and their mean values are reported in Table 4.1, expressed in mg/100 mg of FAME. The results indicate that in extra virgin olive oil (EVOO), the main fatty acid is oleic acid (C18:1), accounting for approximately 70%, followed by much lower percentages of palmitic acid (C16:0) (14.39%) and linoleic acid (C18:2) (9.30%). A similar fatty acid profile has been observed in previous studies on EVOO (Morales *et al.,* 1997; Allouche *et al.,* 2007). As noted by Akil (2015) and Moulodi *et al.* (2015), the abundant presence of oleic acid in EVOO makes it particularly stable during thermal treatments, thanks to the significantly lower oxidation rate of monounsaturated fatty acids, which is approximately 100 times slower than that of polyunsaturated fatty acids. For grape seed oil (GRO), linoleic acid (C18:2) is the most abundant, representing 69.19% of the total, followed by oleic acid (C18:1) at 18.91% and palmitic acid (C16:0) at 6.70%. Stearic acid (C18:0) is also present at a higher percentage than in all other vegetable oils (3.84%). These findings agree with the literature on grape seed oil (Yalcin *et al.,* 2017). Sunflower oil (SO) also exhibits a predominantly unsaturated composition, where, as in grape seed oil, linoleic acid (C18:2) is the main fatty acid, accounting for 53.34%. This is followed by oleic acid (C18:1) at 34.95%, palmitic acid (C16:0) at 5.98%, and stearic acid (C18:0) at 3.67%. In high-oleic sunflower oil (HOSO), the fatty acid composition is markedly shifted towards oleic acid (C18:1), which constitutes nearly the entirety of the fatty acids, with a content of 84.63%. This is followed by linoleic acid (6.20%), palmitic acid (3.57%), and stearic acid (3.33%). This fatty acid profile is also confirmed by Abbas Ali *et al.* (2013). The same authors report a fatty acid composition for sunflower oil consistent with our findings, though with slightly different percentages, particularly for linoleic acid (62.29%) and oleic acid (26.23%). Due to its high linoleic acid content, the authors suggest that sunflower oil is highly susceptible to lipid oxidation. They also argue that genetically modifying sunflower oil to increase oleic acid content at the expense of linoleic acid, as in high-oleic sunflower

oil, could improve its stability during thermal treatments. However, in our study, the lower percentage of linoleic acid in sunflower oil compared to that reported by Abbas Ali *et al.* (2013) might suggest a different oxidative behaviour. In Figures 5.1, 5.2, 5.3 and 5.4 are reported the mean values of fatty acids classified as saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA). Understanding these aspects are crucial for evaluating oxidative stability, as the degree of unsaturation is one of the most significant factors in regulating the rate of lipid oxidation reactions. Regarding EVOO, as shown in Figure 4.1, MUFAs are present in significantly higher amounts than SFAs and PUFAs ($p \leq 0.05$). Allouche *et al.* (2007) highlights that extra virgin olive oil exhibits greater oxidative stability compared to other vegetable oils due to its high MUFA/PUFA ratio.

FAME (mg FA/100 mg FAME)	**EVOO**	**HOSO**	**SO**	**GRO**
C12:0	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
C14:0	0.02±0.00	0.03±0.00	0.06±0.00	0.04±0.00
C15:0	n.d.	0.01±0.00	0.01±0.00	0.01±0.00
C15:1	n.d.	n.d.	n.d.	0.02±0.00
C16:0	14.39±0.2	3.57±0.01	5.98±0.01	6.68±0.00
C16:1 t	0.07±0.00	0.02±0.00	0.01±0.00	0.02±0.00
C16:1 c	0.92±0.02	0.10±0.00	0.09±0.00	0.10±0.00
C17:0	0.04±0.00	0.03±0.00	0.03±0.00	0.05±0.01
C17:1	0.07±0.00	0.04±0.00	0.03±0.00	0.03±0.00
C18:0	3.26±0.17	3.33±0.2	3.66±0.02	3.84±0.08
C18:1t	0.10±0.02	0.07±0.01	0.08±0.00	0.10±0.00
C18:1c	69.96±0.29	84.63±0.18	34.92±0.00	18.87±0.02
C18:2 n6	9.30±0.09	6.20±0.03	53.40±0.02	69.26±0.05
C18:3n3	0.61±0.01	0.02±0.00	0.27±0.00	0.29±0.00
C20:0	0.45±0.01	0.01±0.00	0.18±0.00	0.19±0.03
C20:1	0.23±0.00	0.04±0.00	0.78±0.01	0.25±0.00
C20:2n6	n.d.	n.d.	n.d.	0.04±0.00
C22:0	0.12±0.01	0.30±0.02	0.02±0.00	0.07±0.00
C22:1	n.d.	n.d.	n.d.	0.04±0.00
C22:2	0.39±0.00	0.26±0.00	0.03±0.00	0.02±0.00
C24:0	n.d.	0.93±0.00	0.05±0.01	0.03±0.00
C24:1	n.d.	0.05±0.00	0.25±0.00	n.d.
SFA	**18.35**	**8.55**	**11.08**	**10.92**
MUFA	**71.36**	**85.14**	**35.40**	**19.46**
PUFA	**10.30**	**6.26**	**53.36**	**69.62**

Table 4.1. FAME composition for the 4 different tested oil samples (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil).

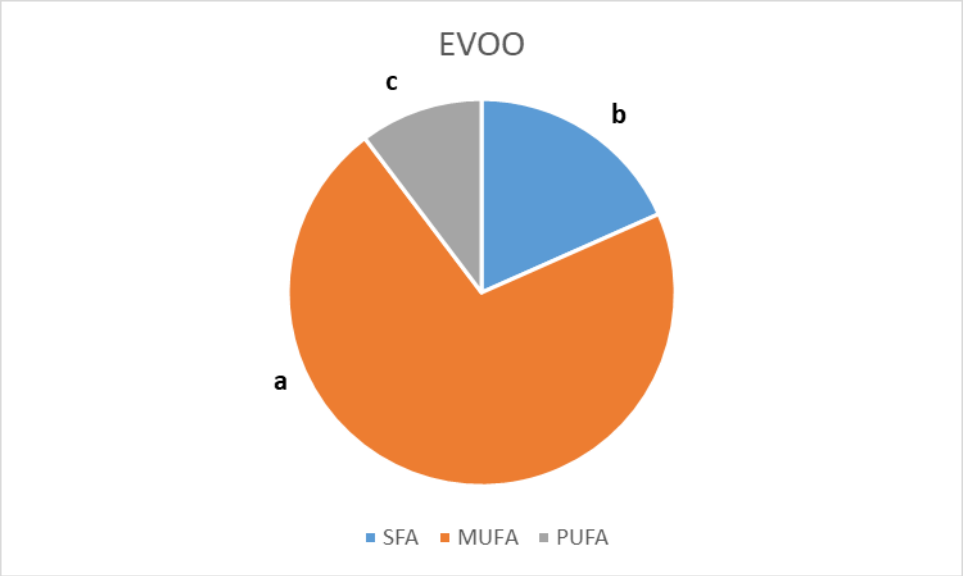

Figure 4.1. Graphical representation of Saturated (SFA), Monounsaturated (MUFA), and Polyunsaturated (PUFA) Fatty Acid composition in extra-virgin olive oil (EVOO) Samples. The fatty acid composition is expressed in mg/100 mg of FAME. Different letters next to the FAME values for each class (SFA, MUFA, PUFA) indicate statistically significant differences ($p \leq 0.05$).

In the case of grape seed oil (GRO), a pattern opposite to that of EVOO is observed, as illustrated in Figure 4.2. There is a clear predominance (significant differences, $p \leq 0.05$) of PUFAs over MUFAs and SFAs, with PUFAs representing 69.6% of the total fatty acid composition in this oil.

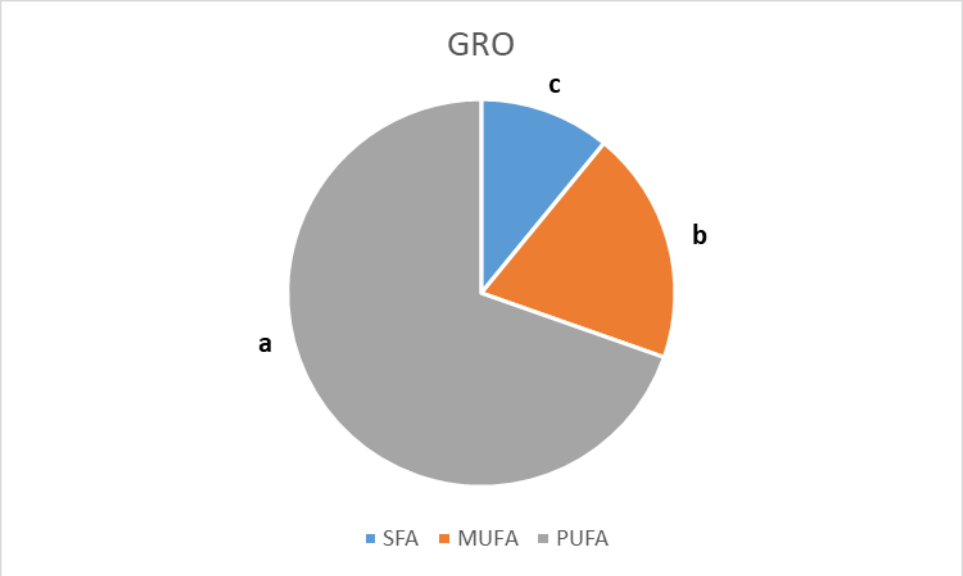

Figure 4.2. Graphical representation of Saturated (SFA), Monounsaturated (MUFA), and Polyunsaturated (PUFA) Fatty Acid composition in grapeseed oil (GRO) Samples. The fatty acid composition is expressed in mg/100 mg of FAME. Different letters next to the FAME values for each class (SFA, MUFA, PUFA) indicate statistically significant differences ($p \leq 0.05$).

For sunflower oil (SO), Figure 4.3 shows significant differences ($p \leq 0.05$) among SFA, MUFA, and PUFA. More than half (53.5%) of the total fatty acids in this oil are PUFAs.

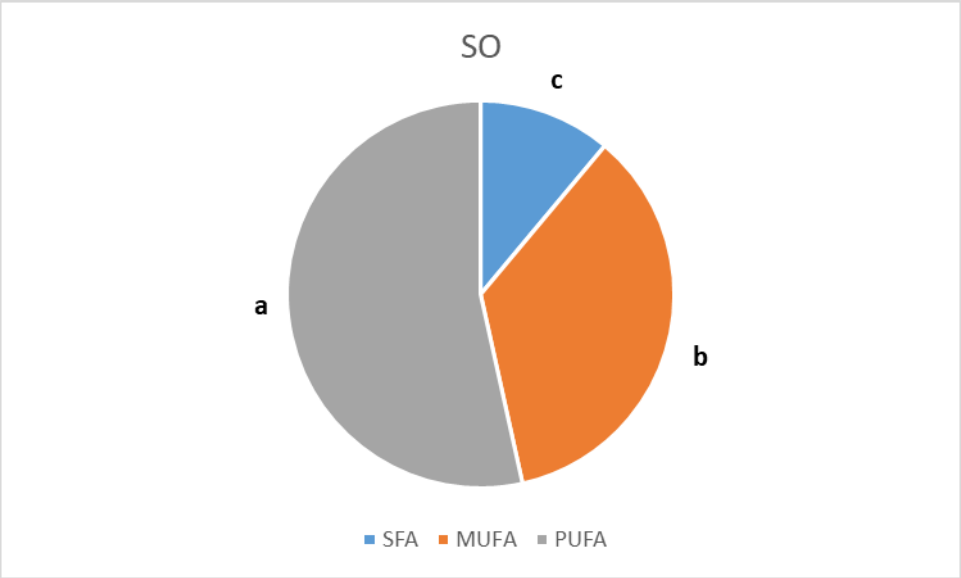

Figure 4.3. Graphical representation of Saturated (SFA), Monounsaturated (MUFA), and Polyunsaturated (PUFA) Fatty Acid composition in sunflower oil (SO) Samples. The fatty acid composition is expressed in mg/100 mg of FAME. Different letters next to the FAME values for each class (SFA, MUFA, PUFA) indicate statistically significant differences ($p \leq 0.05$).

In the high-oleic sunflower oil (HOSO) sample, MUFAs constitute 85.1% of the total fatty acids, as shown in Figure 4.4. As in EVOO, the high MUFA/PUFA ratio is considered an important and favourable factor for the thermal oxidative stability of oils.

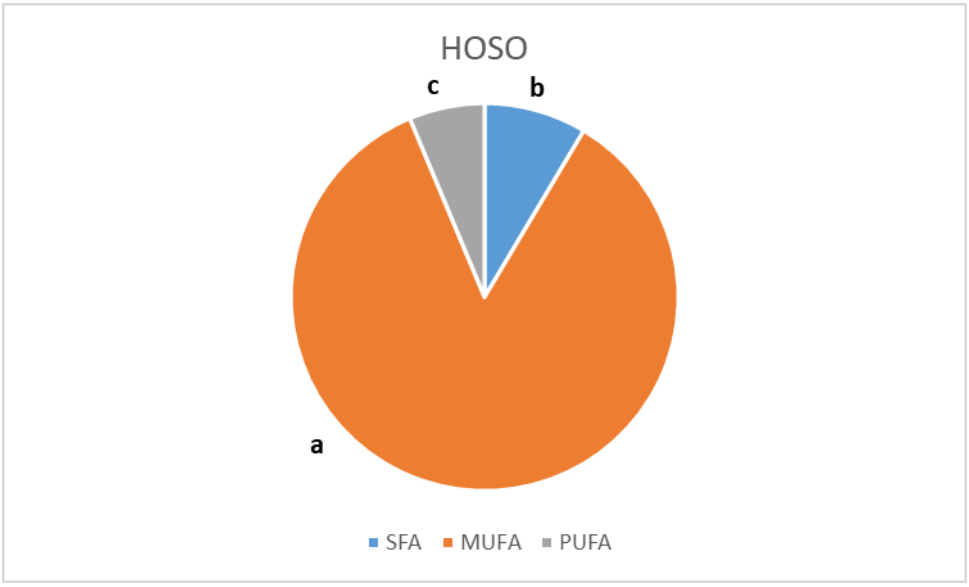

Figure 4.4. Graphical representation of Saturated (SFA), Monounsaturated (MUFA), and Polyunsaturated (PUFA) Fatty Acid composition in high-oleic sunflower oil (HOSO) Samples. The

fatty acid composition is expressed in mg/100 mg of FAME. Different letters next to the FAME values for each class (SFA, MUFA, PUFA) indicate statistically significant differences ($p \leq 0.05$).

These patterns reflect the composition of the main individual fatty acids (Table 4.1), where oleic acid (C18:1), a monounsaturated fatty acid, is the predominant component in EVOO and HOSO, whereas linoleic acid (C18:2), a polyunsaturated fatty acid, is the main constituent in GRO and SO. Figure 29 provides a comparative analysis of fatty acid classes (SFA, MUFA, and PUFA) across the oils under study. Saturated fatty acids generally constitute less than 20% of the total composition, with a significantly higher abundance ($p \leq 0.05$) in EVOO. In contrast, no significant differences in SFA content were observed between GRO and SO. Regarding MUFAs and PUFAs, the overall trend shows an inverse relationship: in oils where MUFAs predominate, PUFAs are present in smaller amounts, and vice versa. For MUFAs, HOSO exhibits the highest value, with a content significantly higher even compared to EVOO, which nonetheless features a substantial proportion of these fatty acids (71.36%). The lowest MUFA content is found in GRO, which, conversely, has the highest PUFA content (69.55%). Following GRO, HOSO has a slightly lower PUFA concentration. EVOO and HOSO, however, exhibit much lower PUFA levels, which are significantly different from each other (10.30% and 8.55%, respectively).

.

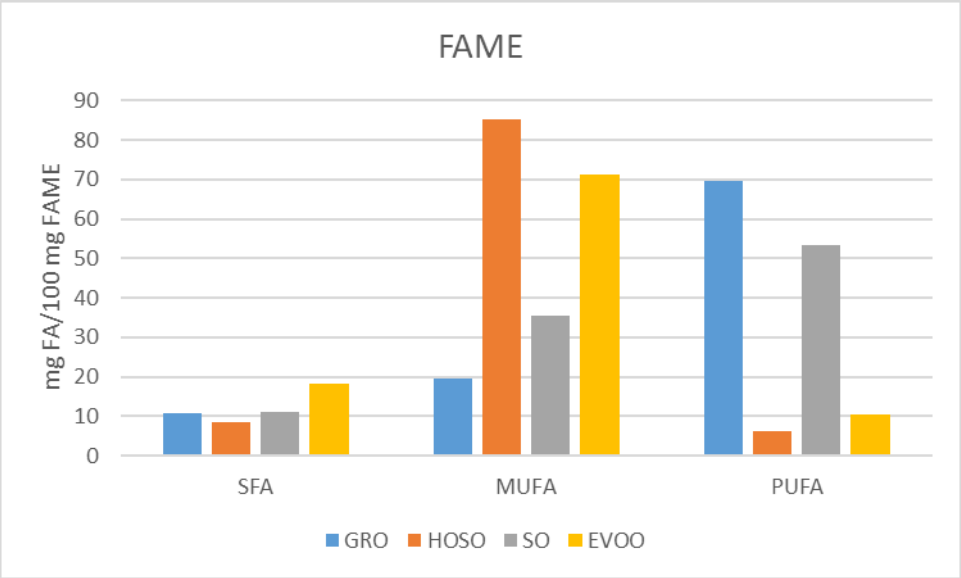

Figure 4.5. Graphical comparison of Saturated (SFA), Monounsaturated (MUFA), and Polyunsaturated (PUFA) Fatty Acid composition in GRO, HOSO, SO, and EVOO samples (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil). The fatty acid composition is expressed in mg/100 mg of FAME. Bars with different letters within each class (SFA, MUFA, PUFA) indicate statistically significant differences ($p \leq 0.05$).

Mild temperature stress

Determination of the peroxide value (PV) by iodometric titration

The quantification of the peroxide value in the analysed samples, determined as described in the Materials and Methods chapter, was conducted for all the four oil samples (EVOO, GRO, SO, HOSO) at the different times of heating at 100°C. The values obtained are expressed in meq of oxygen per kg of oil and are reported in Table 4.2.

Time of treatment (minutes)	EVOO	HOSO	SO	GRO
0	8.7±0.1	2.7±0.4	2.0±0.01	4.9±0.7
30	13.1±0.1	2.9±0.1	1.6±0.2	5.5±0.1
60	11.9±0.3	3.5±0.3	1.9±0.2	6.4±0.2
90	12.7±0.5	3.6±0.2	2.5±0.3	4.5±0.1
120	11.0±0.05	3.9±0.5	1.9±0.2	5.7±0.01
180	13.4±0.2	3.9±0.1	2.1±0.2	5.2±0.7
300	10.5±0.3	4.8±0.1	2.0±0.01	5.3±0.5

Table 4.2. PV values (meq O_2/kg of oil) for the 4 different kinds of thermally treated vegetable oils (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil).

PV is a legal parameter that allows oils to be marketed, but it must be borne in mind that below the legal value, peroxides, which are unstable compounds that can decompose into secondary oxidative products, can have small fluctuations such as those shown in the tab. A decrease in peroxides in a system such as the one studied, in any case, does not indicate a decrease in oxidation. Therefore, PV only provides an indication of the early stages of oxidation. For the EVOO sample, the PV at time zero (0h) is 8.71 meq O_2/kg of oil and it is in accordance with values reported by other authors (Giuffrè *et al.,* 2018). The concentration of peroxides appears to increase significantly after just half an hour of thermal treatment (13.1 meq O_2/kg of oil) and remains relatively stable until the fifth hour, at which point the peroxide value decreases and is no longer significantly different ($p \geq 0.05$) from the initial time. In a study by Allouche *et al.* (2007), where extra virgin olive oil samples were thermally treated at 180 °C, a decrease in PV was observed after 2 hours of treatment. The authors highlight that this coincided with a significant increase in ultraviolet absorption at 270 nm, likely due to the transformation of peroxides into other products such as aldehydes and ketones.

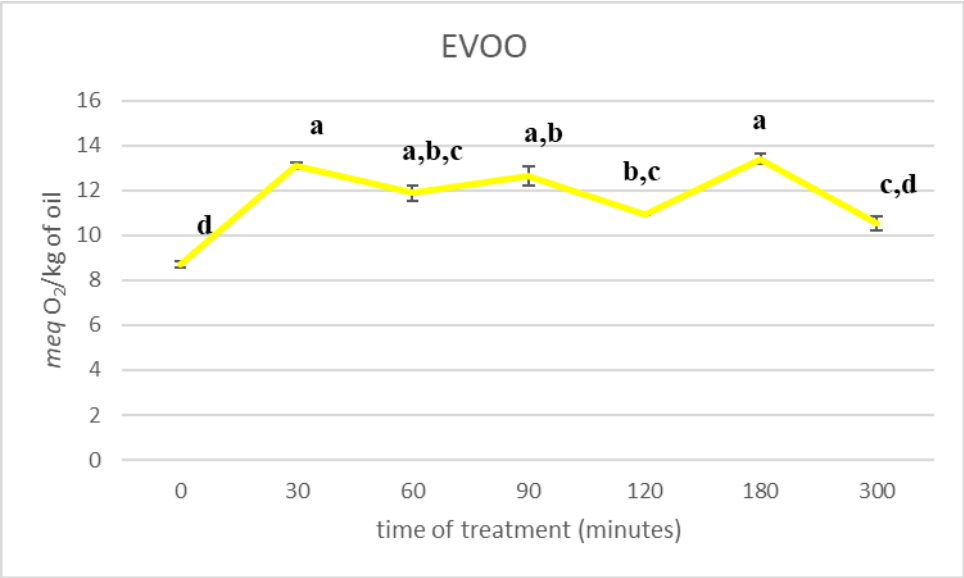

Figure 4.6. Graphical representation of the trend in peroxide value over time for the extra-virgin olive oil (EVOO) sample, expressed as meq O_2/kg of oil. Data points labelled with different letters for the various thermal treatment times are significantly different ($p \leq 0.05$)

As shown in Figure 4.7, the PV values obtained at different thermal treatment times for grape seed oil do not exhibit significant variations ($p \geq 0.05$). The only exception is observed at 1h and 1.5h, where the two PV values are significantly different from each other but not from those at other times. Other studies are in contrast with the findings of this analysis (Tekin, Aday and Yilmaz, 2009), reporting an increasing trend in peroxide value with prolonged treatment time. Specifically, in Tekin's study, the PV for oils thermally treated at 175°C for 25 hours increased from 0.66 to 1.89 meq O_2/kg of oil. It is worth noting, however, that in these cases, the initial peroxide content differs significantly from our findings, which show a much higher baseline value (4.9 meq O_2/kg of oil). Nevertheless, this is consistent with observations reported by Vaidya and Eun (2013).

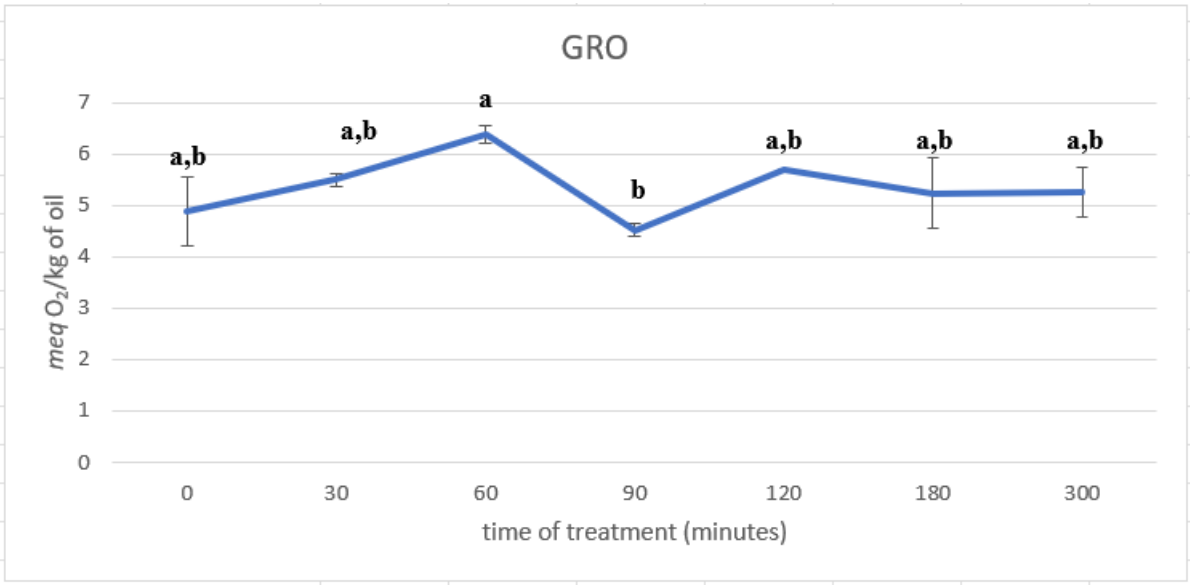

Figure 4.7. Graphical representation of the trend in peroxide value over time for the grapessed oil (GRO) sample, expressed as meq O_2/kg of oil. Data points labelled with different letters for the various thermal treatment times are significantly different ($p \leq 0.05$)

Figure 4.8 reports the peroxide value trend over time for the SO sample. Similarly to previous cases, apart from minor fluctuations, the values obtained at various thermal treatment times do not show significant differences ($p \geq 0.05$). Moreover, the peroxide concentration, both in the fresh product (0 h) and in the treated sample, is lower than what is reported in the literature, where values typically exceed 4 meq O_2/kg of oil on average (Abbas Ali *et al.,* 2013; Sadoudi *et al.,* 2013).

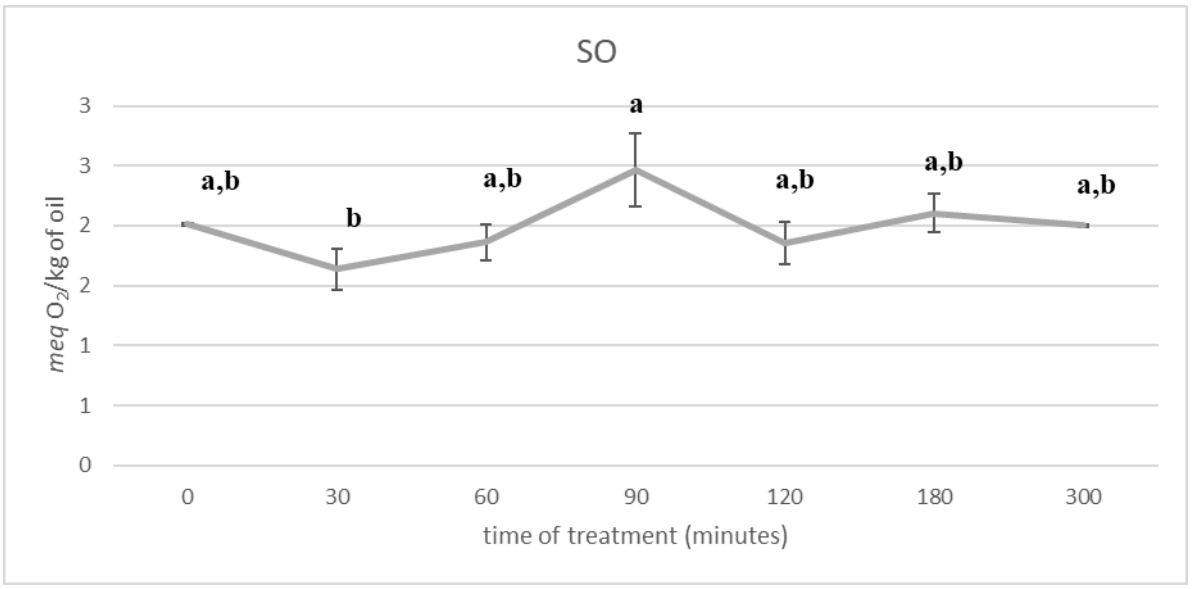

Figure 4.8. Graphical representation of the trend in peroxide value over time for the sunflower oil (SO) sample, expressed as meq O_2/kg of oil. Data points labelled with different letters for the various thermal treatment times are significantly different ($p \leq 0.05$)

As shown in Figure 4.9, high-oleic sunflower oil displays a different trend compared to all other samples, specifically a peroxide concentration that increases slowly with extended thermal treatment time. At time zero, the PV is 2.3 *meq* O_2/kg of oil, confirming the findings of Abbas Ali *et al.* (2013), rising to a PV of 4.8 meq O_2/kg of oil after five hours of treatment at 100°C, which are significantly different results. The intermediate values, from 0.5 hours to 3 hours, although exhibiting increasing absolute values, are not statistically different from each other ($p \geq 0.05$) and range within a PV interval between 2.9 and 3.9 meq O_2/kg of oil.

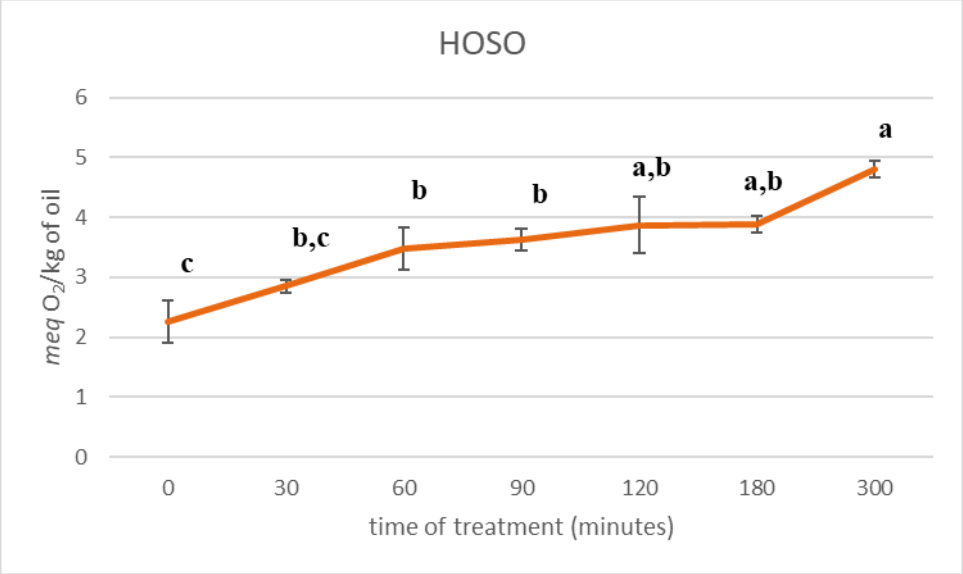

Figure 4.9. Graphical representation of the trend in peroxide value over time for the high-oleic sunflower oil (HOSO) sample, expressed as meq O_2/kg of oil. Data points labelled with different letters for the various thermal treatment times are significantly different ($p \leq 0.05$)

Comparing the peroxide content trends across the oil samples (Figure 4.10), it is evident that EVOO consistently shows higher values than the other samples. This difference may be since, unlike the other three types of oils which are refined oils EVOO does not undergo the refining process. While this allows EVOO to retain higher concentrations of antioxidant substances such as α-tocopherol and phenols, the absence of refining also means that compounds acting as prooxidants (e.g., free fatty acids, phospholipids, trace metals) remain in the product. Nonetheless, the PV values obtained in this study remain below the maximum legal thresholds. For extra virgin olive oil, the legal limit is 20 meq O_2/kg of oil (Codex Alimentarius), while for other vegetable oils, the maximum permissible limit is 10 meq O_2/kg of oil (Codex Alimentarius). Some prior studies (Abbas Ali *et al.,* 2013; Petersen *et al.,* 2012) show trends different from those observed in this study. Specifically, the PV values in high-oleic sunflower oils are usually lower than those in (low-oleic) sunflower oils, attributed to the higher linoleic acid content in the latter. This difference is likely due

to variations in the thermal treatment of the oils, as the cited studies involve oxidative stress tests conducted at higher temperatures and/or for significantly longer periods.

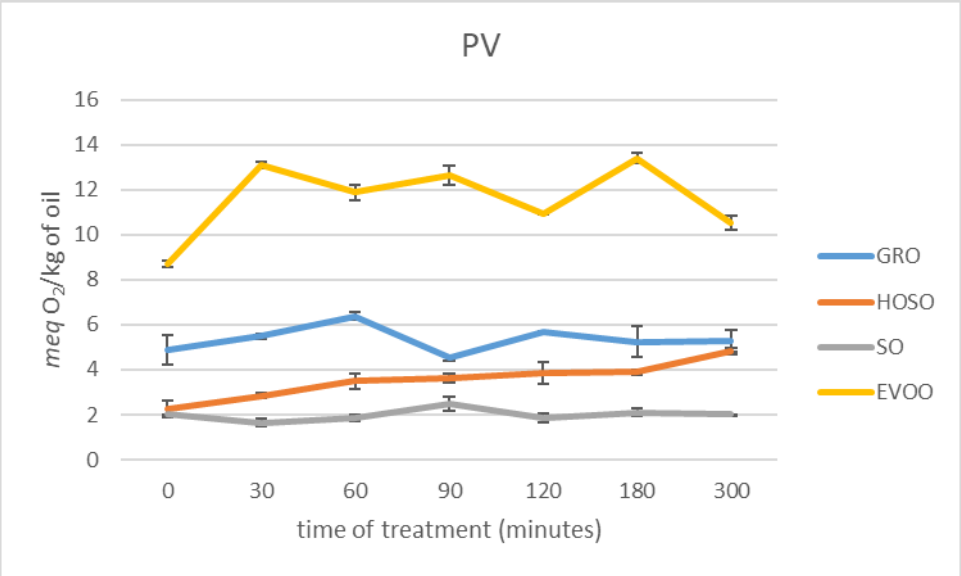

Figure 4.10. Graphical representation of the overall trends in peroxide value over time for the analysed oil samples (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil), expressed as meq O_2/kg of oil.

In Figure 4.11, the PV values of the four oils are also compared through a histogram graph, highlighting more clearly the differences observed at the various applied thermal treatment times. Firstly, EVOO consistently shows significantly higher PV values ($p \leq 0.05$) across all time points, with concentrations averaging 2 (GRO), 3 (HOSO), and 6 (SO) times greater than those of the other oils. Following EVOO, grape seed oil (GRO) exhibits the next highest peroxide value, although its PV does not differ significantly from HOSO at 1.5h, 3h, and 5h. Finally, SO and HOSO initially show similar PV values at time zero (0h) but diverge in subsequent time points, with SO having significantly lower PV. HOSO, on the other hand, starts with lower PV values but then rises to concentrations that do not significantly differ from GRO at the final two time points (3h and 5h). Interestingly, at the intermediate time point (1.5h), the three refined oils (GRO, SO, and HOSO) do not show significantly different PV values ($p \leq 0.05$). Tekin *et al.* (2009), in their study where oil samples were heated at 175°C for 5 hours per day over five consecutive days, observed a greater increase in PV in low-oleic sunflower oil (from 1.84 to 5.87 meq O_2/kg of oil) compared to grape seed oil (from 0.66 to 1.89 meq O_2/kg of oil). The authors attribute this phenomenon, despite both oils being predominantly composed of PUFA, to the higher content of minor components such as phenols, sterols, and tocopherols in grape seed oil. This trend is not observed in our study due to the different experimental conditions: oils were treated at a much

milder temperature (100 °C) and for significantly shorter times (a maximum of 5 hours). This approach was designed to investigate whether and how oxidation progresses under non-extreme conditions. Indeed, in our study, GRO shows higher PV values than SO. This could be related to a more advanced oxidation state in SO, where peroxides may have already degraded into secondary oxidation products. Alternatively, the applied temperature and duration might not be sufficient to induce excessive degradation in the studied oils, despite their compositional differences.

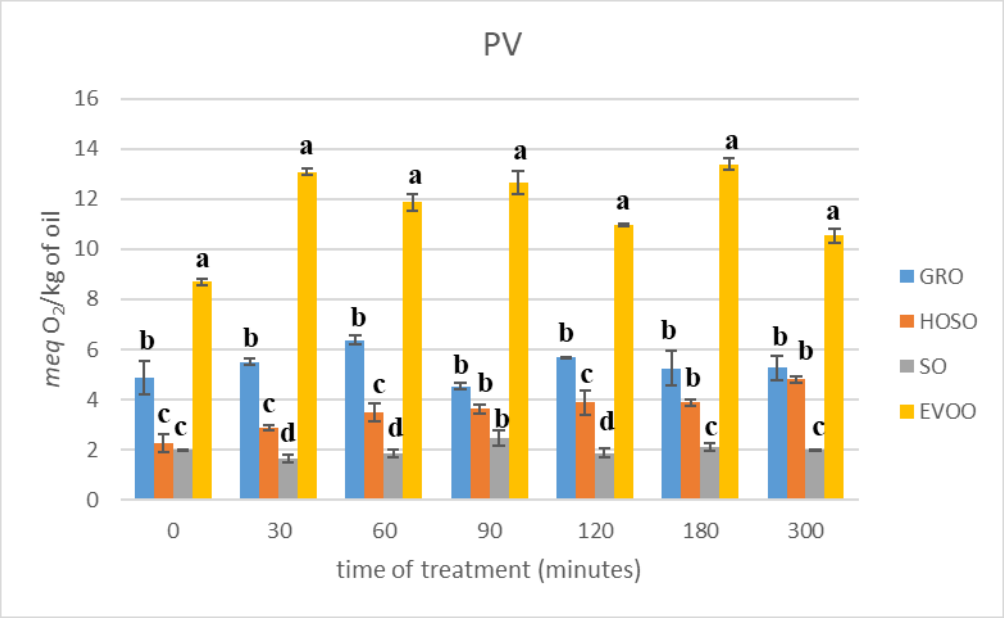

Figure 4.11. Graphical representation of the peroxide value over time for the analyzed oils (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil) expressed as meq O_2/kg of oil. Bars labeled with different letters within each thermal treatment time indicate significantly different PV values among the various oils ($p \leq 0.05$).

Volatile compounds determination by SPME-GC-MS

This analysis was conducted to identify and quantify the compounds characterizing the volatile fraction of the oils under study. The objective was to define the aromatic profile of the samples, enabling the monitoring of potential variations related to the raw materials as well as the thermal treatment durations employed during the analysis. When oil samples undergo oxidation, a significant quantity of aromatic compounds—including alcohols, esters, ketones, and aldehydes—is produced. Unsaturated fatty acids are the primary precursors of volatile compounds in oxidized samples (Morales *et al.,* 1997). The progression of oxidative processes in refined vegetable oils is marked by an increase in both total volatile compounds and specific ones, such as hexanal. As previously mentioned, extra virgin olive oil (EVOO) inherently contains a substantial

amount of volatile compounds, including hexanal. Therefore, characterizing the oxidative trend for EVOO may present additional complexity (Morales *et al.,* 1997). The results regarding hexanal concentration obtained in this study are summarized in Table 4.3, expressed as mcg/g of fat.

Time of treatment (minutes)	EVOO	HOSO	SO	GRO
0	21.0±2.7	5.1±0.3	2.1±0.1	10.8±0.8
30	22.5±0.6	6.4±0.4	1.4±0.2	5.7±0.3
60	21.7±0.5	4.8±0.9	1.8±0.1	7.8±0.1
90	16.0±2.8	8.4±0.1	2.2±0.1	5.4±0.6
120	24.8±0.9	5.5±0.7	2.9±0.2	13.3±0.2
180	23.2±0.9	18.2±0.5	1.8±0.2	12.1±0.9
300	128.3±1.2	9.6±1.0	2.8±0.3	8.9±0.6

Table 4.3. Hexanal content for the 4 different kinds of vegetable oils analysed at the different treatment times, the results are expressed as mcg of hexanal/g of fat (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil).

For the EVOO sample, Figure 4.12 shows that the hexanal concentration remains unchanged during the first three hours of thermal treatment, fluctuating between 15.95 mcg/g. However, it increases significantly after five hours of heating at 100°C, reaching a value of 128.25 mcg/g. Similar results have been reported by Morales *et al.,* (1997), who observed that during up to five hours of thermal treatment at 100°C, the hexanal concentration in EVOO samples remained low but then rose sharply thereafter.

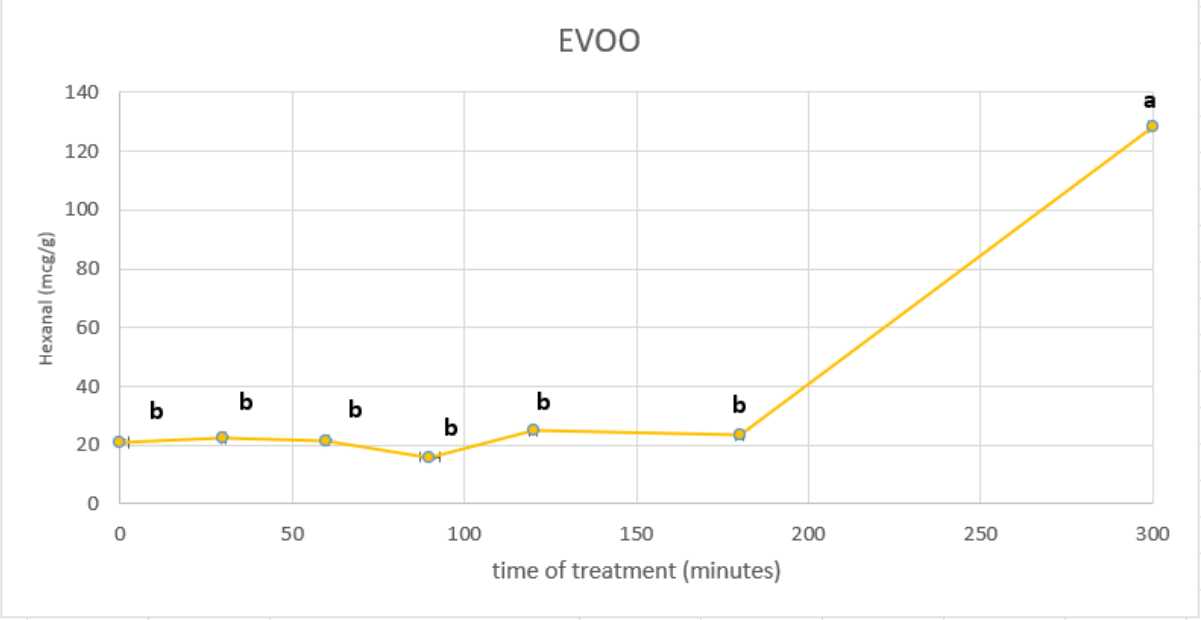

Figure 4.12. Graphical representation of the trend in hexanal content over time for the extra-virgin olive oil (EVOO) sample, expressed as mcg of hexanal/g of fat. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

The trend in hexanal concentration in grape seed oil shows significant fluctuations over the treatment period, as illustrated in Figure 4.13. Specifically, the initial content in fresh oil is 10.78 mcg/g, which then decreases significantly during the next three time points, reaching a minimum value of 5.37 mcg/g after 1.5 hours. At 2 hours of treatment, the hexanal area increases markedly, reaching its maximum value (13.29 mcg/g), and finally decreases again at 5 hours, returning to a level similar to the initial one (8.86 mcg/g). Kiralan *et al.* (2019) investigated the volatile profile of grape seed oil stored at 60°C for several days. Their results align with those observed in our study, although their hexanal trend showed a more linear increase after six days at 60°C.

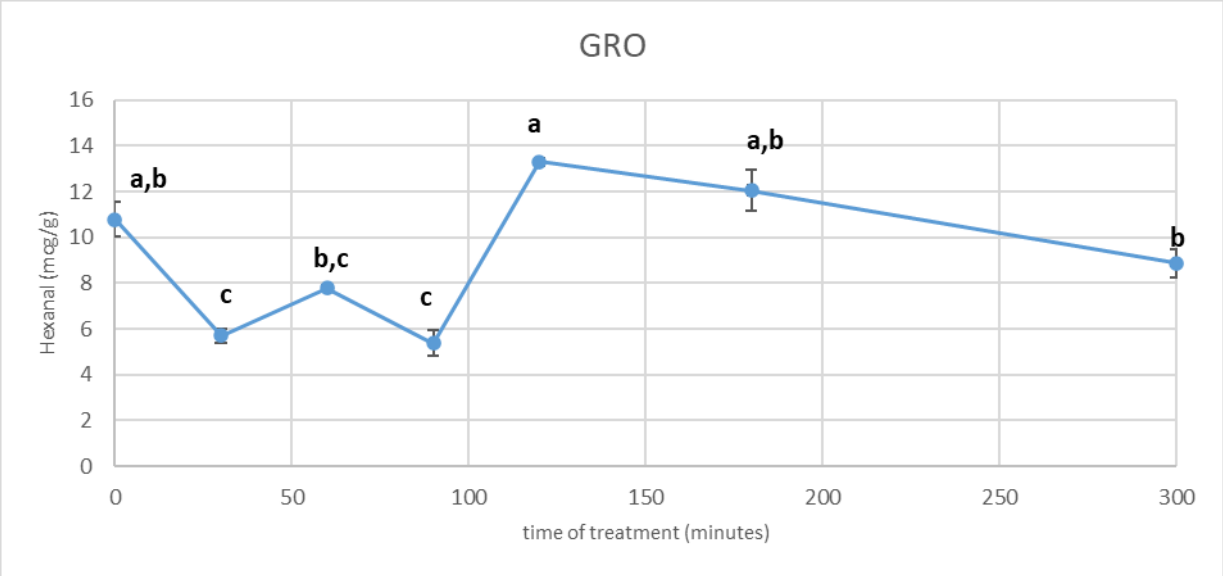

Figure 4.13. Graphical representation of the trend in hexanal content over time for the grapessed oil (GRO) sample, expressed as area. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

The trend in hexanal concentration in SO oil is illustrated in Figure 4.14 The values become significantly higher after 1.5 hours of thermal treatment, peaking at 2.86 mcg/g at 2 hours. At 3 hours, the concentration drops substantially, aligning with the initial levels (1.81 mcg/g), before rising again to another peak at 6 hours.

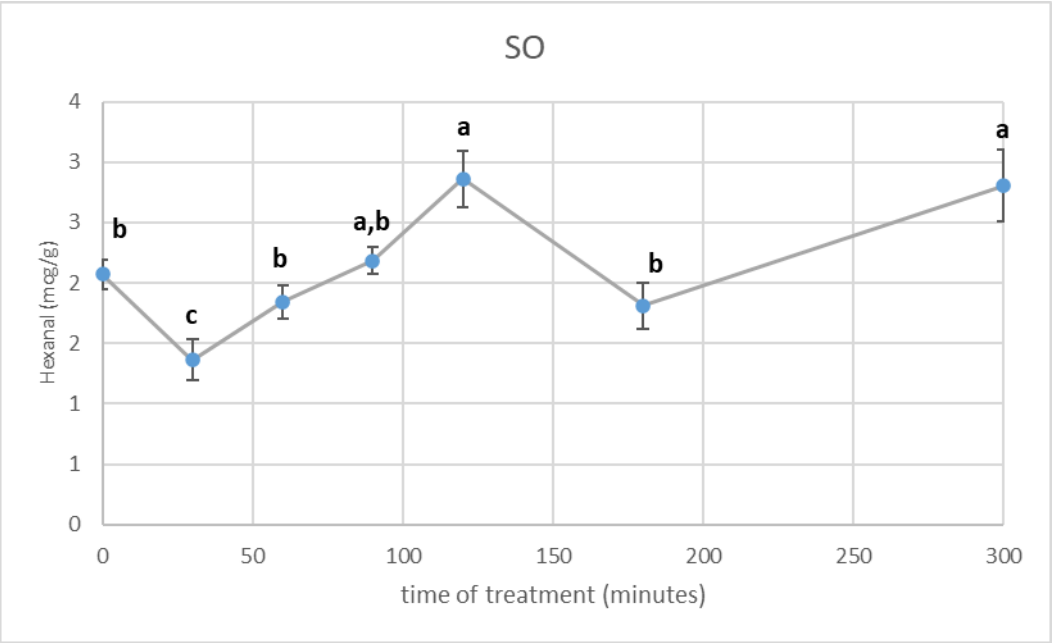

Figure 4.14. Graphical representation of the trend in hexanal content over time for the sunflower oil (SO) sample, expressed as area. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

A different trend was observed for sunflower oil (HOSO), where hexanal concentrations remained relatively low and not significantly different ($p \leq 0.05$) from each other during the first 2 hours of thermal treatment, with values ranging between 4.8 and 8.35 mcg/g at 3 hours of heating at 100°C, however, hexanal levels rose sharply, reaching 18.15 mcg/g, before decreasing again after the fifth hour (5h) of thermal treatment.

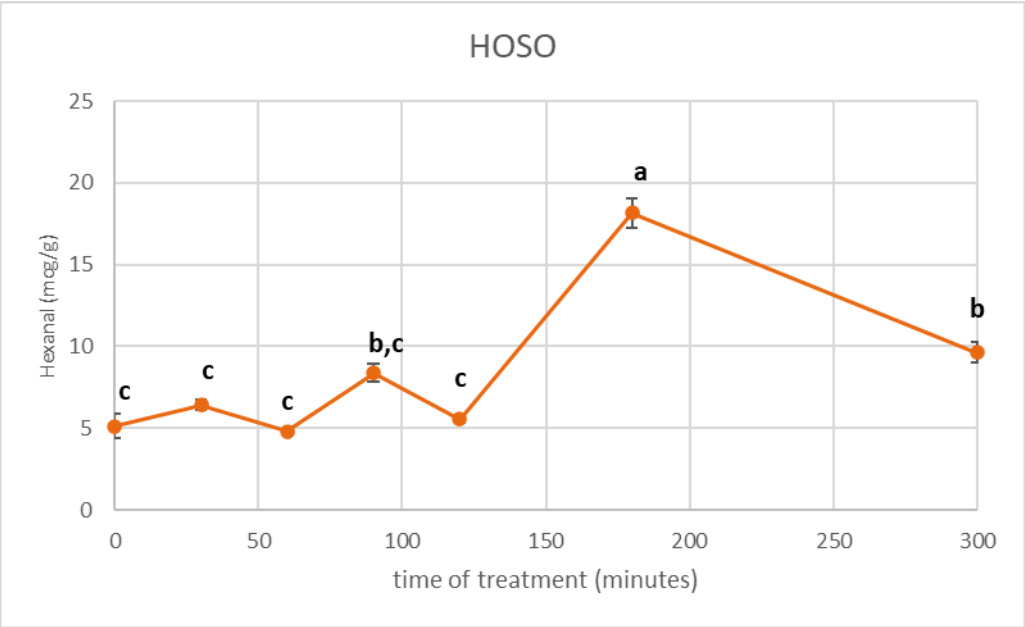

Figure 4.15. Graphical representation of the trend in hexanal content over time for the high-oleic sunflower oil (HOSO) sample, expressed as area. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

Figure 4.16 illustrates the trends for all four oil samples, highlighting that EVOO consistently exhibits a higher hexanal content compared to oils of different vegetable origins. This observation aligns with findings reported by Morales *et al.* (1997), who noted that hexanal, although associated with oil oxidation, is already present in the initial aromatic profile of extra virgin olive oil. This is because it is produced from linoleic acid via the lipoxygenase pathway. From a sensory perspective, hexanal in EVOO is a key aromatic compound that contributes to its sweet aroma, enhancing the oil's quality and uniqueness.

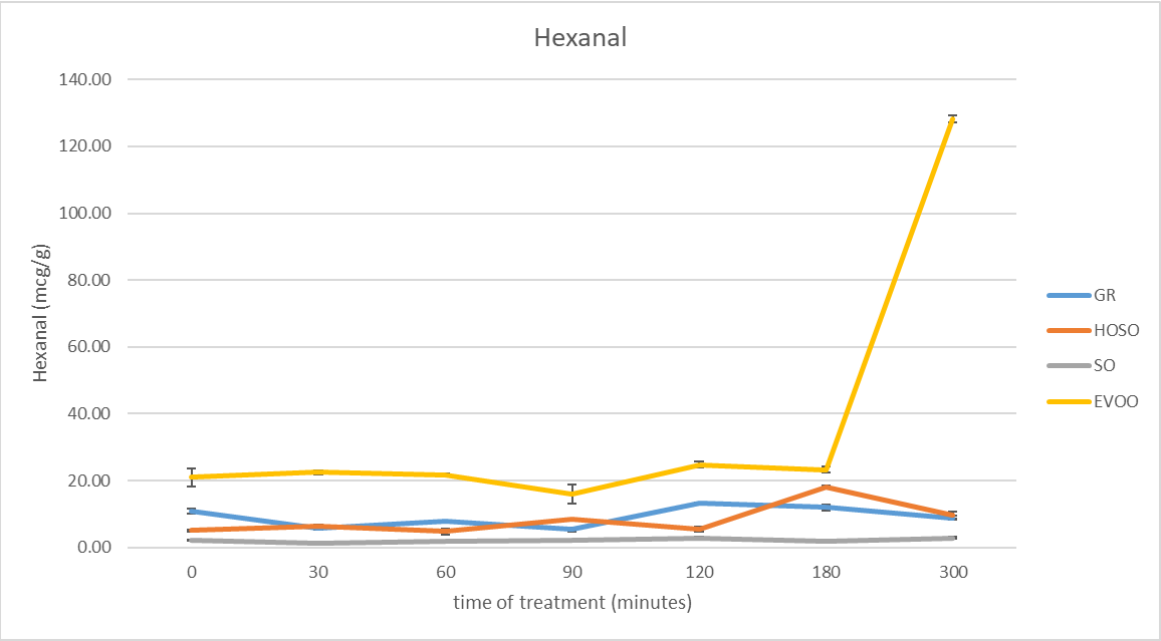

Figure 4.16. Graphical representation of the overall trend in hexanal content over time for EVOO, GRO, SO, and HOSO, expressed as area (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil). Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

Figure 4.17, designed to better visualize the differences between the various samples at different time points, clearly shows that the hexanal concentration is significantly higher ($p \leq 0.05$) in the EVOO sample across all time points. From a content perspective, the trend for hexanal closely mirrors that observed for peroxide values. Similarly, the SO sample consistently shows the lowest hexanal values at all time points, except at the initial time point, where it does not significantly differ ($p \leq 0.05$) from HOSO. In contrast, SO exhibits slightly higher values, often aligning ($p \leq 0.05$) with GRO (e.g., at 0.5 h, 1.5 h, and 5 h). A notable observation occurs at the intermediate time point (1.5 h), where the three refined oils (GRO, SO, and HOSO) do not display significantly different values ($p \leq 0.05$). However, unlike what was observed here, where hexanal values for sunflower oils SO and HOSO are almost always significantly different ($p \leq 0.05$) across all time points, the literature (Petersen *et al.* 2012) concludes that hexanal alone is insufficient to differentiate these two oils. They recommend a combined assessment with other compounds, such as E-2-heptanal and E-2-decenal, for more accurate differentiation.

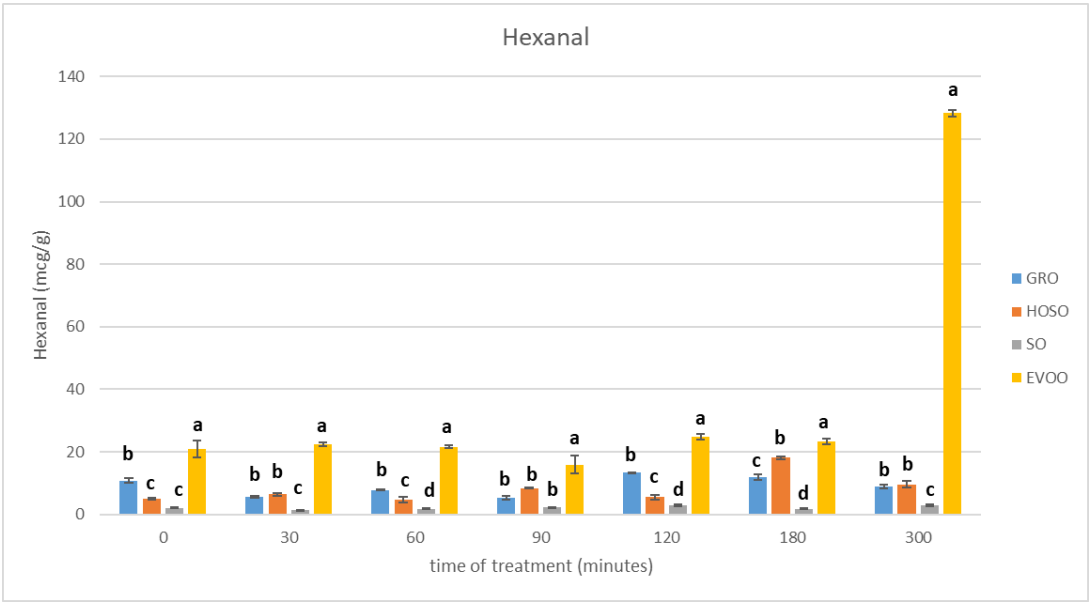

Figure 4.17. Graphical representation of the hexanal content over time for the analysed oils (EVOO, GRO, SO, and HOSO), expressed as mcg of hexanal / g of fat (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil). Bars labelled with different letters within each thermal treatment time indicate significantly different hexanal values among the various oils ($p \leq 0.05$).

Determination of the p-anisidine value (p-AV)

The *p*-anisidine value was determined to assess the secondary oxidation of oils, and the results obtained for the studied samples are reported in Table 4.4.

The determination of the p-anisidine value is an appropriate method for evaluating the secondary products of lipid oxidation and is correlated with the formation of non-volatile aldehydes (2-alkenals) and ketones, which partially contribute to the rancid odour and flavour in fats. The importance of the *p*-anisidine value, especially in refined oils, lies in the minimal effect of deodorization on the removal of secondary oxidation products, whereas it reduces peroxide values. For this reason, if a vegetable oil has undergone severe and prolonged oxidative damage before refining, it can be detected through the determination of the p-anisidine value (Giuffrè *et al.,* 2018). Abbas Ali *et al.,* (2013) consider this index a more reliable and significant test than the peroxide value, as it measures secondary oxidation products, which are known to be more stable during thermal processing.

Time of treatment (minutes)	EVOO	HOSO	SO	GRO
0	7.8±0.8	2.9±0.3	6.5±0.00	7.2±0.4
30	9.6±0.6	3.2±0.1	6.2±0.2	6.5±0.1
60	8.4±0.2	3.6±0.4	5.7±0.01	6.4±0.3
90	7.7±0.05	3.3±0.01	5.9±0.2	6.5±0.02
120	7.2±0.8	3.0±0.02	5.0±0.2	6.1±0.1
180	8.5±0.1	2.9±0.04	6.9±0.02	6.6±0.3
300	9.4±0.03	3.4±0.2	6.3±0.2	6.7±0.12

Table 4.4 p-Anisidine value for the 4 different kinds of vegetable oils analysed at the different treatment times, the results are expressed as Anisidine Value (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil).

Regarding the p-anisidine value for the EVOO sample, no significant differences ($p \geq 0.05$) were observed during thermal treatment (Figure 4.18), with values fluctuating between 7.2 (at 2 h) and 9.6 (at 0.5 h). Giuffrè *et al.* (2018) reported a p-anisidine value of 4.4 in fresh extra virgin olive oil, which increased to 7.4 in samples subjected to thermal treatment at 180°C for 30 minutes. Akil *et al.* (2015) quantified values like those reported by Giuffrè for fresh product. A study by Kar Lin *et al.* (2014), simulating domestic frying at 190°C, reported an initial *p*-anisidine value of 2.12 in fresh oil, which rose to 28.77 after 90 minutes of thermal treatment.

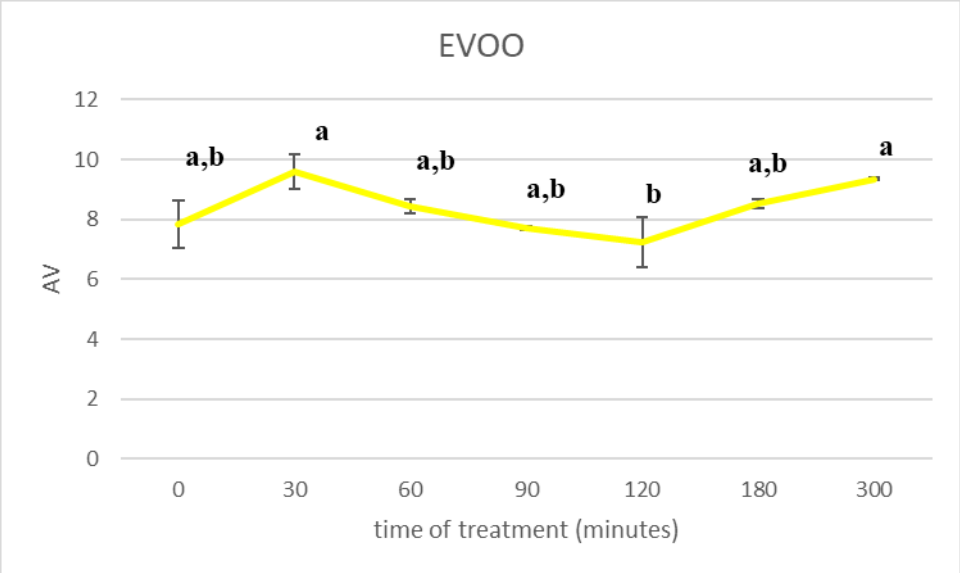

Figure 4.18. Graphical representation of the trend in Anisidine value content over time for the extra-virgin olive oil (EVOO) sample, expressed as AV. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

As shown in Figure 4.19, the p-anisidine value for grape seed oil remains relatively stable, fluctuating between 6.1 (after 2 hours of heating) and 7.2 (recorded at time zero,

i.e., in the fresh product). Other studies report higher p-anisidine values at time zero compared to those observed in this study. For example, Vaydia *et al.* (2013) reported a value of 11.88 in fresh grape seed oil, attributing the elevated value to the high temperatures used during the refining process, particularly during deodorization, which promotes the formation of carbonyl compounds that increase this indicator. Similarly, Kar Lin *et al.* (2014) found a higher p-anisidine value of 9.40 in fresh grape seed oil. Moreover, in their study, thermal treatment of the oil at 180°C for 30 minutes resulted in a significantly elevated value of 28.34, a situation that was not observed in the present study.

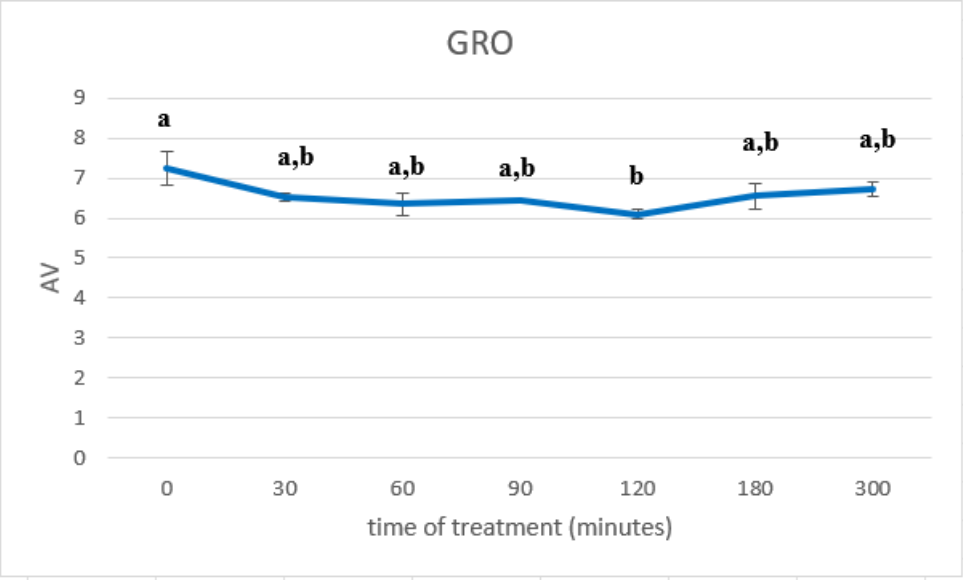

Figure 4.19. Graphical representation of the trend in Anisidine value content over time for the grapeseed oil (GRO) sample, expressed as AV. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

For the SO sample, there appears to be a slight, gradual decrease in the p-anisidine value from 0 to 2 hours of heating (Figure 4.20), reaching a minimum value of 5. At the third hour of treatment, the value rises significantly ($p \geq 0.05$), returning to the initial levels of over 6. Abbas Ali *et al.* (2013) reported a p-anisidine value for fresh high-oleic sunflower oil that aligns with the value observed in this study (5.12). However, following thermal treatment at 185 °C for 4 hours, they recorded values approximately ten times higher (50.01), a substantial increase not observed in this experiment.

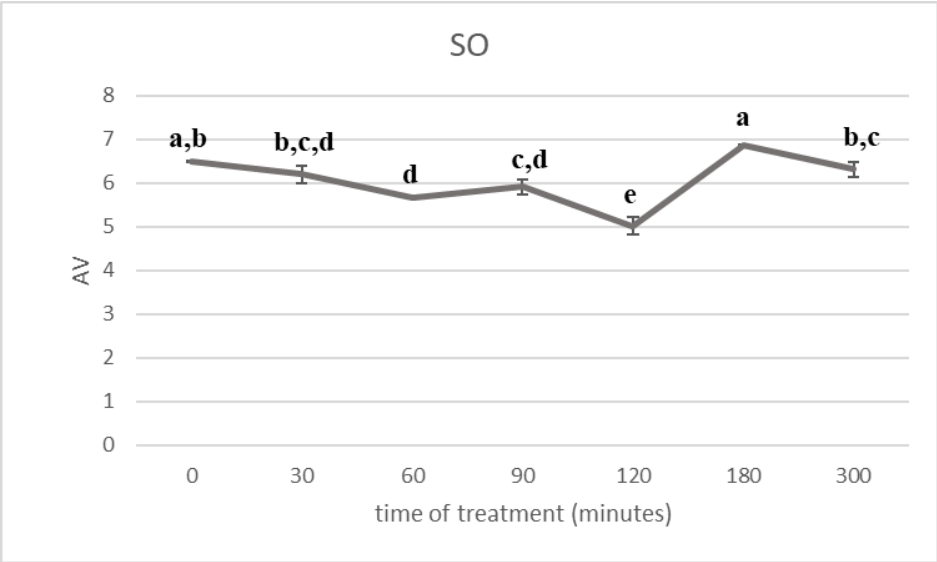

Figure 4.20. Graphical representation of the trend in Anisidine value content over time for the sunflower oil (SO) sample, expressed as AV. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

Finally, as shown in Figure 4.21, the p-anisidine values (*p*-AV) detected in sunflower oil (HOSO) do not exhibit significant differences ($p \geq 0.05$) at any of the time points considered. A similar result was observed by Petersen *et al.,* (2012), who reported a p-AV for fresh high-oleic sunflower oil consistent with the values found in this study. In their shelf-life tests, where the oil was subjected to stress at 80°C for 14 days, no significant changes in p-AV were observed. According to the authors, these findings can be attributed to the fatty acid composition, which is predominantly monounsaturated. This composition provides greater oxidative stability and limits the formation of secondary oxidation products.

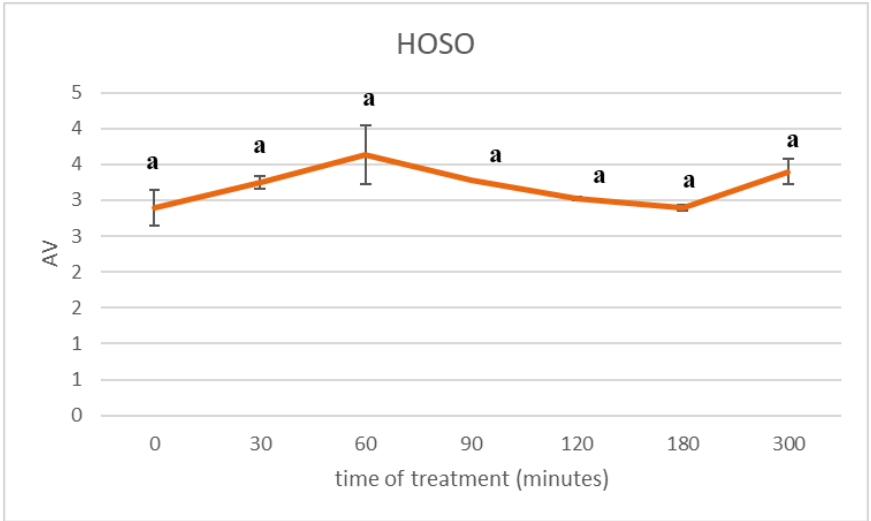

Figure 4.21. Graphical representation of the trend in Anisidine value content over time for the high-oleic sunflower oil (HOSO) sample, expressed as AV. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

Akil *et al.* (2015) evaluated the *p*-anisidine value (*p*-AV) for various cultivars of extra virgin olive oil and some seed oils, specifically soybean oil and low-oleic sunflower oil, treated at 180°C for 75 minutes. Their results showed that EVOO samples consistently had lower *p*-AV values than seed oils across all time intervals considered. As shown in Figures 5.22 and 5.23, in this study, EVOO exhibited higher *p*-anisidine values compared to the other oils tested. This difference may be due to the fact that the *p*-anisidine test quantifies secondary oxidation products derived from the degradation of hydroperoxides. In the peroxide value chapter, it was noted that EVOO had the highest peroxide values across all time intervals considered. Consequently, EVOO contains a greater number of primary oxidation products, which serve as precursors to the secondary products measured by this analysis. Kar Lin *et al.* (2015) also emphasized that in most cases, vegetable oils with high peroxide values tend to show elevated p-anisidine values. From the graphs provided, it is clear that SO and GRO oils exhibit similar *p*-AV content ($p \geq 0.05$), while the sunflower oil (HOSO) shows significantly lower *p*-AV values ($p \geq 0.05$) across all time intervals. A similar finding was reported by Abbas Ali *et al.* (2013), who highlighted that high-oleic sunflower oil, due to its lower PUFA content, experiences a smaller p-AV increase than low-oleic sunflower oil. In their study, oils subjected to thermal treatment at 185 °C for a total of 24 hours showed a p-AV increase ranging from 5.12–94.90 for sunflower oil and 3.35–77.81 for high-oleic sunflower oil. The authors attributed the differing levels of secondary oxidation products to variations in fatty acid composition, which influence the formation of these products from primary oxidation products during heating. Compared to the literature (Abbas Ali *et al.,* 2013; Petersen *et al.,* 2012), Figure 46 shows that the trend in p-anisidine values for all four oils tested in this study resembles a horizontal line. This observation highlights that thermal treatment at much milder temperatures (80–100°C) and for shorter durations (up to 5 hours) does not result in a significant increase in this index, which is often used to evaluate the quality of frying oils exposed to high thermal stress.

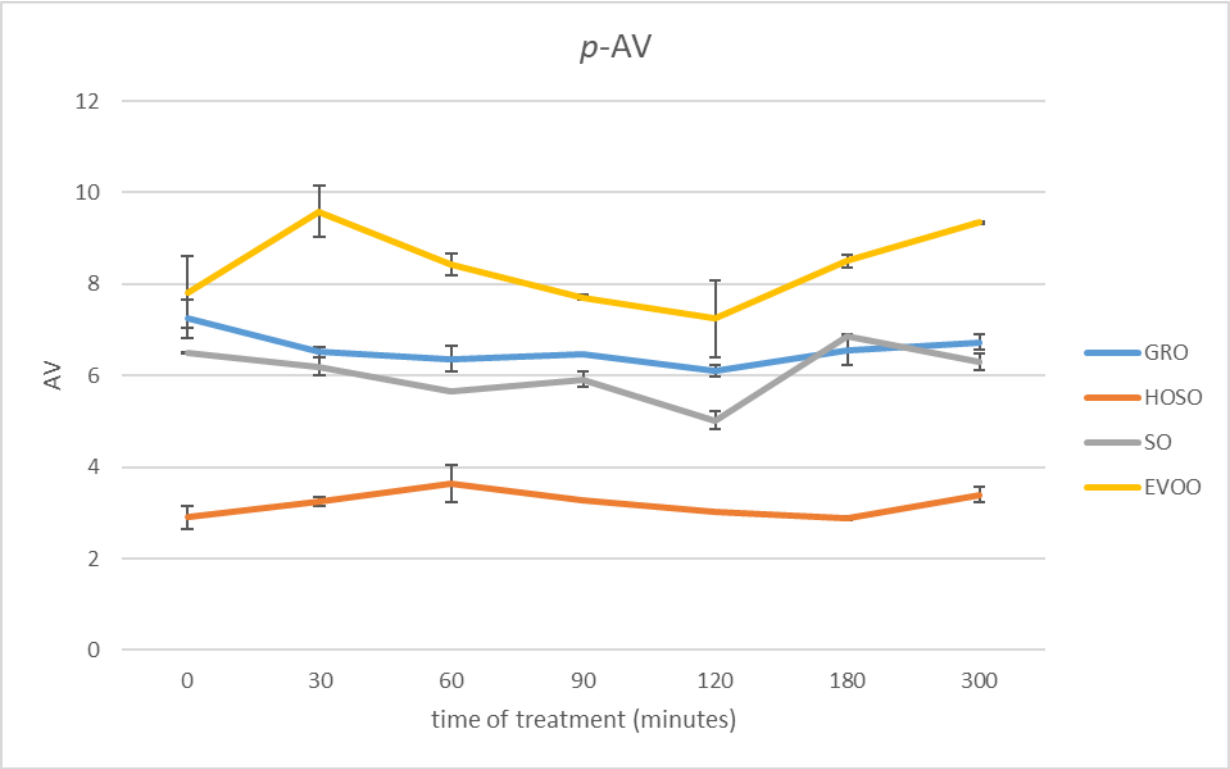

Figure 4.22. Graphical representation of the overall trend in Anisidine value over time for EVOO, GRO, SO, and HOSO, expressed as AV (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil).

In Figure 4.23, the *p*-AV values for the four oils at different treatment times are compared more clearly and directly. As previously mentioned, the EVOO oil consistently shows the highest *p*-anisidine values, although its initial value does not differ significantly from those of GRO and SO. The latter two oils consistently exhibit similar values, which supports the observations previously cited from Abbas Ali *et al.* (2013), that this measure is correlated with the polyunsaturated fatty acid (PUFA) content. Indeed, V and BO oils have comparable PUFA compositions. Finally, the sunflower oil HOSO exhibits the lowest *p*-AV values, which are significantly different ($p \geq 0.05$) from those of the other oils throughout the time interval considered. Notably, these values always remain below 4, the maximum limit permitted according to the Codex Alimentarius (Petersen *et al.,* 2012).

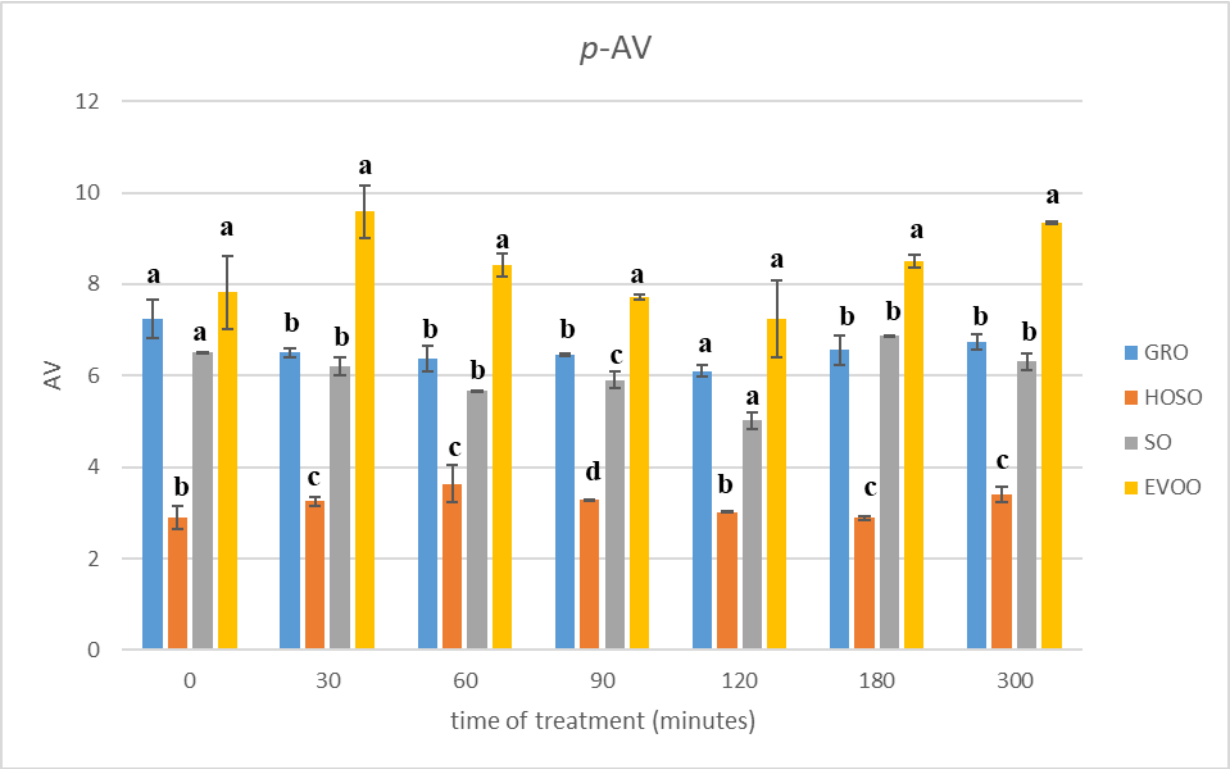

Figure 4.23. Graphical representation of the p-anisidine content over time for the analysed oils (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil), expressed as Anisidine Value. Bars labelled with different letters within each thermal treatment time indicate significantly AV values among the various oils ($p \leq 0.05$).

Determination by gas chromatographic analysis of oxidized fatty acids (OFA)

Analytical techniques for evaluating heated oils and fats, or those used in frying, are essential for elucidating structures and quantifying oxidation compounds that may compromise the nutritional value of lipid-rich foods. New compounds formed during oxidation include triglyceride polymers, dimers, oxidized triglyceride monomers, diacylglycerols, and free fatty acids. Among these, the quantification of oxidized fatty acids (OFA) is prioritized, as they represent the most abundant group of oxidized compounds in foods (Velasco *et al.,* 2004). So far, the impact of mild thermal treatment has been assessed using classical indices and methods aimed at determining primary (peroxides) and secondary (volatiles, aldehydes, and ketones) oxidation products. To complement these methods, the determination of OFA has been applied, enabling the detection of oxygenated compounds that retain the carbon chain length of the corresponding fatty acid, such as aldehydes, ketones, epoxides, and alcohols. This methodology, although not widely applied, offers certain advantages. Unlike the p-anisidine value, this determination is not influenced by the presence of conjugations within the molecule (such as conjugated dienes and trienes), which are known to limit

the application of spectrophotometric methods (such as *p*-anisidine determination) when referencing common threshold values across different matrices. The *p*-anisidine value may lead to overestimation errors, whereas the OFA method avoids this issue as it does not rely on spectrophotometric readings, making the analysis more reliable. The sensitivity of this simple method was tested in this study, particularly its ability to detect even minor increases in oxidation. The limited scientific literature available on the quantification of OFA in oils following thermal treatment complicates comparisons and data interpretation. However, it also highlights the innovative and exploratory nature of this thesis work. The data collected are summarized in Table 4.5, expressed as mcg OFA/mg fat.

Time of treatment (minutes)	EVOO	HOSO	SO	GRO
0	0±0	0±0	0±0	0±0
30	19.4±2.2	21.4±0.9	12.7±0.3	10.2±1.3
60	17.2±1.2	30.6±2.2	32.6±1.2	28.5±2.2
90	15.9±1.8	25.8±1.4	33.3±1.9	24.0±0.9
120	19.0±2.7	28.0±0.5	30.3±0.2	27.0±0.6
180	21.9±2.9	33.3±0.3	26.59±1.0	29.7±0.1
300	20.7±1.3	40.9±0.5	31.21±0.2	34.0±1.2

Table 4.5. OFA content, expressed as mcg/mg of fat, for the four different types of vegetable oils analysed (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil).

For EVOO, as shown in Figure 4.24, the OFA content does not vary significantly during the thermal treatment at 100°C, with values oscillating between 15.9 mcg/mg and 22 mcg/mg.

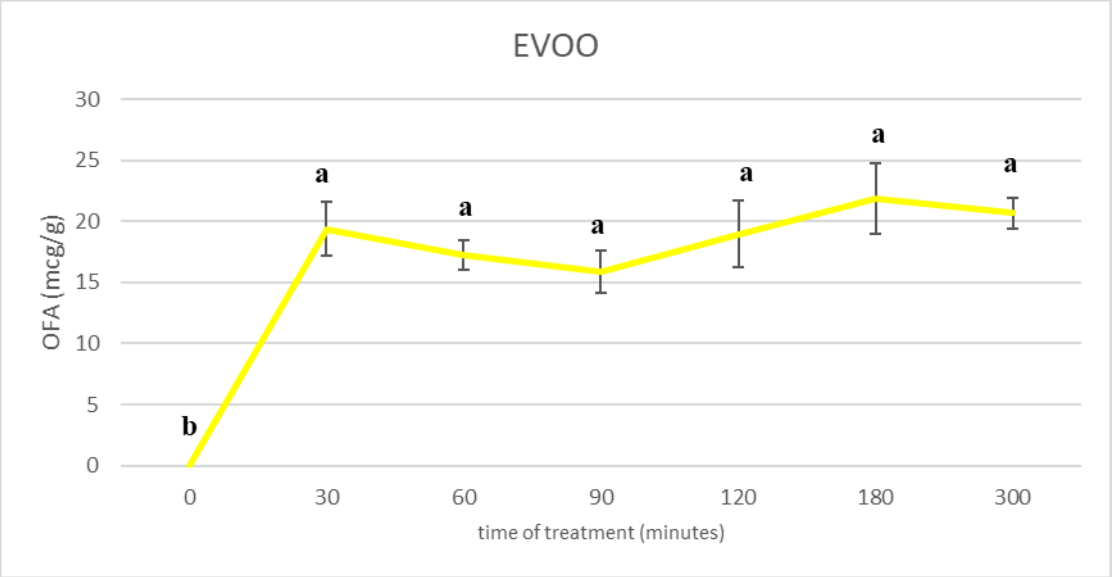

Figure 4.24. Graphical representation of the trend in OFA content over time for the extra-virgin olive oil (EVOO) sample, expressed as mcg/mg of fat. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

For the GRO sample, as shown in Figure 4.25, the initial OFA content is very low, at 9.6 mcg/mg, but increases significantly ($p \leq 0.05$) after 1 hour of treatment, reaching a value approximately three times higher (~30 mcg/mg). This elevated value remains unchanged throughout the subsequent time intervals.

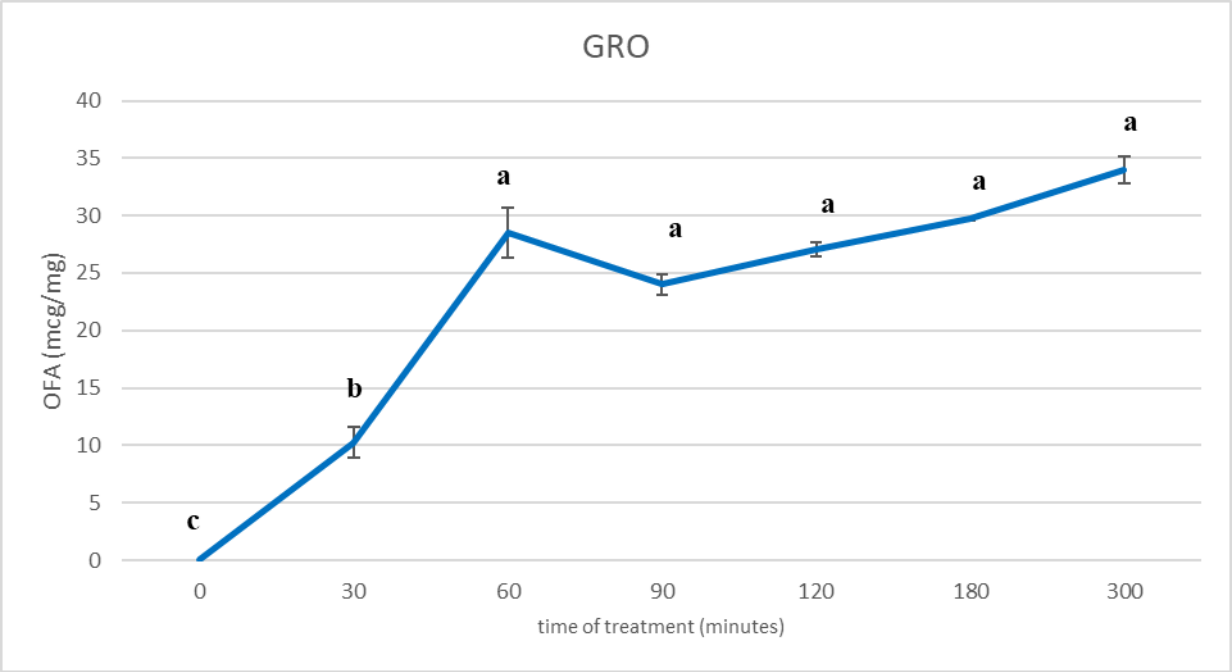

Figure 4.25. Graphical representation of the trend in OFA content over time for the grapeseed oil (GRO) sample, expressed as mcg/mg of fat. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

The SO sample exhibits an identical trend, as shown in Figure 4.26. It also starts with a very low initial OFA content (11.9 mcg/mg) and increases significantly after 1 hour of heating to statistically higher values, which remain unchanged ($p \geq 0.05$) throughout the entire treatment period.

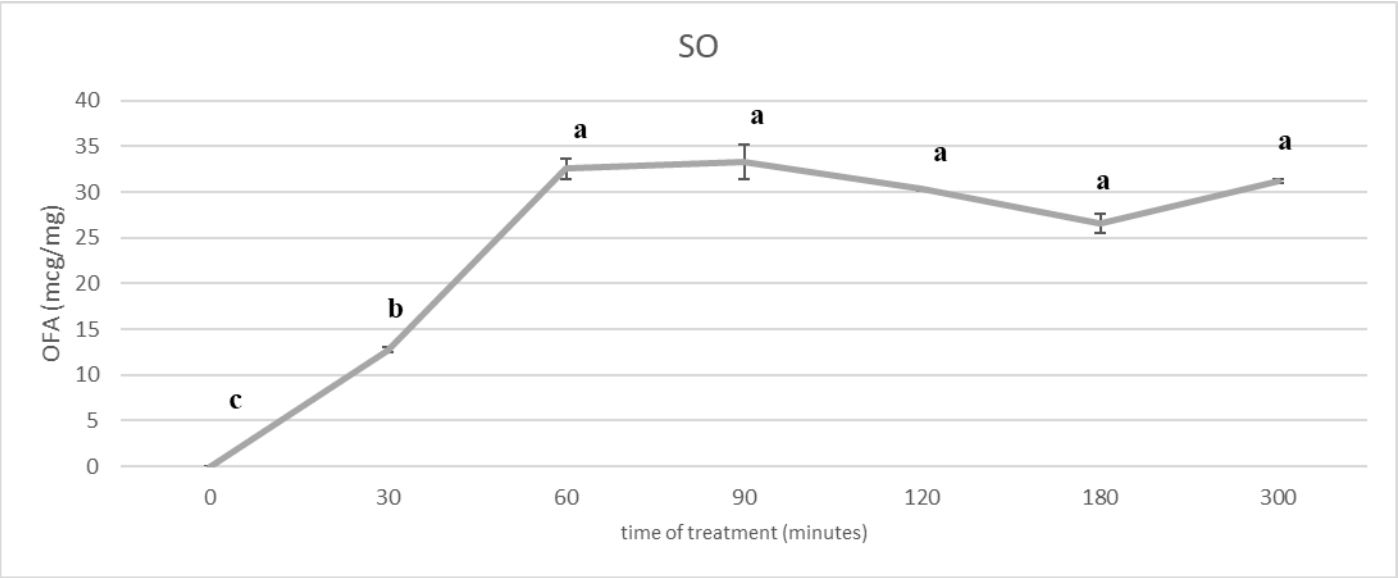

Figure 4.26. Graphical representation of the trend in OFA content over time for the sunflower oil (SO) sample, expressed as mcg/mg of fat. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

For the sunflower oil HOSO sample, as shown in Figure 4.27, there appears to be a slight but gradual significant increase ($p \leq 0.05$) in OFA content from 0 hours to 5 hours of treatment, rising from 20.7 mcg/mg to 40.9 mcg/mg.

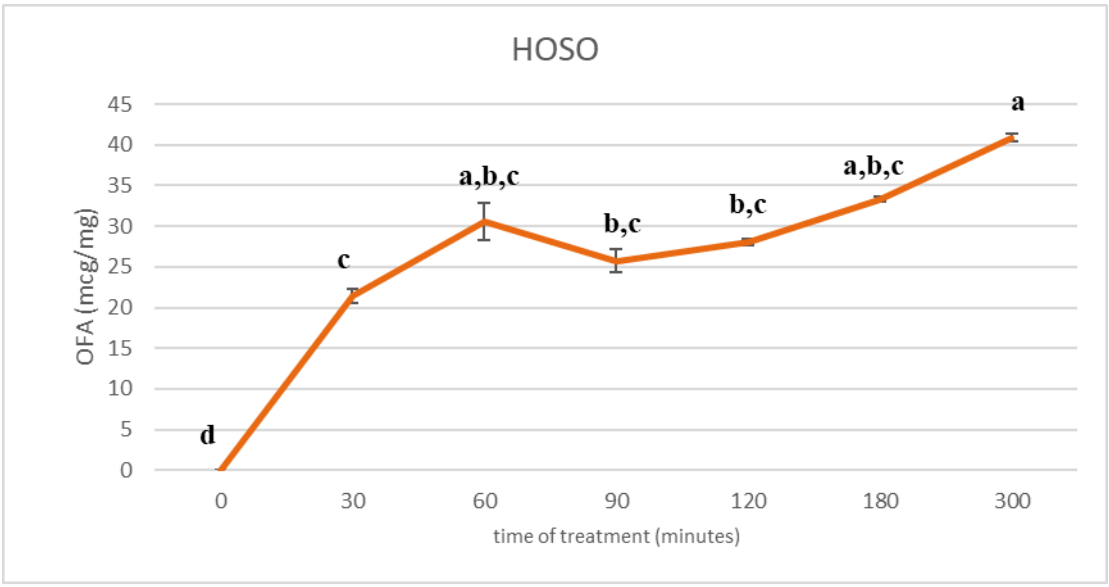

Figure 4.27. Graphical representation of the trend in OFA content over time for the high-oleic sunflower oil (HOSO) sample, expressed as mcg/g of fat. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

Figure 4.28 depicts the temporal trends in oxidized fatty acid (OFA) content across the four oil types under investigation. It is evident that EVOO, despite exhibiting a higher initial OFA concentration compared to GRO and SO, remains relatively stable throughout the treatment period, without undergoing significant increases. In contrast, GRO and SO demonstrate a pronounced increase in OFA levels during the initial hour of thermal treatment. Notably, HOSO, while starting with an initial OFA concentration approximately double that of GRO and SO, reaches OFA levels by the conclusion of the treatment period that are comparable to those observed in the latter oils.

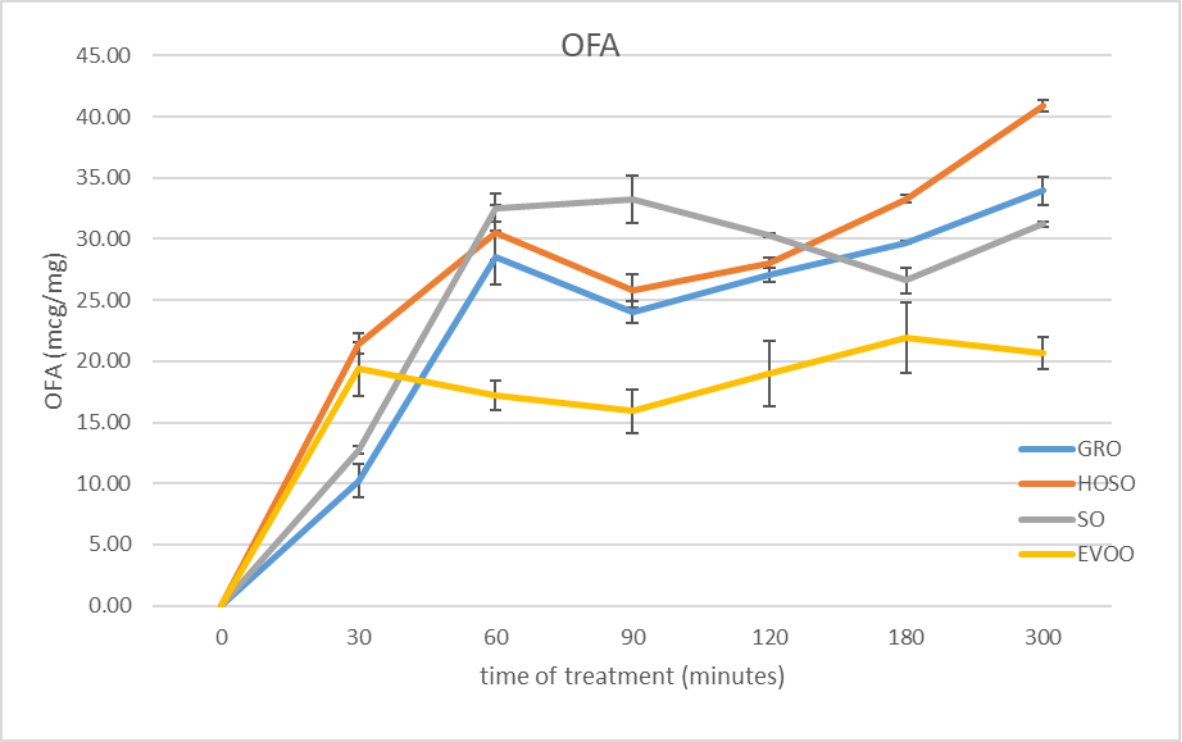

Figure 4.28. Graphical representation of the overall trend in OFA content over time for EVOO, GRO, SO, and HOSO, expressed as mcg/mg of fat (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil).

Figure 4.29 provides a clearer comparative view of the oxidized fatty acid (OFA) content across the four oil types at different time points. Unlike the previously discussed oxidative indices, EVOO consistently exhibits significantly lower OFA levels approximately 1.5 times lower than the other three oils. The only exception is at the initial time point, where the OFA content of EVO does not significantly differ from that of HOSO. However, HOSO demonstrates subsequent increases, with OFA levels that are not significantly different ($p \geq 0.05$) from those observed in GRO and SO across all time intervals. The findings partially align with those of Velasco *et al.* (2004), who reported higher concentrations of oxidized fatty acids in oils with a predominantly

monounsaturated fatty acid (MUFA) profile compared to those richer in polyunsaturated fatty acids (PUFAs). In this study, the HOSO sample, which consists of more than 80% MUFAs (as described in Paragraph 4, *Fatty acids analysis by FAST-GC-FID*), exhibited the highest absolute OFA values. However, these values were often not significantly different from those of SO and GRO.

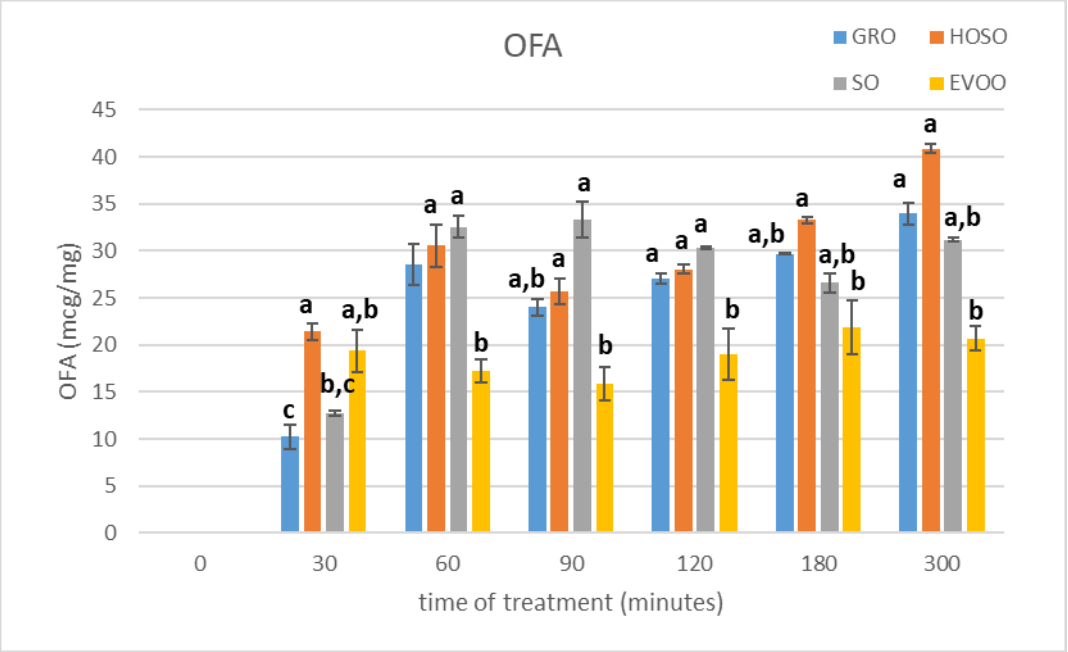

Figure 4.29 Graphical representation of the OFA content over time for the analysed oils (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil), expressed as mcg/g of fat. Bars labelled with different letters within each thermal treatment time indicate significantly different OFA values among the various oils ($p \leq 0.05$).

Conclusions

The oils tested displayed different trends depending on their composition and the oxidative parameter analysed. Overall, it can be concluded that the applied temperature (100°C) and treatment times were insufficient to induce significant or exponential oxidation in most oils, as commonly observed under high thermal stress (e.g., frying). Refined oils, particularly SO and GRO, showed favourable oxidative values, whereas EVOO exhibited the highest concentrations of oxidative markers due to the presence of pro-oxidant compounds retained during its production process. Interestingly, the *p*-anisidine value remained relatively stable in GRO and HOSO, whereas it fluctuated in EVOO and SO without a clear proportional relationship to thermal treatment duration. The differences between HOSO and SO highlight the impact of fatty acid composition and conjugation levels on absorbance and *p*-AV values, as noted by Roman *et al.* (2013). Thus, the *p*-AV appears to vary more with the type of oil than with the thermal

treatment applied. In contrast, OFA values showed greater uniformity across oils when analysed at the same time intervals. Under mild thermal treatment (100°C), the quantification of OFA emerged as a sensitive parameter for detecting thermal effects. However, further validation is needed under more severe oxidative conditions, particularly in oils exceeding acceptability thresholds. This study provides an initial framework for evaluating and understanding the oxidation dynamics of commonly consumed oils under typical domestic use conditions.

Ultra-Violet (UV) light stress

Determination of the peroxide value (PV) by iodometric titration

The PV analysis was carried out for all four types of oil after exposure to ultraviolet (UV) light for different times. The results are expressed in milliequivalents (meq) of oxygen per kilogram of oil and are detailed in Table 4.6.

Time of treatment (minutes)	EVOO	HOSO	SO	GRO
0	7.1±0.9	1.4±0.2	1.1±0.2	3.8±0.5
5	7.4±0.2	1.0±0.01	1.4±0.2	3.7±0.2
10	6.6±0.2	1.9±0.2	1.6±0.2	3.9±0.2
20	6.9±0.2	2.5±0.01	1.4±0.2	3.8±0.3
30	7.6±0.4	1.3±0.01	1.6±0.2	3.8±0.01
60	7.4±0.2	1.5±0.01	1.4±0.2	3.8±0.4
240	7.5±0.0	3.1±0.2	1.2±0.02	5.0±0.01
480	9.0±1.1	3.4±0.2	2.6±0.2	5.3±0.01

Table 4.6 PV (meq O_2/kg of oil) values for the 4 different kinds of vegetable oils analysed at the different treatment times (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil).

In all cases, the PV values remained well below the critical threshold. For grape seed oil (GRO), Figure 4.30 shows that the peroxide concentration remains unchanged during the first hour of treatment but increases significantly after 8 hours of light exposure, reaching 5.3 meq O_2/kg of oil. Hashemi *et al.* (2017) conducted a study in which red grape seed oil samples (*Vitis vinifera cultivar Sardasht*) were exposed to UV light for varying durations (30, 60, 90, and 120 minutes). They observed a smaller increase in PV in crude oil samples (0.8 to 2.8 meq O_2/kg of oil) compared to purified oil samples (1.0 to 4.5 meq O_2/kg of oil). The authors attributed this difference to the high antioxidant content naturally present in crude grape seed oil, including tocopherols and tocotrienols. In the current study, the GRO sample exhibited higher PV values. This discrepancy is likely due to the fact that the oil used in this study was

refined, leading to a significant loss of natural antioxidants. This reduction is especially pronounced during the final step of the refining process, deodorization, which involves high temperatures. Additionally, the PV value measured in this study after 30 minutes of treatment (3.8 meq O_2/kg of oil) exceeds that reported for purified oil without natural tocopherols in the research of Hashemi *et al.* (2017) (1.0 meq O_2/kg of oil). This difference is likely because, as a result of the refining process, the oil in this study started with a higher concentration of fatty acids already degraded into peroxides.

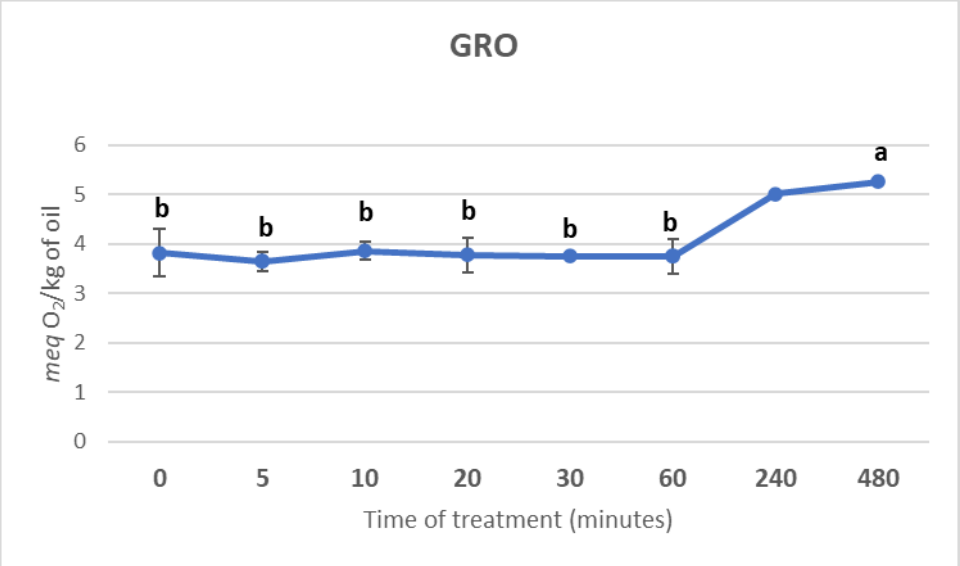

Figure 4.30. Graphical representation of the trend in peroxide value (PV) over time for the grapeseed oil (GRO) sample, expressed as meq O_2/kg of oil. Data points with different letters at various treatment times are significantly different ($p \leq 0.05$).

The peroxide value (PV) trend in high-oleic sunflower oil (HOSO) shows significant fluctuations over the exposure time, as illustrated in Figure 4.31. Specifically, at 5 minutes, the PV is 1.0 meq O_2/kg of oil, followed by a significant increase during the next two-time intervals, reaching a peak value of 2.5 meq O_2/kg of oil after 20 minutes. Subsequently, after 30 minutes of irradiation, the peroxide concentration decreases to 1.3 meq O_2/kg of oil. Finally, after 8 hours of treatment, the PV rises again, reaching a maximum value of 3.4 meq O_2/kg of oil.

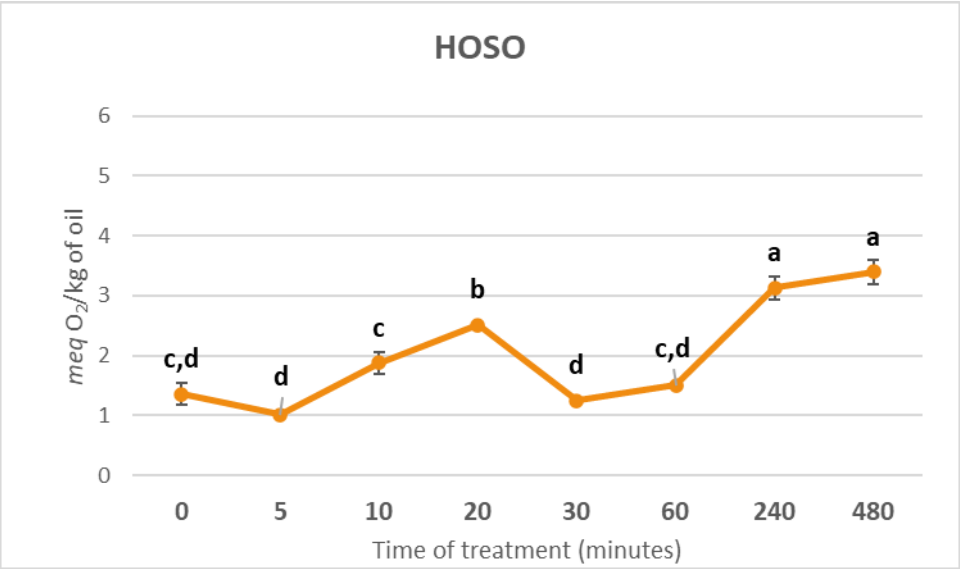

Figure 4.31. Graphical representation of the trend in peroxide value (PV) over time for the high-oleic sunflower oil (HOSO) sample, expressed as meq O2/kg of oil. Data points with different letters at various treatment times are significantly different ($p \leq 0.05$).

In the case of sunflower oil (SO), as shown in Figure 4.32, the peroxide concentration is relatively stable, with minor fluctuations, up until 4 hours of UV light exposure. A significant increase is observed only after 8 hours, reaching a maximum of 2.6 meq O_2/kg of oil. Raza (2009) reported a substantial increase in peroxide value in sunflower oils, from 0.85 to 17.3 meq O_2/kg of oil, which far exceeds the values observed in this study. This discrepancy is likely due to the different experimental conditions, in the same study, the researchers exposed oils to direct sunlight for 10 hours daily over a period of seven weeks, creating extreme photooxidative conditions that led to significantly greater oxidative degradation. In contrast, the UV light source and shorter exposure times applied in the present study were insufficient to trigger such intense and excessive degradation in the sunflower oil samples.

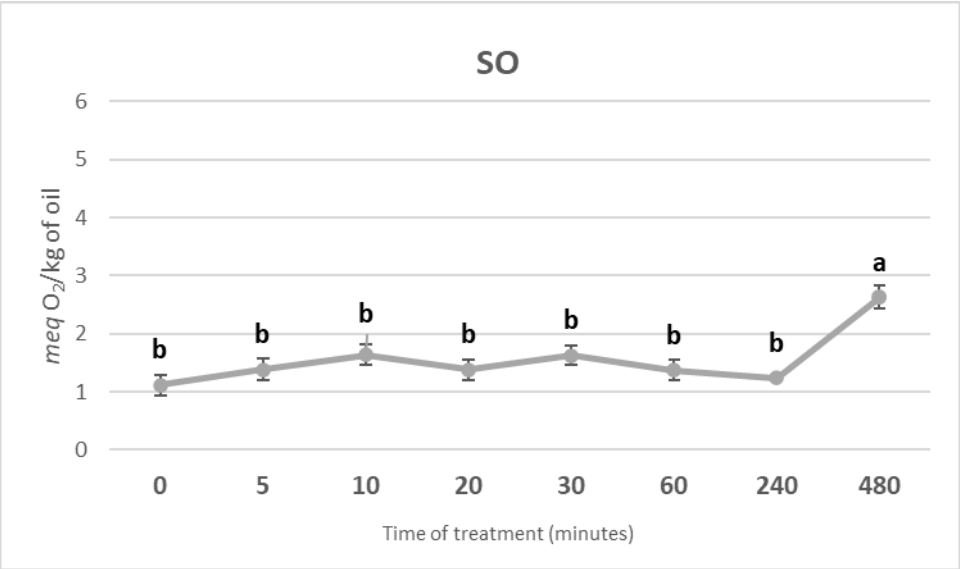

Figure 4.32. Graphical representation of the trend in peroxide value (PV) over time for the sunflower oil (SO) sample, expressed as meq O_2/kg of oil. Data points with different letters at various treatment times are significantly different ($p \leq 0.05$).

For the EVOO sample, as shown in Figure 4.33, there appears to be a slight but gradual and significant increase in peroxide content over the treatment period, rising from 6.6 to 9.0 meq O_2/kg of oil between 10 minutes and 8 hours. Koski *et al.* (2002), in a study where extra virgin olive oil samples were exposed to fluorescent light, observed a similar trend. They reported an increase in PV during the initial hours of treatment, followed by stabilization for the remainder of the exposure period. The authors emphasized that no consistent increase or decrease in PV during light exposure correlated with the decomposition of peroxides. Instead, the sample with the lowest increase in PV was characterized by a lower chlorophyll content. Additionally, the initial PV values reported in their study (6.4 meq O_2/kg of oil and 8.7 meq O_2/kg of oil) align closely with those observed in the current study (7.1 meq O_2/kg of oil), supporting the consistency of findings across different experimental setups.

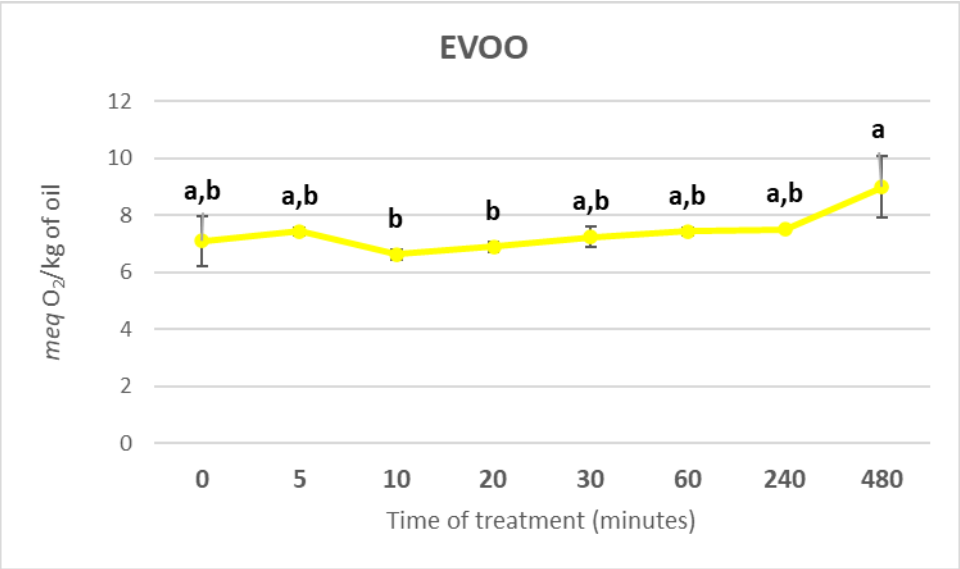

Figure 4.33. Graphical representation of the trend in peroxide value (PV) over time for the extra-virgin olive oil (EVOO) sample, expressed as meq O_2/kg of oil. Data points with different letters at various treatment times are significantly different ($p \leq 0.05$).

When comparing the evolution of peroxide value (PV) across the oil samples (Figure 4.34), it is evident that during the final two sampling times (240 minutes and 480 minutes), all four oil types show an increase in PV with prolonged exposure time. Notably, EVOO consistently exhibits higher PV values compared to the other oils at all time intervals. This can be attributed to the fact that EVOO does not undergo a refining process, unlike the other oil types. As a result, it retains "technological" peroxides formed during the extraction phase, as well as compounds that act as pro-oxidants, such as pigments, free fatty acids, and metals. For example, the presence of chlorophylls significantly contributes to oxidative susceptibility, as these compounds act as photosensitizers. Despite this, the PV values observed in this study remain below the maximum legal thresholds: 20 meq O_2/kg of oil for EVOO and 10 meq O2/kg of oil for refined vegetable oils. In contrast, a previous study by (Esposto *et al.,* 2017) reported PV values exceeding the legal limits. This discrepancy arises from the experimental conditions; in their study, EVOO samples were exposed to light for much longer periods (12 hours per day for 165 consecutive days). These extreme conditions resulted in significant oxidative degradation, unlike the milder exposure used in this study, where the maximum treatment duration of 8 hours was insufficient to cause significant degradation in the oil samples.

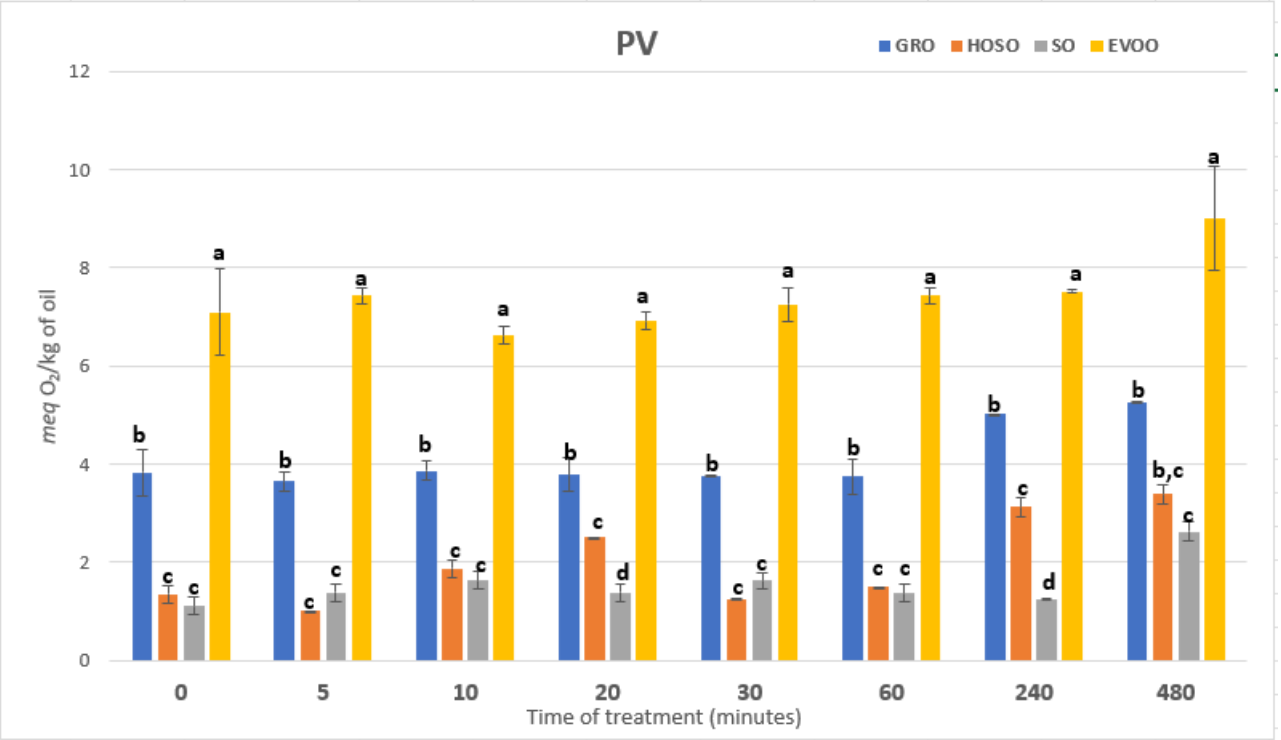

Figure 4.34. Graphical representation of the peroxide value (PV) over time for the analysed oils (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil), expressed as meq O_2/kg of oil. Bars with different letters within each treatment time) indicate significantly different PV values among the oils ($p \leq 0.05$).

Volatile compounds determination by SPME-GC-MS

The analysis of the volatile fraction in the studied oils, as described in the Materials and methods chapter, was conducted to evaluate the aromatic profile of the samples and to examine potential variations due to UV light exposure time and the chemical composition of the vegetable oils. In this study, hexanal content was monitored as an indicator of rancidity. The results are summarized in Table 4.7 and expressed as mcg of analyte on g of fat.

Time of treatment (minutes)	EVOO	HOSO	SO	GRO
0	238.7±7.2	2.6±0.9	0.7±1.5	38.9±5.8
5	211.3±7.2	2.7±0.6	2.3±0.8	40.8±0.6
10	273.4±33.1	2.1±0.6	0.4±0.9	39.9±1.2
20	279.5±23.7	3.3±0.7	0.5±0.8	41.5±1.5
30	263.0±33.7	2.8±0.4	1.9±0.01	43.0±8.4
60	367.8±5.3	3.6±1.7	2.9±0.6	64.7±7.3
240	342.8±9.7	3.4±1.2	2.2±0.9	63.8±1.6
480	204.2±9.0	10.0±1.0	7.6±1.1	53.4±1.4

Table 4.7 Hexanal content for the 4 different kinds of vegetable oils analysed at the different treatment times, the results are expressed as mcg of hexanal/g of fat (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil).

For the grape seed oil (GRO) sample, Figure 4.35 shows that hexanal concentration remained unchanged during the first 30 minutes of treatment. However, a significant increase occurs after 60 minutes, reaching a peak value of 63.84 mcg/g. After 480 minutes, the content decreased slightly to 53.41 mcg/g. Kiralan *et al.* (2018) studied the volatile profile of cold-pressed grape seed oil stored in a photo-oxidation chamber equipped with fluorescent lamps for several days. Their findings align with those of the present study, showing a consistent trend during the first two days of storage, followed by a notable increase reaching a peak on the fifth day. A decline in hexanal content was observed after six days, consistent with the pattern identified in this study.

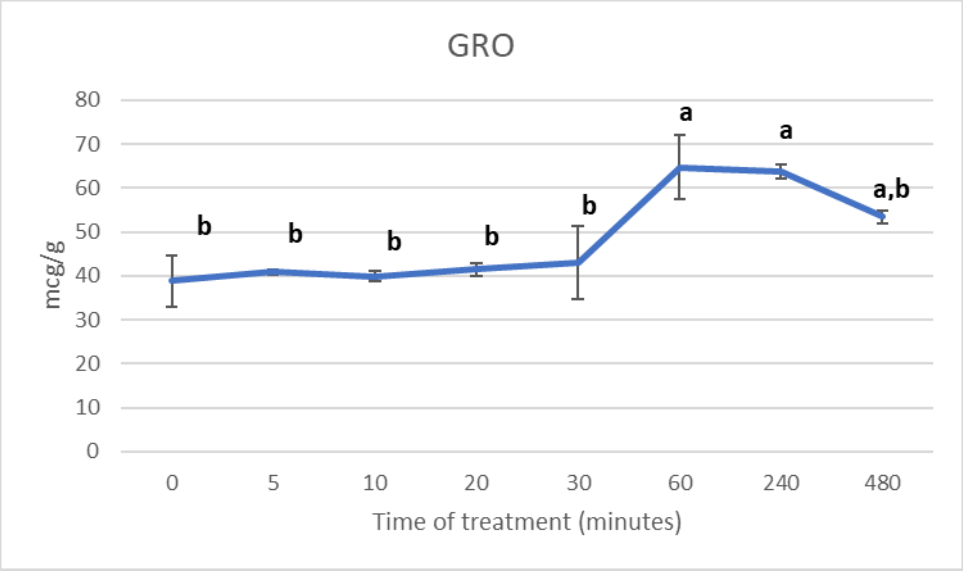

Figure 4.35. Graphical representation of the trend in hexanal content over time for the grapeseed oil (GRO) sample, expressed as mcg of hexanal/g. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

Figure 4.36 illustrates the trend in hexanal concentration in high-oleic sunflower oil (HOSO). The values obtained at various treatment times do not show significant differences ($p \geq 0.05$), fluctuating within a range of 2.8 mcg/g. However, after 8 hours of UV exposure, the hexanal content increases significantly, reaching a value of 10 mcg/g.

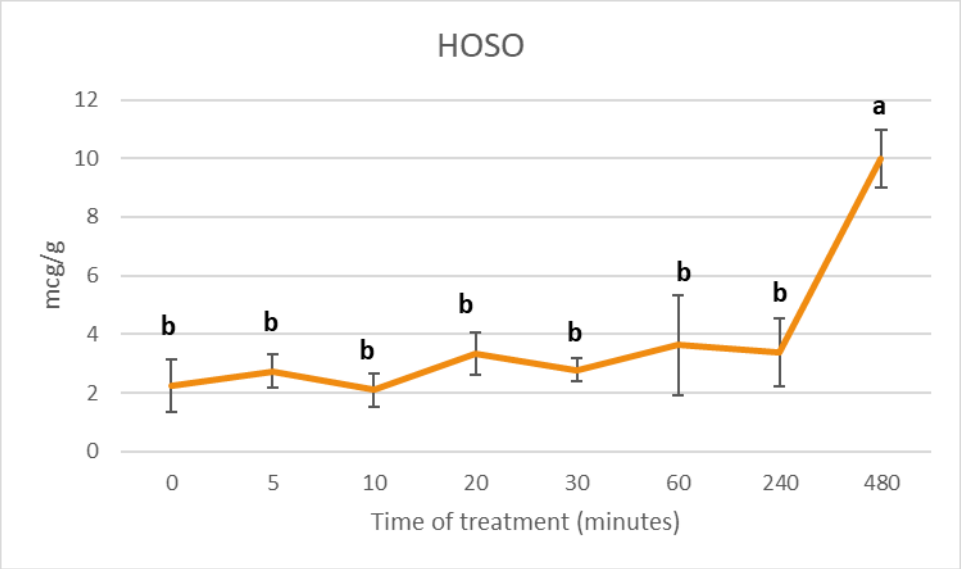

Figure 4.36. Graphical representation of the trend in hexanal content over time for the high-oleic sunflower oil (HOSO) sample, expressed as mcg of hexanal/g. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

The trend, in hexanal concentration, observed in low-oleic sunflower seed oil remained stable over the irradiation period, with only minor fluctuations, as shown in Figure 4.37. Specifically, the hexanal content in the oil measured 2.27 mcg/g after 5 minutes of light exposure, then decreased over the next two intervals 0.51 mcg/g after 20 minutes. By 60 minutes, the hexanal area showed a slight increase to 2.94 mcg/g, and finally, following a minor dip, reached a maximum value of 7.62 mcg/g after 8 hours of UV lamp exposure.

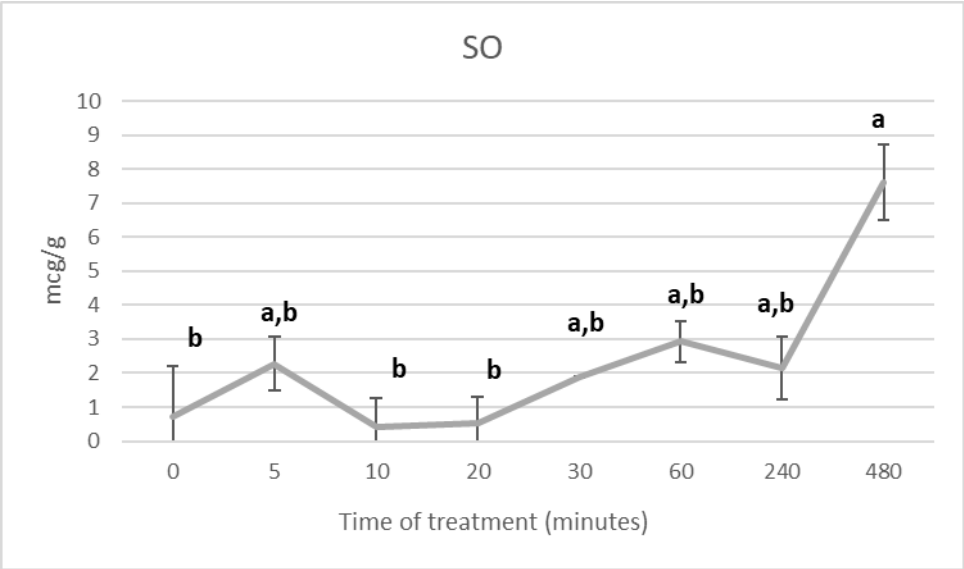

Figure 4.37. Graphical representation of the trend in hexanal content over time for the sunflower oil (SO) sample, expressed as mcg of hexanal/g. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

As clearly shown in Figure 4.38, extra virgin olive oil exhibited a different trend compared to the other samples, with a rather fluctuating behaviour over the treatment period. Specifically, the initial hexanal content in fresh oil was 238.69 mcg/g, which then dropped to a value not significantly different after 5 minutes of treatment. The values, once again, began to increase significantly after 60 minutes of treatment, reaching a maximum of 367.89 mcg/g, and finally, decreased again after 480 minutes, returning to a level similar to the initial value (204.19 mcg/g).

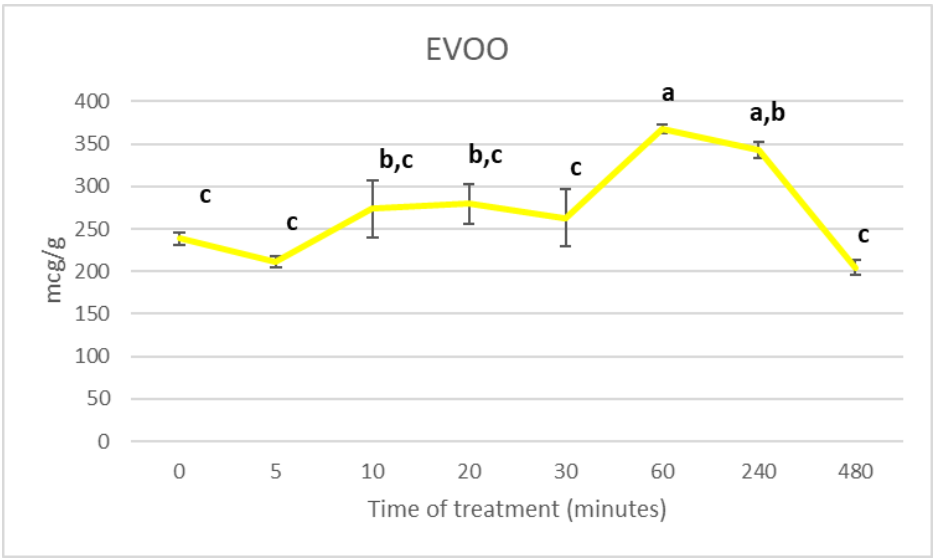

Figure 4.38. Graphical representation of the trend in hexanal content over time for the extra-virgin olive oil (EVOO) sample, expressed as mcg of hexanal/g. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

Figure 4.38 illustrates the overall trends in hexanal content across the four types of oil examined. It is evident that grape seed oil starts with a higher initial value compared to high-oleic and low-oleic sunflower oils, maintaining a relatively stable level over time. In contrast, HOSO and SO showed no significant differences throughout the treatment period. Finally, extra virgin olive oil (EVOO) consistently exhibited the highest hexanal content. This can be attributed to the fact that, unlike the other three oil types, EVO contains a wide variety of volatile compounds, including a substantial amount of hexanal in its initial aroma profile. These compounds are formed from polyunsaturated fatty acids through the lipoxygenase pathway during the extraction process, contributing to its sweet and fruity aroma (Morales *et al.,* 1997). (Luna, Morales and Aparicio, 2006) conducted a study where EVOO samples were exposed to UV radiation over several days. They observed a progressive reduction in the intensity of the fruity attribute, correlating this with a decrease in (E)-2-hexenal concentration—a compound, as previously noted, naturally present in fresh EVOO. Similarly, (Kalua *et al.*, 2006) monitored EVOO stored in light over several months and reported a decrease in volatile C6 compounds, including (E)-2-hexenol, indicative of a loss in freshness. However, hexanal levels increased during this period. These authors noted that while hexanal is associated with oil oxidation, its increase alone was insufficient to classify an oil as oxidized. Furthermore, they concluded that measuring hexanal is not a reliable method for distinguishing oxidized oils from virgin oils, as hexanal is also produced from linoleic acid via the lipoxygenase pathway.

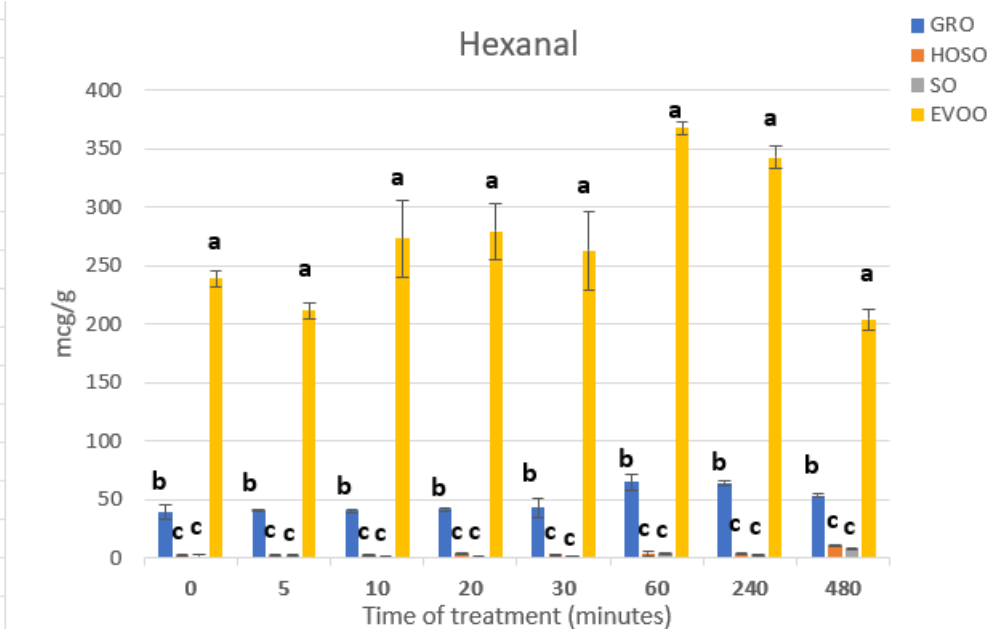

Figure 4.38. Graphical representation of the hexanal content over time for the analysed oils (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil), expressed as mcg of hexanal / g of fat. Bars labelled with different letters within each thermal treatment time indicate significantly different hexanal values among the various oils ($p \leq 0.05$).

Determination of the p-anisidine value (p-AV)

The p-anisidine value was determined to assess the secondary oxidation of oils, and the results obtained for the studied samples are reported in Table 4.8.

Time of treatment (minutes)	EVOO	HOSO	SO	GRO
0	8.2±0.4	8.1±0.6	7.3±0.2	8.7±0.6
5	9.6±0.1	8.8±0.08	8.2±0.05	9.6±0.1
10	10.2±0.3	8.8±0.3	8.3±0.1	9.9±0.07
20	8.9±0.6	7.8±0.5	7.3±0.5	5.7±0.2
30	6.5±0.2	7.0±0.3	6.3±0.5	7.6±0.7
60	7.9±0.6	7.8±0.2	7.0±0.4	8.4±0.02
240	8.0±0.2	7.6±0.7	5.1±0.06	9.1±0.2
480	8.4±0.2	8.0±0.4	6.9±0.4	8.6±0.1

Table 4.8 p-Anisidine value for the 4 different kinds of vegetable oils analysed at the different treatment times, the results are expressed as Anisidine Value (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil).

Looking at the GRO sample, as shown in Figure 4.39, the *p*-anisidine value is very high during the first 10 minutes of treatment, reaching 9.9, but then drops significantly ($p \leq 0.05$) after 20 minutes to 5.7. At subsequent time points, the p-AV goes back up considerably, returning to a value like the initial one (8.6).

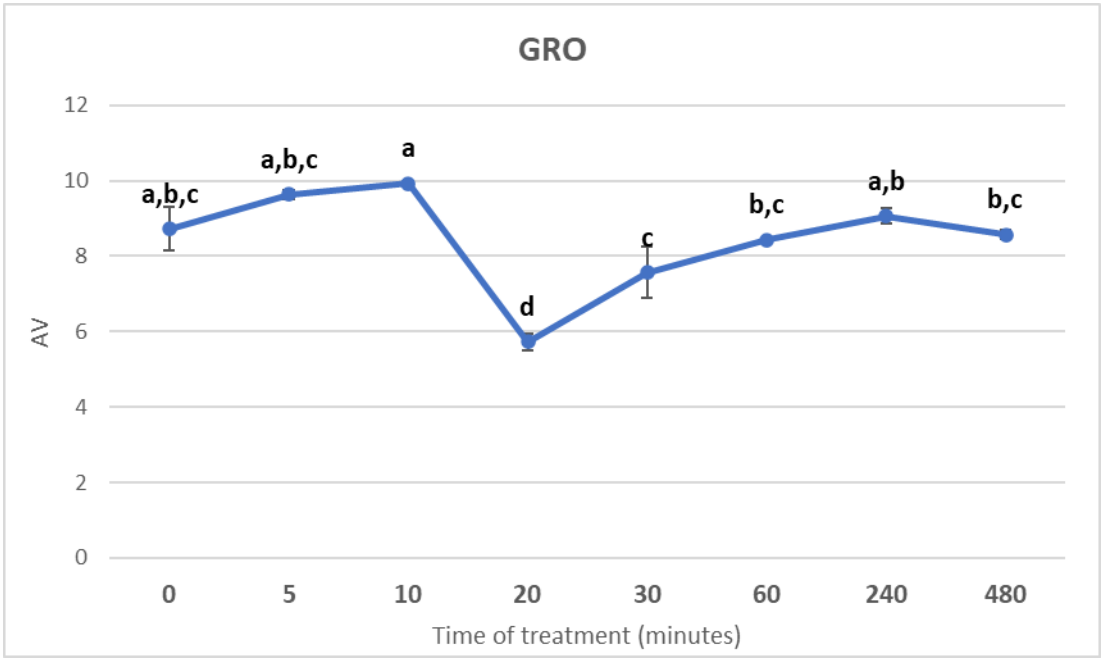

Figure 4.39. Graphical representation of the trend in anisidine value over time for the grapeseed oil (GRO) sample. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

As shown in Figure 4.40, the HOSO sunflower oil does not display any significant differences ($p \geq 0.05$) during UV exposure, with values fluctuating between 7.0 (after 30 minutes of treatment) and 8.8 (recorded after 10 minutes).

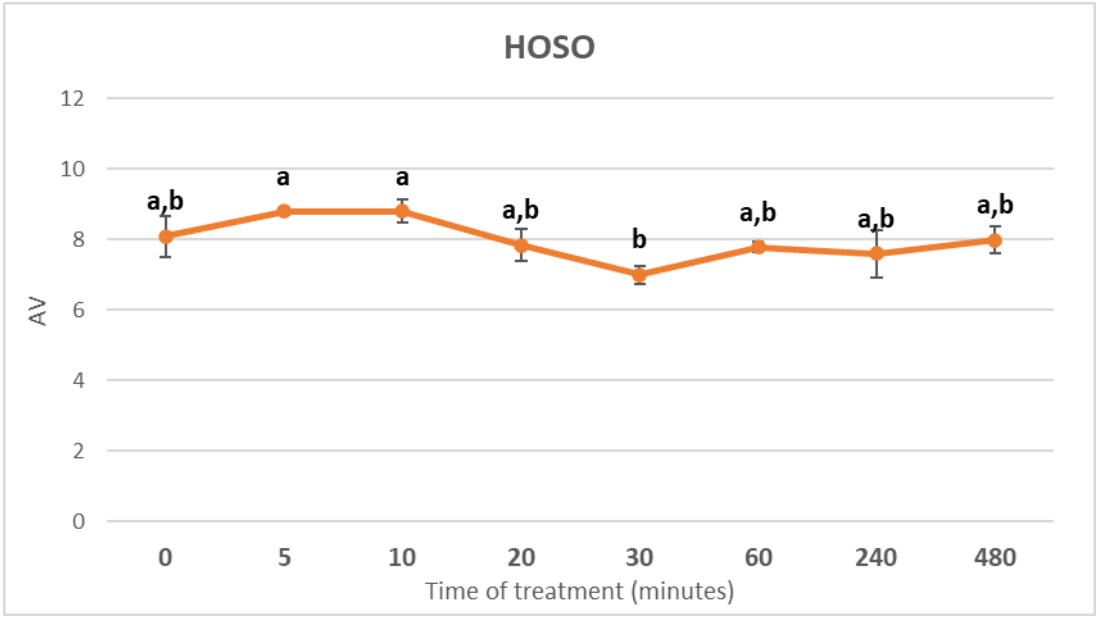

Figure 4.40. Graphical representation of the trend in anisidine value over time for the high-oleic sunflower oil (HOSO) sample. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

As shown in Figure 4.41, the p-anisidine trend in low-oleic sunflower oil fluctuates significantly over the treatment period. Initially, the values are quite high at 8.3, then decrease over the next two time points to 6.3 after 30 minutes. After 4 hours of treatment, the p-anisidine value drops again, this time significantly ($p \leq 0.05$), reaching a minimum of 5.1, and finally rises back up, aligning with the initial value (6.9).

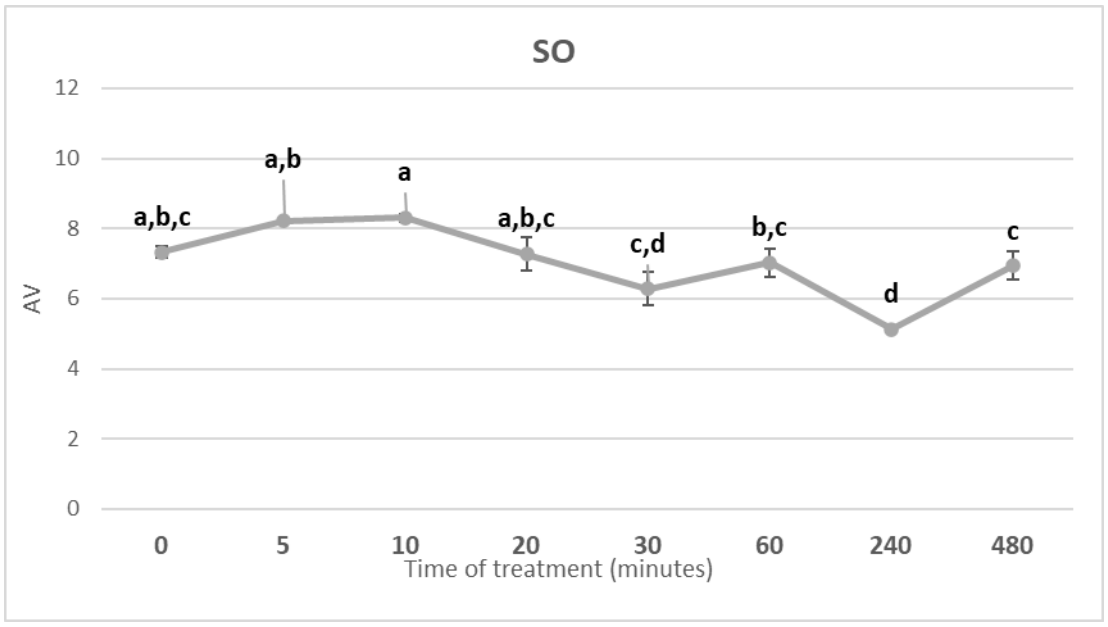

Figure 4.41. Graphical representation of the trend in anisidine value over time for the sunflower oil (SO) sample. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

Regarding the EVOO sample (Figure 4.42), the trend is similar to that observed for the GRO sample. In this case as well, during the first 10 minutes of treatment, the p-anisidine value is quite high (10.2), then it decreases significantly ($p \leq 0.05$) to a minimum of 6.5, and subsequently rises again over the following three intervals, returning to a level statistically similar to the initial value (8.4) after 8 hours of treatment.

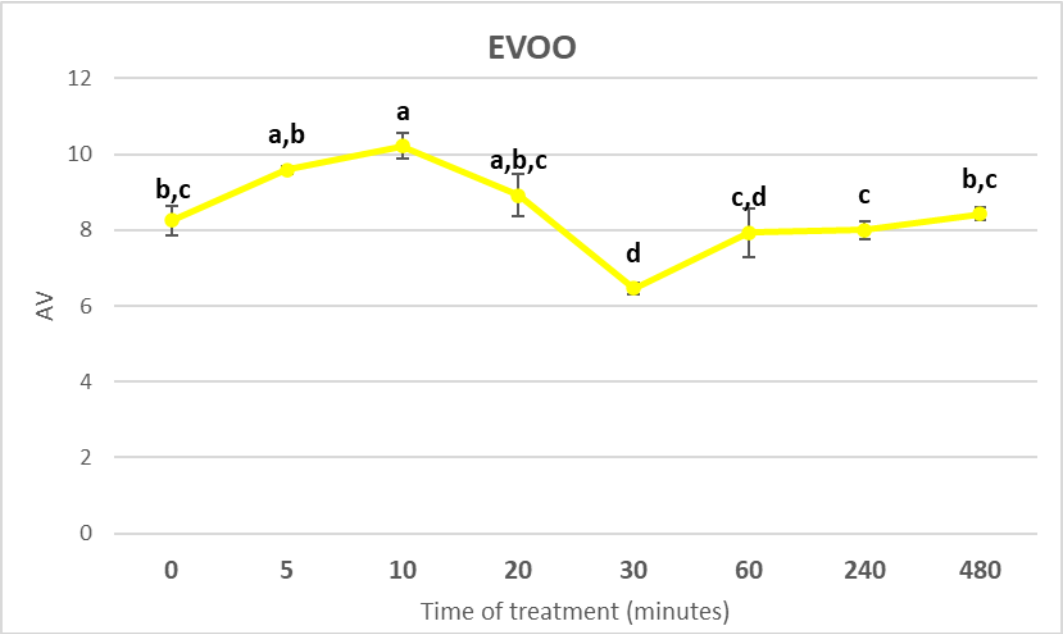

Figure 4.42. Graphical representation of the trend in anisidine value over time for the extra-virgin olive oil (EVOO) sample. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

As shown in Figure 4.43, grape seed oil consistently exhibits significantly lower p-anisidine values ($p \geq 0.05$) across all the time intervals considered, while extra virgin olive oil records higher p-AV values than the other oils. As we can clearly see, the p-anisidine trend for all four tested oils remains steady and linear over time without any substantial changes. This suggests that the photo-oxidative stress conditions used, UV light with a wavelength of 150 nm and relatively short exposure times (up to 8 hours), did not lead to a significant increase in this index. The p-anisidine test estimates the content of secondary and tertiary oxidation products and is generally considered the most accurate and reliable method for evaluating the oxidative state of frying oils, which typically undergo much higher oxidative stress.

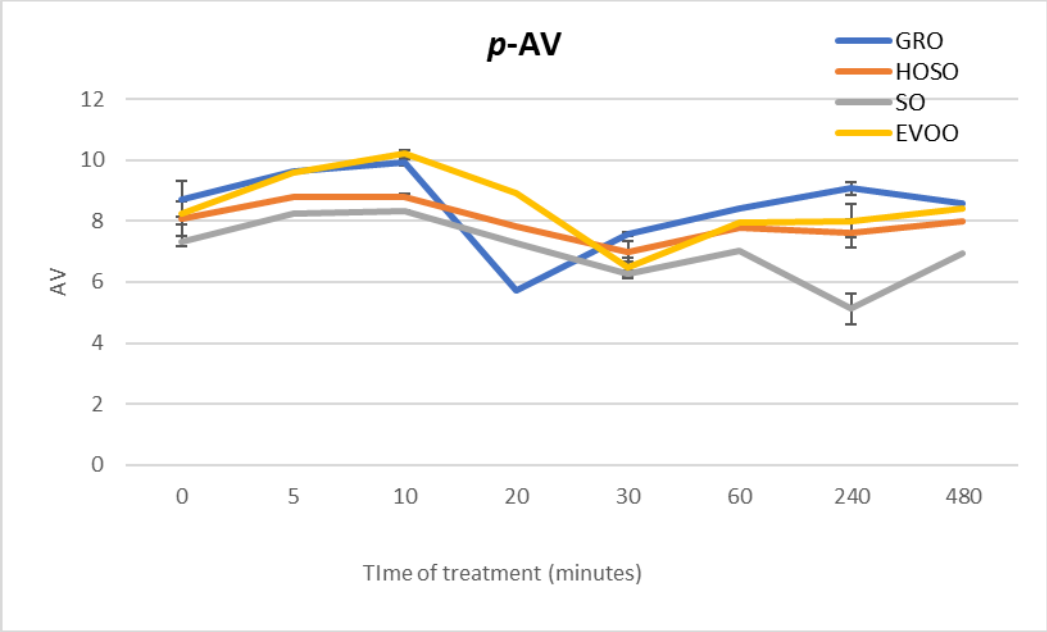

Figure 4.43. Graphical representation of the overall trend in p-AV content over time for EVOO, GRO, SO, and HOSO, expressed as anisidine value (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil).

Determination by gas chromatographic analysis of oxidized fatty acids (OFA)

The results for the OFA content in the photo-oxidized oils were different and not simple to interpret, given the low number of references and photo-oxidation studies performed on similar matrices, such as refined vegetable oils. Results are reported in Table 4.9.

Time of treatment (minutes)	EVOO	HOSO	SO	GRO
0	1.9±0.1	3.4±0.4	2.1±0.9	3.0±02
5	1.7±0.1	6.0±0.5	25.9±0.6	8.0±0.1
10	1.6±0.4	7.4±0.6	4.3±1.4	13.6±1.2
20	2.2±0.4	20.2±2.8	11.3±2.0	8.4±1.8
30	2.0±0.1	1.5±0.3	24.0±0.2	2.6±0.2
60	1.8±0.3	1.9±0.3	1.8±0.2	2.5±0.1
240	1.5±0.2	1.8±0.1	1.7±0.5	3.0±0.7
480	5.3±0.5	3.1±1.4	1.2±0.1	2.3±0.2

Table 4.9. OFA content, expressed as mcg/mg of fat, for the four different types of vegetable oils analysed (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil).

For the GRO samples, as shown in Figure 4.44, the content first established itself at a value of 2.97 mcg/mg of OFAs at time 0, then after 10 minutes the concentration reached the highest value (13.59 mcg/mg) to then stabilize again after 30 minutes of treatment at 2.56 mcg/g and keep similar values all over the treatment. This trend could

be linked to the immediate reaction, catalysed by UV light that take place with the PUFA present in the GRO, such as the linoleic acid (C18:2).

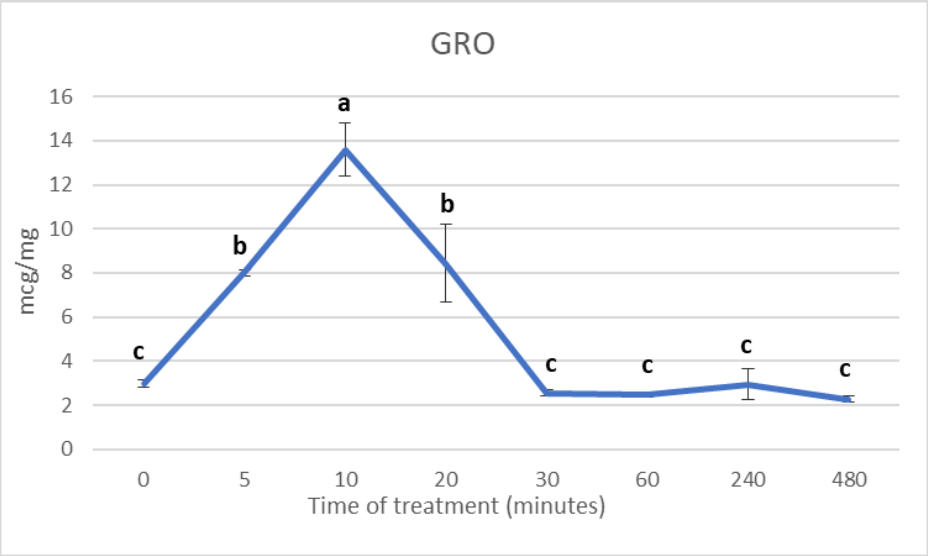

Figure 4.44. Graphical representation of the trend in the OFA content over time for the grapeseed oil (GROO) sample, results are expressed in mcg of OFA/mg of fat. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

If a look is taken at the EVOO samples, as shown in Figure 4.45, it can be seen how the values for the OFA content tend to stay stable during the first 4 hours of treatment, with a mean of 1.82 mcg/mg, to then increase to values of 5.29 mcg/mg after 8 hours of treatment. The high stability of the EVOO could be linked with the higher presence of solutes and particulate matter, that increase the murkiness of the oil, with a better blockage of the UV rays.

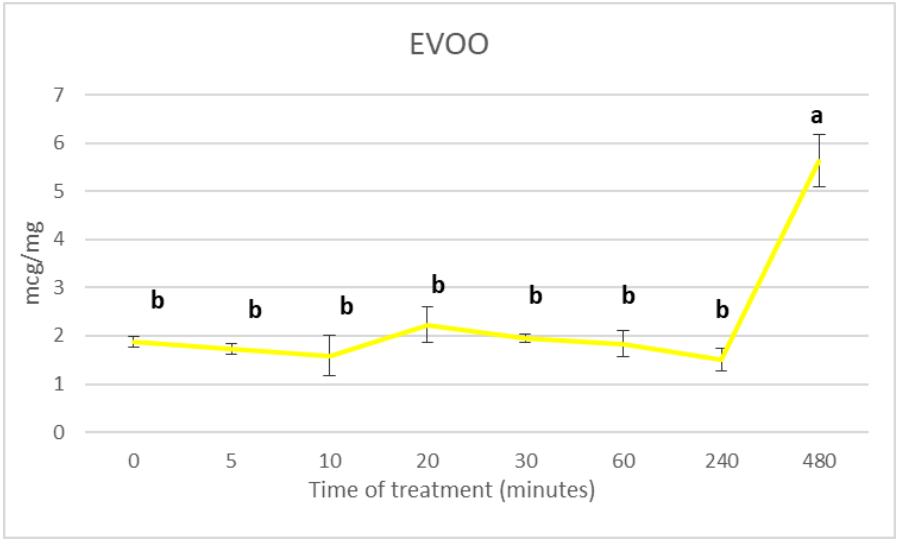

Figure 4.45. Graphical representation of the trend in the OFA content over time for the extra-virgin olive oil (EVOO) sample, results are expressed in mcg of OFA/ mg of fat. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

The results regarding the HOSO show a trend similar to the GRO samples, with a delayed increase in the OFA concentration, which starts at 3.42 mcg/mg, and then increases steadily up to 20.19 mcg/mg after 20 minutes of treatment. After 20 minutes the OFA content decreases to the lowest value, 1.50 mcg/mg at 30 minutes, to then stabilize at similar values for the remaining of the treatment. The results are shown in Figure 4.46.

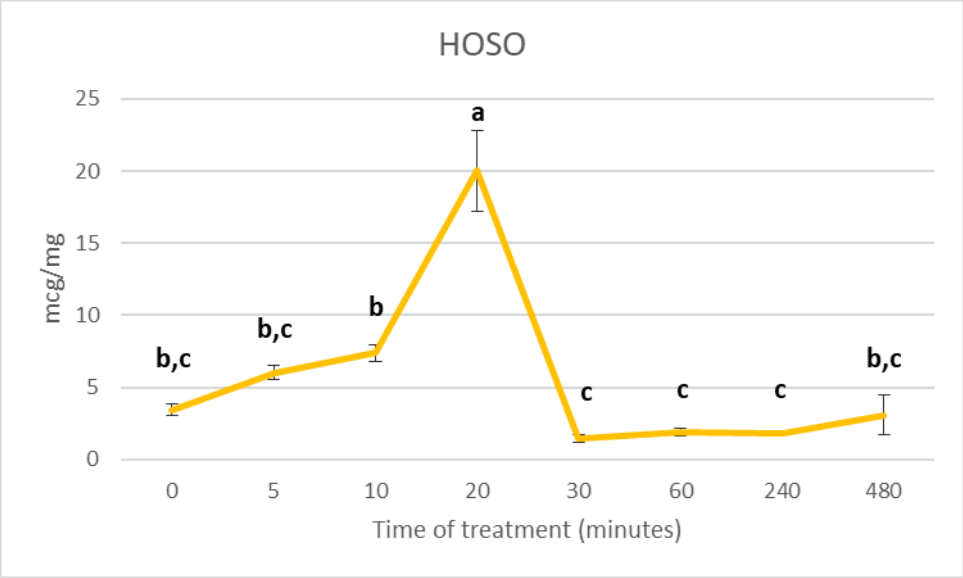

Figure 4.46. Graphical representation of the trend in the OFA content over time for the high-oleic sunflower oil (HOSO) sample, results are expressed in mcg of OFA/ mg of fat. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

SO had the most peculiar development of all the samples, as reported in Figure 4.47, it is possible to observe how the OFA content immediately rises to values of 25.91 mcg/mg after 5 minutes of treatment, to then decrease to values of 4.26 mcg/mg after 10 minutes, and then rise again to values of 23.60 mcg/mg after 30 minutes of treatment. This double OFA spike could be linked to the rapid reaction of firstly the PUFA, such as the linoleic acid, followed by the reaction of the MUFA acid fraction. In fact, it is to bear in mind that the OFAs are unstable molecules, which undergo rapid deterioration when conditions of high temperature or UV-light exposures are reached.

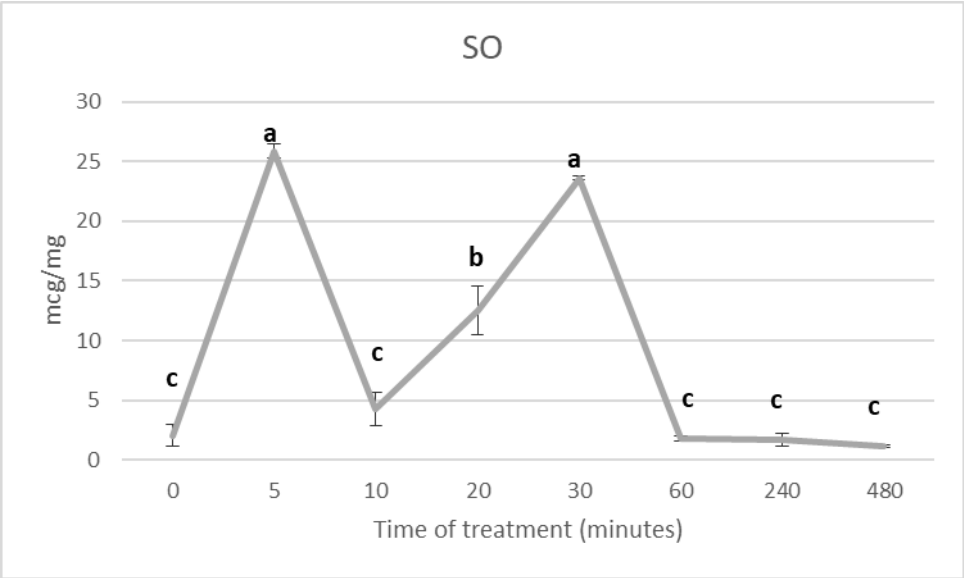

Figure 4.47. Graphical representation of the trend in the OFA content over time for the sunflower oil (SO) sample, results are expressed in mcg of OFA/ mg of fat. Data points labelled with different letters at various thermal treatment times are significantly different ($p \leq 0.05$).

In Figure 4.48 the samples are shown grouped in comparison; it is clear how out of the four samples the sunflower oils have the highest concentrations. Being highly studied, and fractioned oils, the industry, in the latest years provided for products with higher purity and more neutral flavour and aspect, which often translate for good adaptability in food formulations. But, the high transparency, and neutral colour of the sunflower oils tends to provide less "protection" to the UV rays, enticing major oxidation reactions, linked to the increase of the OFA content. Reverse reasoning can be made for the EVOO samples, in which the relative murkiness and higher concentration of particulate matter, pectins, proteins, polyphenols and tocopherols offer higher barrier to the UV rays. GRO is in a position similar to the sunflower oils, but in contrast to them, the higher concentration of antioxidants, and natural colouring agents provide a lower concentration of OFAs.

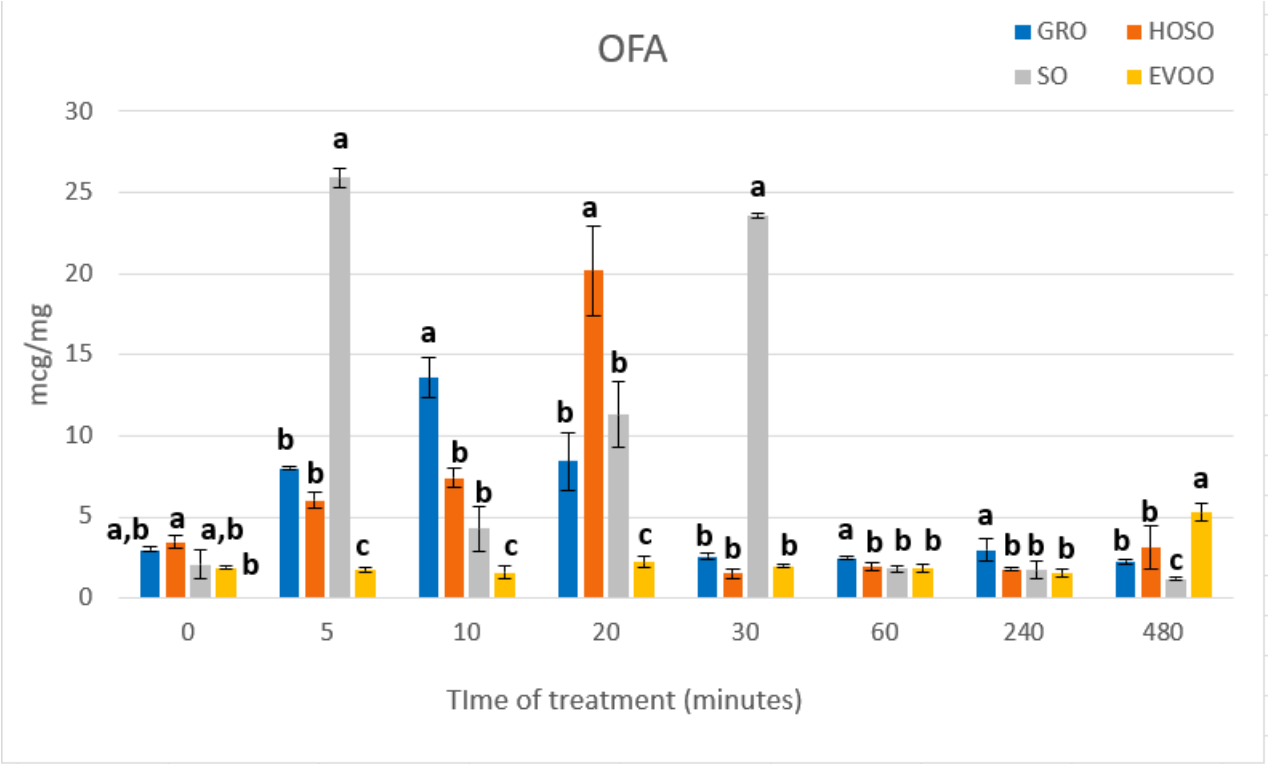

Figure 4.48. Graphical representation of the OFA content over time for the analysed oils (EVOO, extra-virgin olive oil; HOSO, high-oleic sunflower oil; SO, sunflower oil; GRO, grapeseed oil), expressed as mcg of OFA / mg of fat. Bars labelled with different letters within each thermal treatment time indicate significantly different hexanal values among the various oils ($p \leq 0.05$).

Conclusions

The analyses conducted on the four oils examined in this study aimed to evaluate the effect of low-intensity, low-power ultraviolet (UV) light on their resistance to photo-oxidative stress, with the goal of understanding whether brief exposure times (up to a maximum of 8 hours) would significantly compromise oil quality. Current scientific literature provides extensive evidence on the photo-oxidative stability of vegetable oils exposed to light under realistic shelf-life conditions, typically involving much longer exposure times and utilizing the ambient light of storage environments. Furthermore, when discussing oxidative damage due to prolonged light exposure, the literature frequently focuses on the applicability and effectiveness of packaging materials that serve as barriers against UV radiation. Conversely, fewer studies address the photo-oxidation of vegetable oils under shorter exposure intervals (e.g., less than 1 hour), and most of these employ higher-intensity fluorescent or UV lamps. In this study, we specifically evaluated the effect of light in isolation, without adding the influence of temperature or extended time beyond 8 hours, factors that could be investigated in subsequent research. Thus far, the analytical parameters examined have shown very

modest changes throughout the light exposure. Data analysis revealed that, overall, despite differences in unsaturation levels and the presence of natural antioxidants, the impact of light exposure was relatively similar. Although grape seed oil (GRO) showed significantly lower values than extra virgin olive oil (EVOO), it still ranked second highest in terms of PV and hexanal content. The higher initial peroxide concentration in GRO compared to the other refined oils may be attributed to incomplete removal of polar compounds (e.g., phospholipids) during degumming. These compounds may remain during subsequent high temperature refining steps, acting as pro-oxidants and slightly increasing peroxide levels. The presence of these polar compounds can be inferred from the fact that grape seed oil displays a slightly more pronounced pigmentation than sunflower oils. High-oleic sunflower oil (HOSO), despite having a predominantly monounsaturated fatty acid composition, shows only minimal differences in peroxide and hexanal values compared to low-oleic sunflower oil (SO) across all exposure times, even though SO contains more polyunsaturated fatty acids (PUFAs). The lower peroxide values observed in HOSO and SO may be related to a more rigorous refining process than that applied to grape seed oil. For example, the bleaching step, which targets the removal of pigments like carotenoids, chlorophylls, and related compounds known to catalyse oil oxidation when exposed to light (Gotor and Rhazi, 2016), is likely more effective in sunflower oils. This is consistent with the less intense colour of sunflower oils. In low-oleic sunflower oil, the peroxide value remains low and constant at all time points considered, differing significantly from the higher values observed in other oils, such as grape seed oil. Even though both oils are rich in PUFA, low-oleic sunflower oil maintains significantly lower PV, likely due to the persistence of antioxidant compounds through refining and into the shelf-life period. For *p*-anisidine (*p*-AV), the values are similar among the three refined oils, displaying only minor fluctuations toward significantly lower values at intermediate time points. Although grape seed oil may appear slightly more oxidized than HOSO and SO, it is not to the extent that would justify the formation of secondary and tertiary oxidation products. This is because the photo-oxidative conditions necessary for forming these compounds have not yet been reached. It is important to note that p-anisidine values are derived from spectrophotometric readings influenced by the molar extinction coefficient, which varies with the degree of unsaturation and thus aldehyde conjugation. EVOO consistently exhibits higher values for both peroxide and hexanal, although still below the maximum legal limits. This can be attributed to the fact that, unlike the other oils, EVOO does not undergo refining processes. Consequently, it retains antioxidant compounds (such as tocopherols and phenolics) but also pro-oxidant substances like chlorophylls and pheophytins, which are potent photo-oxidation promoters. As a result, one might expect higher *p*-AV in EVOO, given its

elevated peroxide values that serve as precursors to the secondary compounds measured by this index. However, in our study, the *p*-anisidine values for EVOO are not significantly different from those of the three refined oils. Moreover, EVOO contains a high level of aromatic compounds since it is not deodorized, and its high hexanal content is part of its initial aromatic profile. In future work, it may be useful to assess the long-term impact of this treatment on oils by subjecting them to a storage period following exposure, potentially including a technological application of the treated oils.

b. Second goal: chemical and sensorial characterization of different kinds of baked goods ("tarallini" and "frollini")

(results reported in the article "Effect of replacing olive oil with oil blends on physicochemical and sensory properties of taralli" - Federica Pasini, Silvia Marzocchi, Cesare Ravagli, Francesca Cuomo, Maria Cristina Messia, Emanuele Marconi & Maria Fiorenza Caboni - International Journal of Food Science and Technology 2024, 59, 2697–2706)

Taralli

Fatty acids analysis by FAST-GC-FID

As shown in Table 4.10, a total of 14 fatty acids were identified and quantified in oil, oil blends and in the respective taralli. Monounsaturated fatty acids (MUFA) represented the principal class of FA in all samples, with a content in the range of 72–83% for the lipid matrices and 68–81% for taralli. Saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) were detected in the range of 8–16% and 7–15% respectively. In general, all taralli samples showed an increase in PUFA compared to their respective raw lipid matrices, especially in Tctrl, TC and TD. The TA, TB and TC samples also showed an increased SFA concentration in relation to the corresponding lipid fraction. The same samples showed a significant decrease (about 3–6%) in MUFA content, while Tctrl and TD did not show significant differences with their lipid matrices. These changes could be related to the presence of fatty acids in wheat flour (in taralli formulation) as well as to the influence of the process, especially the cooking step. The individual fatty acid profile of the taralli fully reflected the profile of the raw lipid matrices used for their formulation. Indeed, lauric acid (C12:0) was only found in the HOSO:CO (4.4%) lipid blend and in the corresponding final taralli, TD (4.3%). Oleic acid (C18:1cis9) was the most abundant fatty acid in all the raw lipid matrices and the final samples; in particular, HOSO and TA taralli made with 100% HOSO have

the highest contents of C18:1cis9 (82.5% and 80.5% respectively). Palmitic acid (C16:0) and linoleic acid (C18:2n6) were the most abundant SFA and PUFA, respectively, found in the samples. In particular, palmitic acid content was higher in EVOO (13.4%) and EVOO:RO (12.4%) blend and in taralli in which these lipid matrices were used, i.e., Tctrl (12.1%) and TC (13.6%), respectively, while the EVOO:SO blend showed the highest percentage of linoleic acid (13.5%) as did the corresponding taralli, TB (14.3%). The fatty acid profiles of these vegetable oils were consistent with the results already found in the literature (Chowdhury *et al.,* 2007; Ichihara *et al.,* 2021).

FA	EVOO (100%)	HOSO (100%)	EVOO:SO (87.5%:12.5%)	EVOO:RO (75%:25%)	HOSO:CO (87.5%:12.5%)	Tctrl (100% EVOO)	TA (100% HOSO)	TB (87.5% EVOO + 12.5% SO)	TC (75% EVOO + 25% RO)	TD (87.5% HOSO + 12.5% CO)
C12:0	n.d.	n.d.	n.d.	n.d.	4.4 ± 0.0a	n.d.	n.d.	n.d.	n.d.	4.3 ± 0.0a
C14:0	n.d.	n.d.	0.1 ± 0.0c	0.1 ± 0.0c	1.8 ± 0.0b	n.d.	n.d.	n.d.	0.1 ± 0.0c	2.1 ± 0.0a
C16:0	13.4 ± 0.0a	4.4 ± 0.0i	10.2 ± 0.0e	12.4 ± 0.0b	5.3 ± 0.0 g	12.1 ± 0.0c	4.9 ± 0.0 h	11.6 ± 0.2d	13.6 ± 0.0a	5.6 ± 0.0f
C16:1 *cis*	0.8 ± 0.0a	0.1 ± 0.0d	0.8 ± 0.0a	0.7 ± 0.0b	0.1 ± 0.0d	0.8 ± 0.0a	0.2 ± 0.0c	0.7 ± 0.0b	0.7 ± 0.0b	0.1 ± 0.0d
C17:0	0.1 ± 0.0a	n.d.	0.1 ± 0.0a	0.1 ± 0.0a	n.d.	0.1 ± 0.0a	n.d.	0.1 ± 0.0a	0.1 ± 0.0a	n.d.
C17:1	0.1 ± 0.0a	n.d.	0.1 ± 0.0a	0.1 ± 0.0a	n.d.	0.1 ± 0.0a	n.d.	0.1 ± 0.0a	0.1 ± 0.0a	n.d.
C18:0	2.7 ± 0.0b	2.6 ± 0.0c	2.8 ± 0.0a	2.6 ± 0.0c	2.7 ± 0.0b	2.7 ± 0.0b	2.7 ± 0.0b	2.8 ± 0.0a	2.5 ± 0.0d	2.7 ± 0.0b
C18:1 *cis9*	75.1 ± 0.1c	82.5 ± 0.1a	71.2 ± 0.0e	71.4 ± 0.2e	75.3 ± 0.0c	74.6 ± 0.0c	80.5 ± 0.0b	68.8 ± 0.3f	67.3 ± 0.4 g	72.9 ± 0.1d
C18:2n6	6.1 ± 0.0 L	9.1 ± 0.1 g	13.5 ± 0.0c	11.3 ± 0.1d	8.6 ± 0.0 h	7.8 ± 0.0i	10.5 ± 0.0e	14.3 ± 0.1a	13.9 ± 0.2b	10.3 ± 0.0f
C18:3n3	0.6 ± 0.0b	0.1 ± 0.0d	0.6 ± 0.0b	0.6 ± 0.0b	0.1 ± 0.0d	0.7 ± 0.0a	0.2 ± 0.0c	0.7 ± 0.0a	0.7 ± 0.0a	0.2 ± 0.0c
C20:0	0.3 ± 0.0b	0.2 ± 0.0c	0.3 ± 0.0b	0.4 ± 0.0a	0.2 ± 0.0c	0.3 ± 0.0b	0.2 ± 0.0c	0.3 ± 0.0b	0.4 ± 0.0a	0.2 ± 0.0c
C20:1	0.2 ± 0.0b	0.2 ± 0.0b	0.2 ± 0.0b	0.3 ± 0.0a	0.2 ± 0.0b	0.2 ± 0.0b	0.2 ± 0.0b	0.2 ± 0.0b	0.3 ± 0.0a	0.2 ± 0.0b
C22:0	0.1 ± 0.0c	0.7 ± 0.0a	n.d.	n.d.	n.d.	0.1 ± 0.0c	0.6 ± 0.0b	0.1 ± 0.0c	0.1 ± 0.0c	n.d.
C22:2	0.6 ± 0.0a	n.d.	n.d.	n.d.	n.d.	0.4 ± 0.0b	n.d.	0.3 ± 0.0c	0.3 ± 0.0c	n.d.
SFA	16.6 ± 0.0a	7.9 ± 0.0 h	13.7 ± 0.0f	15.5 ± 0.0c	15.6 ± 0.0c	15.3 ± 0.0d	8.5 ± 0.0 g	14.9 ± 0.2e	16.8 ± 0.1a	15.8 ± 0.1b
MUFA	76.2 ± 0.0c	82.9 ± 0.1a	72.3 ± 0.0e	72.5 ± 0.2e	75.7 ± 0.0 cd	75.8 ± 0.0c	80.9 ± 0.0b	69.8 ± 0.3f	68.3 ± 0.3 g	73.5 ± 0.1d
PUFA	7.2 ± 0.0 h	9.2 ± 0.1f	14.0 ± 0.0b	12.0 ± 0.1c	8.7 ± 0.0 g	8.9 ± 0.0f	10.6 ± 0.0e	15.3 ± 0.1a	14.9 ± 0.3a	10.7 ± 0.0d

Table 4.10. Fatty acid composition and content (mg FA/100 mg of FAME) of raw lipid matrices and taralli, Different letters in the same row show significantly different mean values (Tukey HSD $p < 0.05$). (Pasini *et al.,* 2024).

Tocol analysis

Tocols are important for their health roles as powerful anticancer, antioxidant, immunostimulant, anti-inflammatory and nephroprotective agents (González Belo *et al.,* 2017). As shown in Table 5, six tocols were identified and quantified in all lipid matrices and taralli samples in this order of elution: α-tocopherol, α-tocotrienol, β-tocopherol, γ-tocopherol, β-tocotrienol and δ-tocotrienol. α-tocopherol was the main tocol in all samples, with a content ranging from 17 to 35 mg/100 g of oil. Among the lipid matrices, the EVOO:SO blend reported the highest level of a-tocopherol (34.9 mg/100 g of oil), followed by the HOSO:CO and EVOO:RO samples with similar contents (~30 mg/100 g of oil) and HOSO and EVOO with concentrations of 27.7 and 27.4 mg/100 g of oil respectively. Sample TC formulated with EVOO:RO had the highest ($p < 0.05$) concentration of total tocols (40.2 mg/100 g of oil), reflecting the trend in the lipid matrices, where the EVOO:RO blend had the highest content (46.3 mg/100 g of oil) among lipids. The high total tocol content of this blend and of the corresponding taralli is mainly due to the contribution of γ-tocopherol, and α- and γ-

tocotrienol particularly abundant in rice oil (Wen *et al.,* 2020). TB, formulated with EVOO:SO, was the sample with the second highest total tocol content (29.2 mg/100 g of oil), followed by TD (HOSO:CO), Tctrl (100% EVOO) and TA (100% HOSO) with concentration of 27.6, 26.1 and 24.0 mg/100 g of oil respectively. β-tocotrienol was only found in all final formulated taralli and not in the lipid matrices, as it is a typical tocol of cereal flour as reported in the literature (Panfili, Fratianni and Irano, 2003; Engelsen and Hansen, 2009). However, the results showed a general decrease in tocol content from the lipid matrices to the final products, where a-tocopherol showed a reduction of about 40%, probably linked to its thermolability (Kiczorowska *et al.,* 2019).

	EVOO (100%)	HOSO (100%)	EVOO:SO (87.5%:12.5%)	EVOO:RO (75%:25%)	HOSO:CO (87.5%:12.5%)	Tctrl (100% EVOO)	TA (100% HOSO)	TB (87.5% EVOO + 12.5% SO)	TC (75% EVOO + 25% RO)	TD (87.5% HOSO + 12.5% CO)
α-tocopherol	27.4 ± 0.4c	27.7 ± 0.4c	34.9 ± 0.2a	29.5 ± 0.2b	29.8 ± 0.2b	17.0 ± 0.4 fg	16.6 ± 0.2 g	21.0 ± 0.4d	17.8 ± 0.2ef	18.8 ± 0.5e
α-tocotrienol	n.d.	n.d.	n.d.	2.2 ± 0.1a	0.5 ± 0.0b	n.d.	n.d.	n.d. c	1.9 ± 0.3a	0.4 ± 0.1b
β-tocopherol	0.1 ± 0.0f	0.7 ± 0.0d	0.3 ± 0.0e	0.9 ± 0.1bc	0.6 ± 0.0d	0.8 ± 0.0c	0.6 ± 0.0d	0.9 ± 0.0bc	1.2 ± 0.3a	0.6 ± 0.0d
γ-tocopherol	2.5 ± 0.1c	0.5 ± 0.1e	2.3 ± 0.02c	6.2 ± 0.2a	1.1 ± 0.0d	2.3 ± 0.1c	n.d.	2.0 ± 0.1 cd	5.5 ± 0.3b	1.5 ± 0.2d
β-tocotrienol	n.d.	n.d.	n.d.	n.d.	n.d.	6.0 ± 0.2b	6.8 ± 0.2a	5.3 ± 0.1c	6.1 ± 0.0b	6.3 ± 0.1ab
γ-tocotrienol	n.d.	n.d.	n.d.	7.5 ± 0.9a	n.d.	n.d.	n.d.	n.d.	7.7 ± 0.1a	n.d.
Total	30.0 ± 0.5e	28.9 ± 0.1 fg	37.5 ± 0.2c	46.3 ± 1.3a	32.0 ± 0.1d	26.1 ± 0.8 h	24.0 ± 1.4i	29.2 ± 0.6ef	40.2 ± 0.9b	27.6 ± 1.0 g

Table 4.11. Tocol composition and content (mg/100 g of oil) of raw lipid matrices and taralli, Different letters in the same row show significantly different mean values (Tukey HSD $p < 0.05$). (Pasini et al., 2024)

Sterol analysis

Sterols represent a group of important health compounds and powerful antioxidants in vegetable oils, and it is known that their consumption can significantly reduce serum LDL cholesterol levels (Moreau, 2015). In line with the reported literature on plant phytosterols, a total of seven sterols were identified and quantified in the raw lipid matrices and in the final taralli samples (Table 4.12). Like tocols content, the blend EVOO:RO and the corresponding taralli TC were the richest samples in total sterols (354.7 and 427.6 mg/100 g of oil respectively), reporting the highest ($p < 0.05$) content of the major compounds, such as β-sitosterol (216.3 and 241.3 mg/100 g of oil, respectively), campesterol and avenasterols (Δ^5 and Δ^7 - avenasterol). These results are consistent with the high sterol content reported in the literature for rice oil (Moreau, 2015; Yang *et al.,* 2019), which improved the sterol content in the new blend and in the corresponding final taralli. TA, formulated with 100% HOSO, was the sample with the second highest total sterol content (255.8 mg/100 g of oil), followed by TB (EVOO:SO) and TD (HOSO:CO) with concentrations of 237.0 and 245.9 mg/100 g of oil respectively; whereas sample Tctrl, formulated with 100% EVOO, reported the

lowest ($p < 0.05$) total phytosterol content (205.9 mg/100 g of oil) among all the taralli samples. This trend observed for the taralli reflects the results obtained for the respective oils and blends used in formulation and is in line with other studies on sterols in vegetable oils(Liu *et al.,* 2020; Bai, Ma and Chen, 2021). In fact, 100% EVOO and the EVOO: SO blend reported the lowest sterol content due to the low percentage of these substances in olive oil compared to rice and sunflower oil. Coconut oil was also poor in sterols, but in the HOSO:CO blend, the total content increased by the greater contribution of sunflower oil which has a considerable sterol content (Schwartz *et al.,* 2008; Bai, Ma and Chen, 2021). The taralli samples had a slightly higher concentration of phytosterols than their corresponding lipid fractions used for their formulation. This increase is probably due to the contribution of the other ingredients, especially wheat flour, as well as to the hydrolysis of sterols from steryl esters or γ-oryzanol during baking (Mandak and Nyström, 2013). As widely reported in literature, wheat is a good source of sterols and it is mainly characterised by sitosterol, campesterol and the corresponding saturated forms of stanol and stigmasterol (Rajhi, 2020; Loskutov and Khlestkina, 2021).

	EVOO (100%)	HOSO (100%)	EVOO:SO (87.5%:12.5%)	EVOO:RO (75%:25%)	HOSO:CO (87.5%:12.5%)	Tctrl (100% EVOO)	TA (100% HOSO)	TB (87.5% EVOO + 12.5% SO)	TC (75% EVOO + 25% RO)	TD (87.5% HOSO + 12.5% CO)
Campesterol	15.1 ± 0.2e	20.4 ± 0.2d	9.7 ± 0.2f	62.1 ± 0.2b	15.6 ± 0.4e	21.2 ± 2.0d	33.0 ± 0.8c	19.8 ± 0.3d	74.6 ± 2.1a	30.8 ± 1.1c
Campestanol	8.4 ± 0.3a	5.5 ± 0.5bcd	2.5 ± 0.1e	4.6 ± 0.4cde	4.4 ± 0.3de	8.2 ± 1.0ab	7.2 ± 0.1abc	7.9 ± 0.1ab	8.2 ± 1.7ab	6.5 ± 0.4abcd
Stigmasterol	n.d.	13.5 ± 0.6d	1.7 ± 0.2 g	16.1 ± 0.2c	7.6 ± 0.2e	n.d.	17.9 ± 0.2b	5.2 ± 0.1f	29.8 ± 0.1a	15.7 ± 0.1c
β-sitosterol	111.0 ± 0.8 g	139.8 ± 1.1e	111.1 ± 0.7 g	216.3 ± 1.8b	119.4 ± 1.2f	147.9 ± 0.0d	161.0 ± 0.5c	150.7 ± 0.1d	241.3 ± 0.0a	155.8 ± 3.6d
Sitostanol	10.8 ± 0.3abcd	9.3 ± 0.3 cd	7.7 ± 0.1d	7.3 ± 0.3d	9.7 ± 0.1bcd	14.8 ± 2.2ab	13.5 ± 1.5abc	13.9 ± 1.0abc	12.9 ± 1.8abc	15.1 ± 2.4a
Δ^5-avenasterol	9.7 ± 0.2ef	8.0 ± 0.1f	9.8 ± 0.2def	11.4 ± 1.7cdef	8.5 ± 0.1f	13.8 ± 0.4bcd	10.8 ± 1.3 cdef	15.8 ± 0.7ab	19.6 ± 0.1a	12.9 ± 2.3bcdef
Δ^7-avenasterol	n.d.	8.1 ± 0.1e	15.9 ± 0.9d	36.9 ± 1.0b	4.6 ± 0.0e	n.d.	12.4 ± 0.1de	23.7 ± 2.8c	41.2 ± 2.6a	9.1 ± 0.9e
Total	155.0 ± 2.3 h	204.6 ± 1.8e	158.4 ± 0.8 g	354.7 ± 4.1b	169.8 ± 0.5f	205.9 ± 5.6e	255.8 ± 2.6c	237.0 ± 3.8d	427.6 ± 0.9a	245.9 ± 6.9d

Table 4.12. Sterol composition and content (mg/100 g of oil) of raw lipid matrices and taralli, Different letters in the same row show significantly different mean values (Tukey's HSD $p < 0.05$). (Pasini et al., 2024).

Oxidative stability with OXITEST

The oxidative stability of taralli was tested to evaluate the impact of the different lipid fractions used in the formulation on the oxidative quality of the final product. The results obtained with OXITEST (Table 4.13) are expressed as the induction period (IP) in hours (h), which is the time required for a complete oxidation cycle of the samples. Tctrl and TC, formulated with EVOO and EVOO:RO, respectively, exhibited the highest ($P < 0.05$) IP values, which were 38.7 and 40.4 h respectively. The other samples, TA, TB and TD, reported significantly lower ($p < 0.05$) IP values than Tctrl and TC and with no significant differences among them (23.0, 20.7 and 23.5 h respectively). The high stability of the sample TC is probably due to its maximum

content in sterols and tocols, natural bioactive compounds that preserve the lipid fraction with a synergic antioxidant effect (Cheng *et al.,* 2022), together with its pattern in fatty acids, rich in SFA and mainly in palmitic acid. On the other hand, considering the results in tocols and sterols of the Tctrl sample, its high oxidative stability is unexpected, except for its high content in SFA and low percentage in PUFA compared to the other samples. These results for TC and Tctrl are probably also linked to the high concentration of other compound with well-known antioxidant properties that are typically present in these oils: the c-oryzanol in rice oil and the polyphenols in EVOO (Jung *et al.,* 2017; Massarolo *et al.,* 2017), of which both sunflower and coconut oils are poor.

Taralli	IP (h)
Tctrl	38.7 ± 3.1a
TA	23.0 ± 0.33b
TB	20.7 ± 0.44b
TC	40.4 ± 1.15a
TD	23.5 ± 0.40b

Table 4.13. IP (induction period) values recorded for the different taralli samples, Different letters in the same row show significantly different mean values (Tukey's HSD $p < 0.05$).

Hardness measurement and sensory analysis

The taralli samples were tested for their texture characteristics using the penetration test. The heights of the products were very similar, with values varying between 11.68 (0.89) and 12.02 (0.26) mm. Figure 4.49 shows the response to the test in terms of fracturability and hardness. No differences were detected among samples for the above parameters. The high variability of samples was confirmed by the high values of SD, which was mainly attributable to the hand-made process of taralli production. The sensory data were analysed using a three-way ANOVA that evidenced the significant differences for taralli relative to the different attributes. The results of the statistical analysis, expressed as the output of Fisher's least significant difference (LSD) test, are shown in Table 4.14.

	Samples				
Attributes	Tctrl	TA	TB	TC	TD
Overall aroma	4.8a	4.9a	5.1b	5.1b	5.1b
Wine aroma	4.25a	4.2a	4.8b	4.75b	5.05c
Overall flavour	5.30d	4.45b	4.85c	3.75a	5.45d
Flavour of cereals	4.85b	4.75b	4.40a	4.45a	5.00c
Crispiness	5.10b	4.85b	4.95b	5.05b	4.35a
Consistency	5.35c	5.15b	4.85b	4.60a	5.10b
Friability	5.70a	6.15b	5.65a	5.60a	5.55a
Fat perception	5.15b	4.80a	5.65c	5.20b	5.55c
Palatability	5.30a	5.65b	5.25a	5.35a	5.35a

Table 4.14. Sensory data on taralli samples, Different letters in the same row indicate significant differences among samples (Fisher's LSD P < 0.05).

As shown in Table 4.14, the overall aroma was perceived differently for Tctrl and TA compared to TB, TC and TD, as was the flavour of wine. The overall flavour was perceived as more intense in Tctrl and TD compared to the other samples, the cereal flavour was perceived as stronger in TD taralli, while the crispiness was lower compared to the other samples. The consistency was perceived as lower in the TC sample, while it was higher for Tctrl. Higher scores for friability and palatability were assigned to TA taralli, and the perception of fat on the palate was stronger for taralli made with fat blends (TB, TC and TD). The results of the appreciation test for the different taralli samples are reported in Figure 4.50 in the form of a spider plot representation. As can be seen from the figure, Tctrl, TB and TD were rated similarly for overall appearance and shape, while TA and TC scored lower on the same attributes (TC had a lower score than TA). The flavour of Tctrl and TB received the highest scores, followed by those of TA, TD and TC. The latter still has the lowest score for both aroma and flavour. The flavour score of Tctrl and TA was higher than that of TC but lower than that of TD and TB. The product with the highest crispiness score was TB, and the others had similar scores. Despite the higher intensity of palatability, the TA sample scored lowest on this attribute, while TD was the most valued. Finally, Tctrl, TA, TB and TD received a similar overall rating for acceptability, while TC received a slight score. Overall, samples TB (87.5% EVOO and 12.5% SO) and TD (87.5% HOSO and 12.5% CO) were rated better than Tctrl on several parameters, such as appearance, shape and palatability. Consequently, TC (75% EVOO and 25% RO) and TA (100% HOSO) are further away from Tctrl on most parameters. Looking at texture attributes measured instrumentally (hardness) and by sensory analysis, the samples did not show any significant differences in the first case, while some differences were perceived among samples in sensory analysis. This discrepancy is consistent with the observations of (Barbieri *et al.,* 2018), who attributed the low correlation between the two methods, both to the heterogeneity of the samples and to the different way in which the evaluation was carried out, as the samples are exposed to different humidity and temperature conditions in the sensory test than in the penetration test.

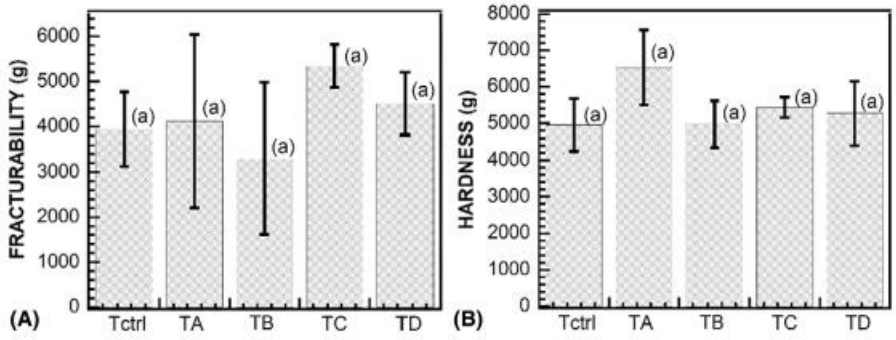

Figure 4.49. Fracturability (A) and hardness (B) of taralli samples measured through penetration test. Different superscript letters indicate significant difference (Tuckey's HSD $p < 0.05$).

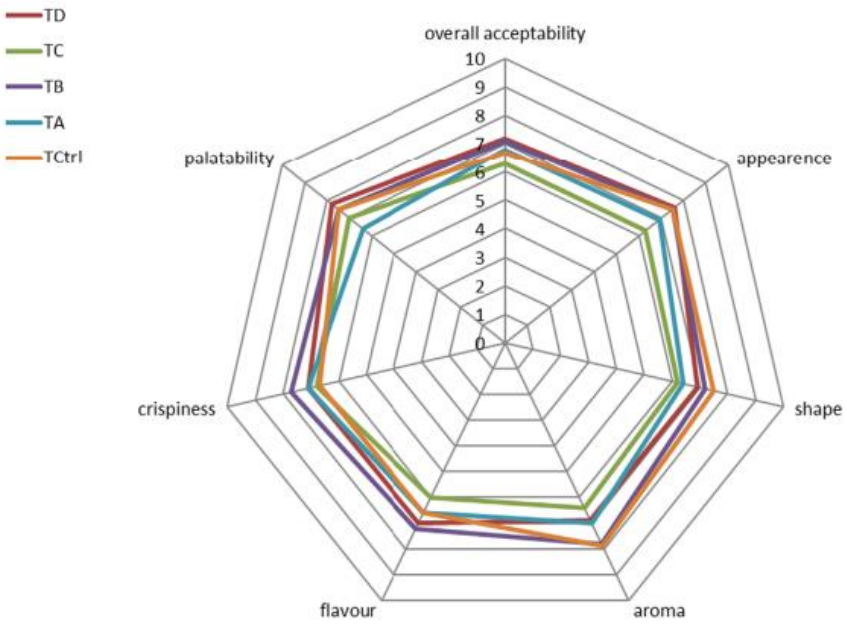

Figure 4.50. Spider plot illustrating the appreciation of the products through mean score assigned by the assessors to different descriptors.

Shelf-life trials and analyses

From the analysis of the various matrices, the choice regarding which samples to test in conditions of shelf-life simulations, was made upon the samples TC and TD. Not only the samples showed good adaptability in manner of texture and sensorial analysis, but also, the increased content of saturated fatty acids lead to the reasoning that said fats could partially stop the oxidative phenomena that normally affects the taralli, which are baked products with low shelf-life (≈2 months).

Determination of the Peroxide value by spectrophotometric detection

Results for the peroxide value are reported in Table 4.15 reported in meq O_2 /kg of oil.

Shelf-life (Days)	TCtrl	TC	TD
0	13.3±1.7	17.2±1.1	2.7±0.2
7	18.6±0.7	18.3±2.0	5.8±0.3
14	23.6±2.1	22.8±0.6	7.3±0.4
21	17.9±0.3	24.3±0.7	7.5±0.03
28	29.4±0.4	23.6±2.2	12.0±0.01
35	16.9±0.02	24.5±1.2	16.0±0.2
42	17.8±1.2	26.0±0.5	18.8±0.03
50	31.9±0.5	36.1±2.1	17.3±0.03
75	27.6±2.6	31.9±3.5	28.5±0.5

Table 4.15. Peroxides values for the 3 different formulations of taralli at different shelf-lifes. Results are expressed in meq O_2 /kg of oil.

By looking at the values for the TCtrl formulation, it is evident that the progression is somewhat erratic, as the peroxide concentration initially appeared to increase up to 14 days of storage, only to decrease at 21, 35, and 42 days, returning to values comparable to the initial level. After 28 days of storage, however, a peak of 29.4 meq O_2/kg of oil was reached, which then recurred at 50 and 75 days, with values that were not significantly different from each other ($p \leq 0.05$). Even before 14 days of storage, the sample had already surpassed 20 meqO_2/kg of oil, beyond which the fat can be considered altered. Nevertheless, the peroxide value is subject to fluctuations in this test because peroxides undergo degradation.

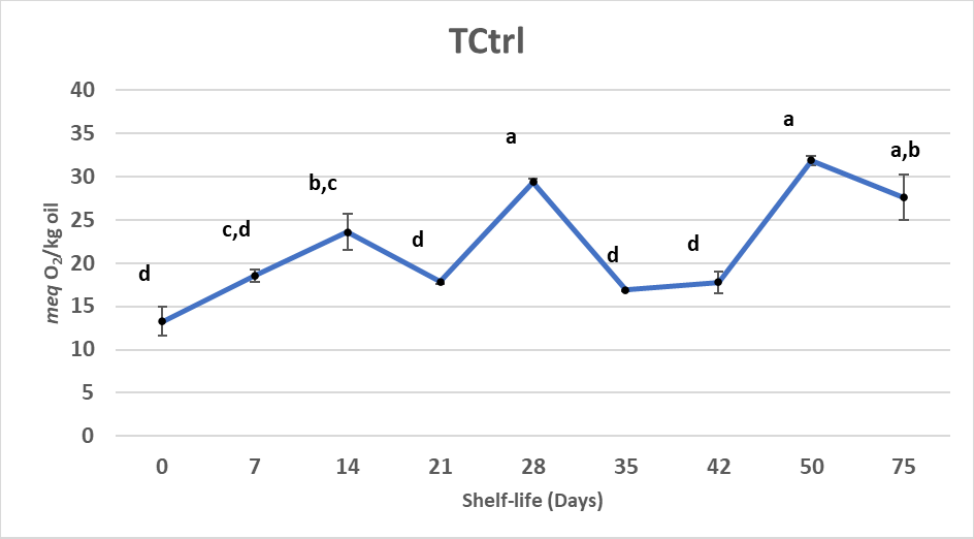

Figure 4.51. Trend of peroxide values over time for the TCtrl sample. Values are expressed as meqO_2/kg of oil. Different letters within each category represent significantly different values ($p \leq 0.05$).

In Figure 4.52 the peroxide value results for the TC sample are presented. Although in this case the graph displayed a more linear trend, with the peroxide content increasing very gradually from day zero to day 42, the sample exceeded the 20 *meq*O_2/kg threshold at around 10 days. Up to that point, the peroxide value did not show significant variations (fluctuating between 22 and 26 meqO_2/kg of oil, but then rose more sharply at 50 days, reaching 36.13 *meq*O_2/kg of oil. Similarly, the final value at 75 days of shelf life did not differ significantly from the preceding measurement.

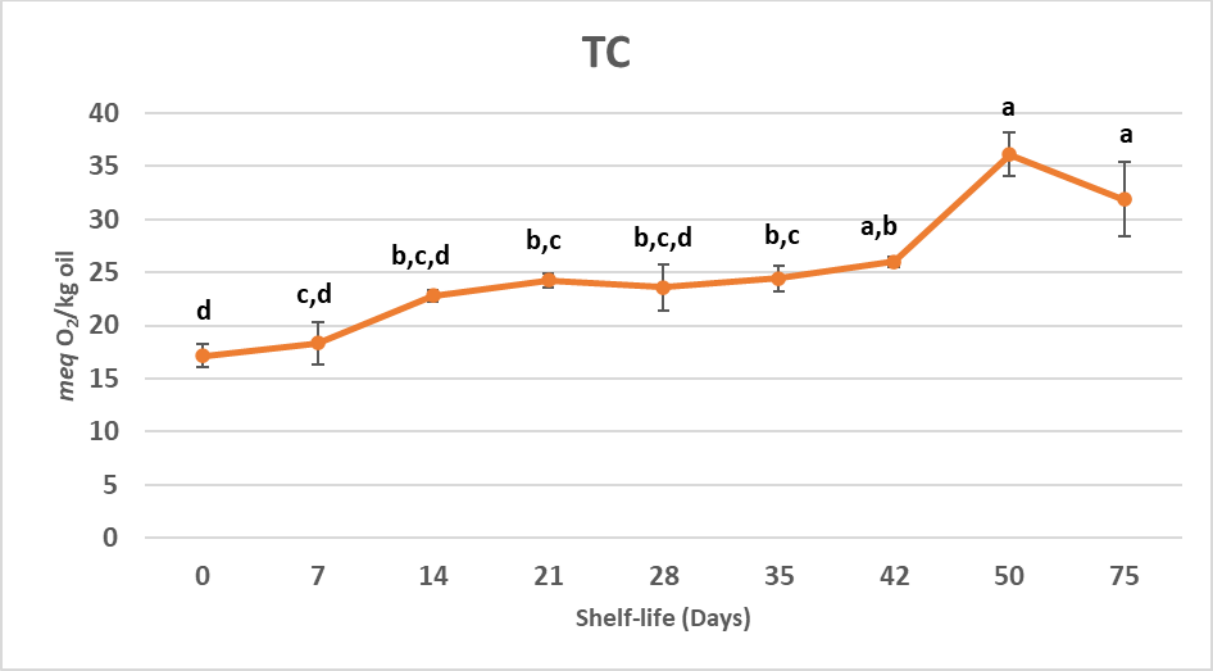

Figure 4.52. Trend of peroxide values over time for the TC sample. Values are expressed as meqO2/kg of oil. Different letters within each category represent significantly different values ($p \leq 0.05$).

As clearly shown in Figure 4.53, the TD sample maintained peroxide values within the legal limit of 20 meqO_2/kg of oil for more than 50 days of storage. The increasing trend was even more pronounced and evident; in this case, the peroxide number rose significantly ($p \leq 0.05$) from day zero to day 75, increasing from 2.67 meqO_2/kg of oil (at 0 days) to 28.51 meqO_2/kg of oil. This represented more than a tenfold increase in PV content.

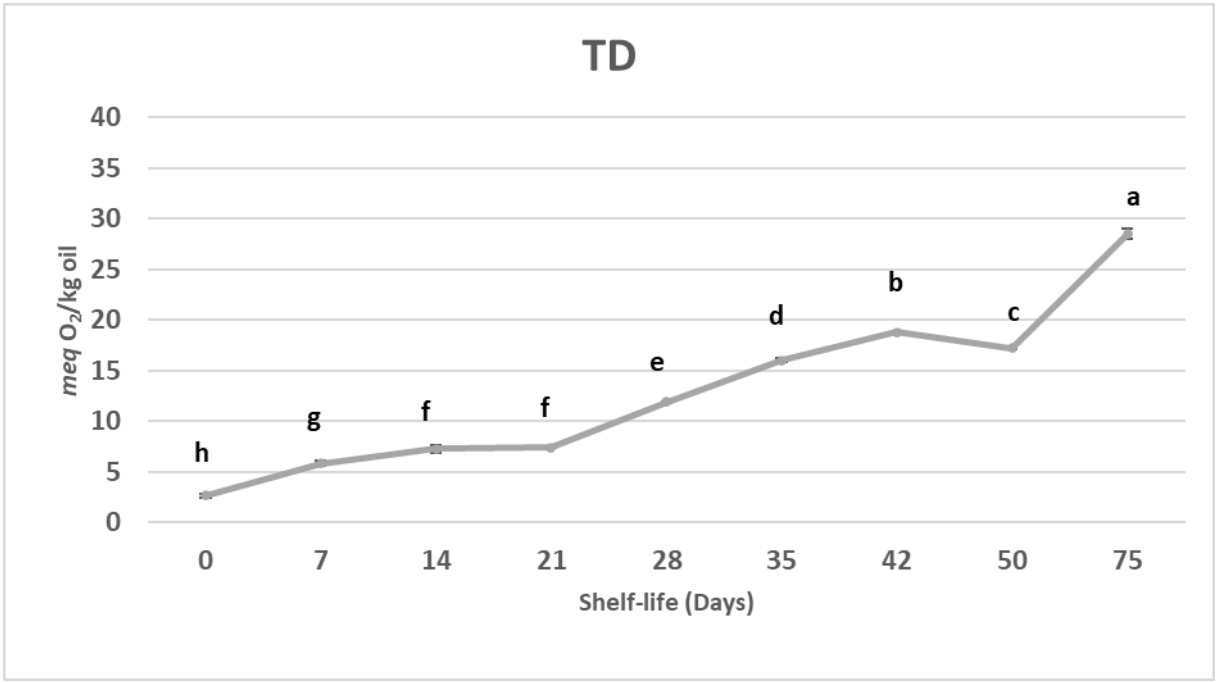

Figure 4.53. Trend of peroxide values over time for the TD sample. Values are expressed as meqO_2/kg of oil. Different letters within each category represent significantly different values ($p \leq 0.05$).

Figure 4.54 presents a bar chart that better highlights the differences in peroxide content among the three samples under study at the various analysed shelf-life times. First and foremost, it is evident that the TD sample displayed significantly lower values ($p \leq 0.05$) than the other two taralli formulations, with values ranging from 2.67 meqO_2/kg of fat at time zero to 17.25 meqO_2/kg of oil at 50 days. At the final shelf-life point (75 days), however, TD showed a significantly higher value (28.25 meqO_2/kg of oil), bringing it into line with the other two samples. The TCtrl and TC samples, on the other hand, exhibited very similar peroxide levels, fluctuating respectively from 13.25 to 27.59 meqO_2/kg of oil and from 17.16 to 31.91 meqO2/kg of fat. These two taralli only differed at 21 and 35 days, when TC displayed a significantly higher PV. At 28 days, the situation reversed, with TC's peroxide number decreasing and TCtrl's increasing, making TCtrl's value significantly higher at that point. It is important to note that by law, the peroxide value of a food is tolerable only up to a maximum of 20 meqO2/kg of oil. Applying this condition to the behaviour of the taralli samples, both TCtrl and TC containing substantial amounts of EVO—started from relatively high values and exceeded the 20 *meq*O_2/kg of oil limit after just 14 days of storage at room temperature. In contrast, the TD taralli, composed of 87.5% high-oleic sunflower oil and the remainder coconut oil, remained significantly lower and below the legal limit for all tested times, except at the end of the storage period (75 days), when it reached values comparable to the other two samples. This trend underscores that despite registering low values initially, TD underwent a more accelerated oxidation after two

and a half months, showing more than a tenfold increase in PV compared to the fresh sample. A study by Comandini and co-workers on taralli revealed a very similar situation (Comandini *et al.,* 2009). When comparing taralli formulated with different lipid sources among them an EVOO based tarallo and another tarallo formulated with refined palm oil, the EVOO based sample displayed a higher peroxide value. The tarallo with refined palm oil thus showed greater resistance to lipid oxidation. In that study, as in the present one, the EVOO-based sample also exhibited somewhat fluctuating PV values over time. In this present study, the greater resistance of taralli formulated with coconut oil (or palm oil in Comandini's work) can be partly attributed to the fatty acid composition of the lipid fraction, which contains a higher proportion of saturated fatty acids (SFA). Additionally, the method of oil processing used in formulation may have repercussions. Because TCtrl and TC contained high percentages of EVOO (100% and 75%, respectively), and EVOO is not subjected to any refining processes, it retains naturally occurring antioxidant molecules (e.g., tocopherols and phenolic compounds) but also certain pro-oxidant compounds (e.g., free fatty acids, phospholipids, trace metals) and any hydroperoxides already present. This combination likely influenced the observed oxidation trends.

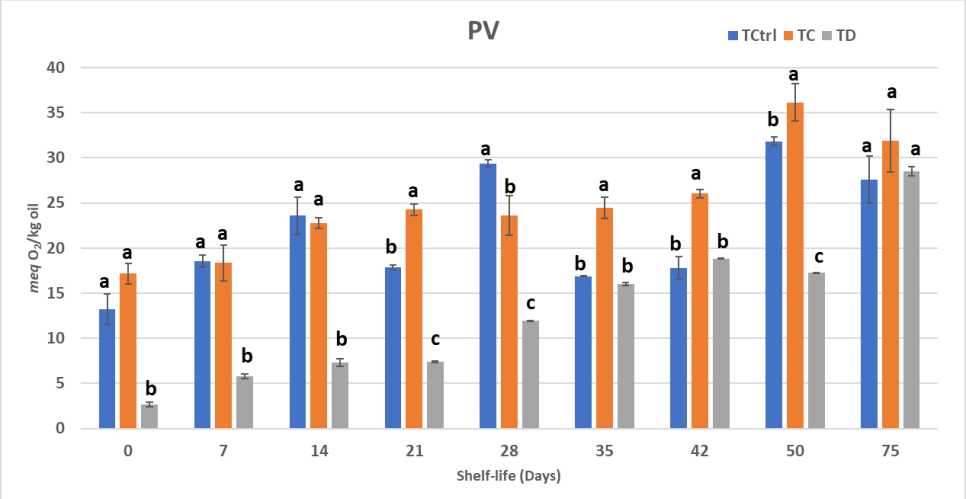

Figure 4.54. Comparison of the PV for all three samples (TCtrl, TC, and TD) at 20°C, at equivalent shelf-life times, expressed as meq O_2/kg of oil. Bars marked with different letters within each shelf-life interval indicate significantly different ($p \leq 0.05$) oxidized fatty acid values among the various taralli

Volatile compounds determination by SPME-GC-MS

The results for the quantification of the hexanal content are reported in Table 4.16, and are expressed in mcg/g.

Shelf-life (Days)	TCtrl	TC	TD
0	40.0±2.4	22.8±5.6	15.8±2.3

7	30.7±3.7	38.1±9.8	8.8±2.2
14	34.5±1.3	50.5±13.7	5.0±0.2
21	44.3±3.3	67.8±1.1	21.8±1.9
28	91.2±18.6	90.6±14.7	39.4±0.7
35	55.6±2.3	136.8±8.8	74.0±15.8
42	112.2±18.0	113.5±6.8	88.0±3.5
50	133.8±7.2	167.9±9.0	57.0±11.1
75	213.0±0.8	118.5±8.0	109.8±3.3

Table 4.16. Results of the hexanal content for the 3 different formulations at different storage periods. The results are expressed in mcg of hexanal/g of product.

Except for day 28, the TCtrl sample (100% EVO) exhibits a relatively flat and linear trend up to day 35 of shelf life, during which the hexanal content does not show any significantly different values ($p \leq 0.05$), fluctuating between a concentration of 30.66 and 91.20 mcg/g (Figure 4.55). From day 42 onward, however, the hexanal content begins to undergo a rapid and significant increase ($p \leq 0.05$), doubling its value (at 42 and 50 days) and reaching its peak at the end of the shelf life (75 days), with a hexanal content of 212.97 mcg/g, almost five times higher than the value recorded at time zero. The unusual spike in the value at day 28 aligns with what was observed for the peroxide values.

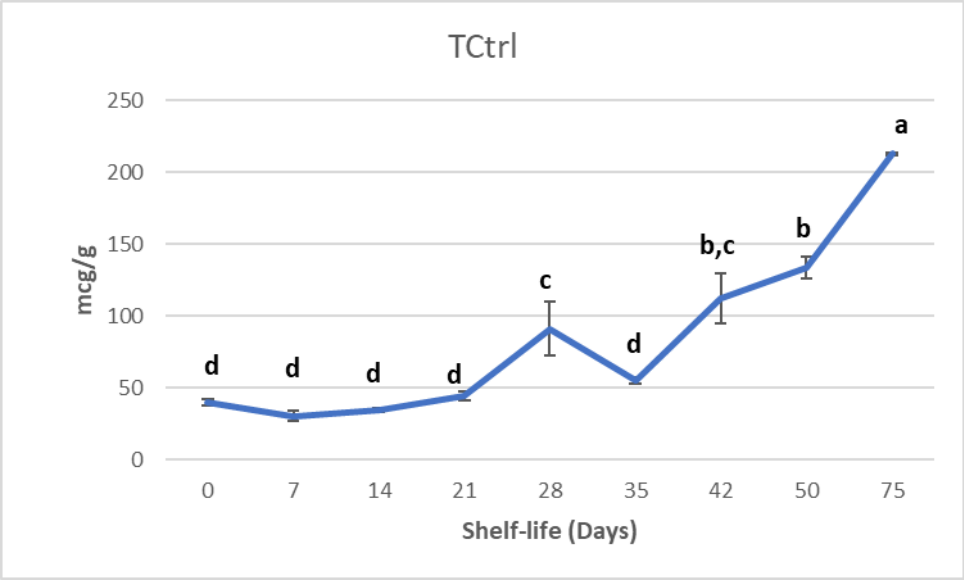

Figure 4.55. Trend of the hexanal content over time for the TCtrl sample. Values are expressed as mcg of hexanal/g of product. Different letters within each category represent significantly different values ($p \leq 0.05$).

For the TC sample, composed of EVOO oil and rice oil, a rising trend can be observed up to day 35, where the values increase gradually and reach a maximum of almost 140 mcg/g (Figure 4.56), which is significantly different ($p \leq 0.05$) from the values recorded in the previous sampling times. Subsequently, there is a slight, albeit not significant, decrease at day 42 (113.48 mcg/g), followed by an increase after 50 days

of storage, returning to a maximum concentration comparable to that at day 35. Finally, at 75 days, there is another decline to a value not significantly different from that at 42 days of shelf life. Despite these intermediate fluctuations, the hexanal content in the tarallo increases by over four times at the end of the storage period compared to time zero.

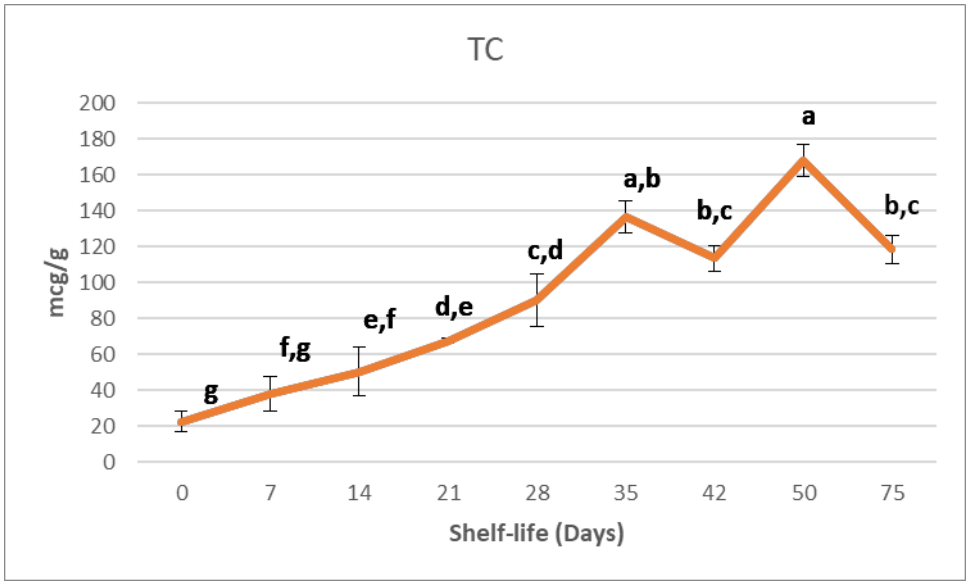

Figure 4.56. Trend of the hexanal content over time for the TC sample. Values are expressed as mcg of hexanal/g of product. Different letters within each category represent significantly different values ($p \leq 0.05$).

In the TD sample, formulated with high-oleic sunflower oil and coconut oil, the amount of hexanal present was much more contained, and, as observed for TCtrl, it remained practically stable during the first three weeks of storage (Figure 4.57). From day 28 onward, the hexanal content increased gradually and significantly ($p \leq 0.05$), reaching a maximum at 42 days with a value of 88.02 mcg/g. At 50 days, there was a significant decrease in this compound, followed by another rise, culminating in the highest value at the end of the storage period (75 days) at 109.81 mcg/g. In this case, the increase over time was even more pronounced, as the final value was more than 5.5 times higher than that recorded at time zero.

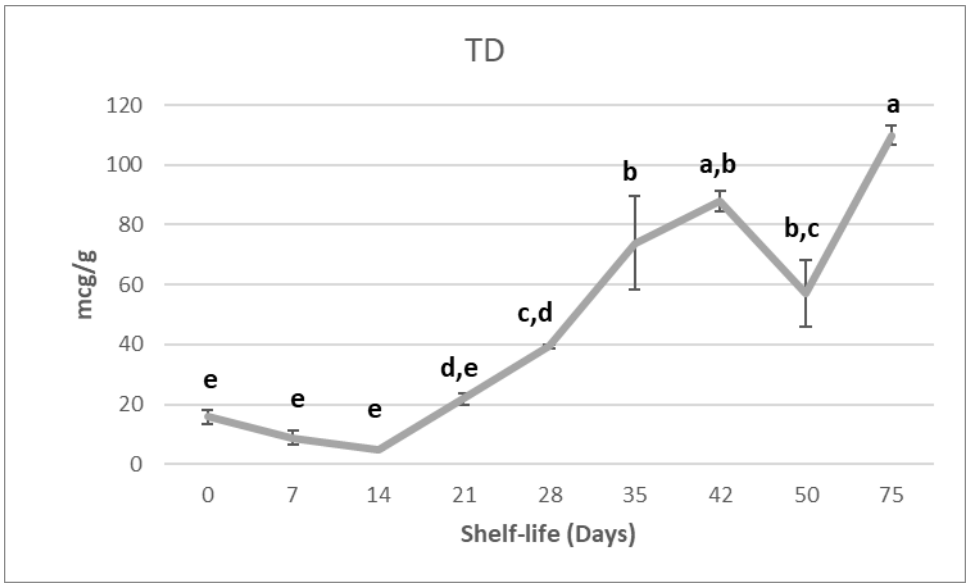

Figure 4.57. Trend of the hexanal content over time for the TD sample. Values are expressed as mcg of hexanal/g of product. Different letters within each category represent significantly different values ($p \leq 0.05$).

For various storage times (7, 14, 21, 35, and 50 days), a trend emerged that closely resembled what was observed for PV. Indeed, in this case as well (Figure 4.58) the TD sample, formulated with high-oleic sunflower oil and coconut oil, always exhibited the lowest hexanal content. Its progression started from a very low hexanal content in the first days of shelf life; at 14 days it presented the lowest recorded value. However, even here, although less markedly than for PV, the TD sample underwent more accelerated oxidation over time, ultimately reaching values at the end of shelf life (75 days) that were 5.5 times higher than the initial ones. The TC sample, composed of EVOO and rice oil, often showed a hexanal content very similar to TCtrl but with a much more regular increase over time and a significant decrease ($p \leq 0.05$) at the end of shelf life. On the other hand, the TCtrl sample, formulated exclusively with EVOO, displayed a somewhat more fluctuating trend, similar to that observed for PV, culminating in a significant rise at 75 days of storage and reaching the highest overall value. Previous studies on "tarallini" formulated with different lipid fractions (EVOO, olive oil, refined palm oil, and olive-pomace oil) had shown that the EVOO-based sample actually exhibited a lower hexanal content, followed by the refined palm oil sample (Caponio *et al.,* 2011).

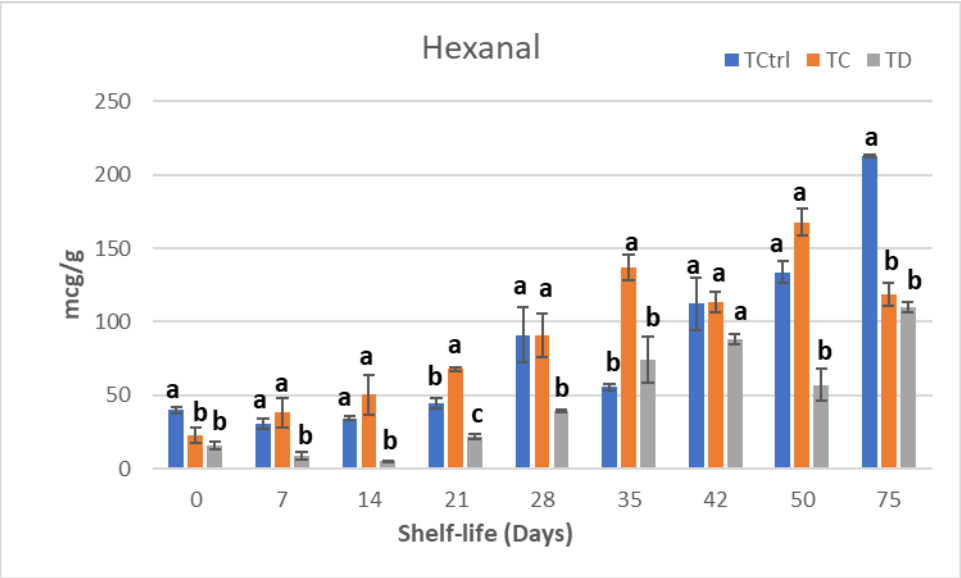

Figure 4.58. Comparison of the hexanal content for all three samples (TCtrl, TC, and TD), at equivalent shelf-life times, expressed as mcg of hexanal/g of product. Bars marked with different letters within each shelf-life interval indicate significantly different ($p \leq 0.05$) oxidized fatty acid values among the various taralli.

Determination of the general oxidative stability – OXITEST

Results regarding the Induction period (IP) for the different samples are shown in Table 4.17.

Shelf-life (Days)	**TCtrl**		**TC**		**TD**	
	IP (minutes)					
0	1782.5±136.5	A; c	2664.0±200.8	A; b	3432.5±4.9	A; a
7	1397.0±96.2	B,C; b	2418.0±202.2	A,B; a	2051.5±119.5	C; a
14	1055.5±48.8	C; b	2274.0±9.9	A,B; a	2244.0±19.8	B,C; a
21	1437.0±147.1	A,B; b	2175.5±146.4	A,B; a	2279.0±145.7	B,C; a
28	1338.5±53.0	B,C; b	2145.0±189.5	A,B; a	2337.0±97.6	B,C; a
35	1630.0±17.0	A,B; c	1884.5±58.7	B; b	2498.0±11.3	B; a
42	1461.5±112.4	A,B; c	1943.5±180.3	B; b	2218.0±118.8	B,C; a
50	1386.5±64.3	B,C; b	1963.5±111.0	B; a	2308.5±123.7	B,C; a
75	1474.5±94.0	A,B; b	1855.0±114.6	B; b	2297.0±86.3	B,C; a

Table 4.17. IP values registered for the 3 different formulations (TCtrl, TC and TD). Letters in uppercase show significant differences ($p \leq 0.05$) in the same sample among different days of shelf-life; letters in lowercase showcase differences in different samples and same days of shelf-life.

The TCtrl sample, formulated exclusively with EVOO, displayed a trend in the forced oxidation test that closely resembled a straight line, with IP values not significantly

different ($p \leq 0.05$) from one another. Initially, the sample showed a significantly higher ($p \leq 0.05$) IP value (approximately 30 hours) than at the other time points. This was followed by a slight decrease until day 14 (17.5 hours), and from day 21 of shelf life onward, it maintained IP values that did not differ significantly until the end of storage. Although these IP values were not extremely low, this trend indicated a certain degree of oxidative stability that remained constant throughout the entire tested storage period. The TC sample, formulated with EVOO and rice oil, also displayed a trend similar to that observed for TCtrl, with IP values ranging from approximately 44 hours at time zero to 30.9 hours at the final sampling. From the seventh day of storage onward, the values were no longer significantly different ($p \leq 0.05$) from one another, which, as in the previous case, indicated a certain oxidative stability over time. The TD sample showed a very high IP at time zero, around 57 hours of resistance to forced oxidation. Despite this, after just one week of shelf life, the IP decreased markedly and significantly ($p \leq 0.05$) to about 34 hours, then remained stable during subsequent storage times, with IP values that did not exhibit significant differences ($p \leq 0.05$). It can be clearly seen that the TCtrl sample, formulated only with EVO, had a significantly lower IP ($p \leq 0.05$) at time zero than the other two samples, approximately 30 hours, which was about 1.5–2 times lower than TC and TD. Although its trend remained constant over time, its IP was always significantly lower than that of the other two taralli. This finding partially aligns with the previously recorded PV and hexanal values, which were often significantly higher in TCtrl compared to TC and TD. As previously mentioned, EVOO's disadvantage lies in the fact that it undergoes no refining before use and, therefore, is not stripped of certain components that may promote lipid oxidation. The TC sample, despite also being formulated with 75% EVOO, showed a significantly higher IP ($p \leq 0.05$) than the sample made solely with EVOO for all tested times except at the end of the shelf life (75 days). This outcome was somewhat surprising, considering that among the three samples, TC's lipid fraction was the most unsaturated and thus more readily susceptible to oxidation. Indeed, the PV and hexanal values for TC were very similar to TCtrl, yet here it exhibited a forced oxidation resistance that was almost always significantly higher than TCtrl and not significantly different from TD. This result could be partly explained by the fact that, in addition to the EVOO's antioxidant compounds, such as tocopherols and phenols, there might have been contributions from the compounds present in rice oil, known to contain other antioxidants like tocopherols and γ-oryzanol. Overall, the TC sample had high IP values, generally above 30 hours. Regarding the TD sample, it displayed high IP values at every time interval, ranging from 57.2 to 38.3 hours, which were either in line with those of T3 or significantly higher ($p \leq 0.05$), as observed at time zero and at the end of storage. This stability and resistance to forced oxidation

were undoubtedly related to its fatty acid composition, which included fewer PUFAs and more SFAs than the other two formulations. Coconut oil, in fact, contains a very high percentage of saturated fatty acids (C10, C12, C14, and C16, with approximately 44.5% C12:0) and, combined with high-oleic sunflower oil, provides a substantial content of monounsaturated fatty acids. Although TD displayed the highest IP values, it is worth emphasizing that it was the only one of the three samples to experience a drastic and significant drop in resistance to oxidation as early as one week into shelf life, showing a value 1.6 times lower (in contrast, TCtrl and TC showed a decrease in IP of only 1.3 and 1.1 times, respectively, from day 0 to day 7). Taking the entire shelf life into consideration, it is important to note that both TC and TD experienced more than a 30% reduction in IP and thus in forced oxidation resistance, while the TCtrl sample showed a decrease of only about 17%.

Conclusions

This work identified the oil blend made of 75% extra virgin olive oil and 25% rice oil and the blend of 87.5% high oleic sunflower oil and 12.5% coconut oil as to be the most promising for being used in the taralli formulation. The first blend can be considered the best compromise in terms of lipid bioactive compound content, such as tocols and sterols. It results in a combination that shows improved resistance to the accelerated oxidation test, comparable to the reference control containing only extra virgin olive oil. The second blend, the unusual combination of 87.5% high oleic sunflower oil and 12.5% coconut oil, provided taralli that, despite the lower induction time, have a fatty acid composition (in terms of SFA, MUFA and PUFA), tocol and sterol content very close to that of EVOO and are also better accepted than the latter on the basis of the sensorial test. At the end of all the analyses carried out during the storage period, we can conclude that the results indicate that, after approximately two and a half months of storage at room temperature, the TD sample exhibited a better oxidative state compared to the other taralli. This sample featured a lipid blend composed of 87.5% high-oleic sunflower oil and 12.5% coconut oil—two fats whose fatty acid composition was highly favorable, as it was particularly rich in both MUFAs, provided by the high-oleic oil, and SFAs, provided by coconut oil. On the other hand, however, this sample also showed a faster rate of oxidative degradation, which could be related to a lower content of antioxidant compounds compared to the other samples. In the TCtrl and TC taralli, where EVOO served as the main fat with a wealth of natural antioxidants, its initial free acidity was higher than that of refined oils. Free fatty acids were far more susceptible to oxidation than their esterified counterparts, and the presence of these elevated oxidative indices in EVOO was partly linked to its production technology, which did not allow the removal of possible pro-oxidant

compounds. On the other hand, it showed a more gradual progression of the oxidative process, thanks to its high concentration of substances such as tocopherols and phenols. In the TC sample, in addition to the antioxidant substances present in EVOO, the contribution of those in rice oil—characterized by a high concentration of tocopherols, tocotrienols, and γ-oryzanol—was also evident. These results, however, suggested that the fatty acid composition might outweigh the presence of antioxidant substances in determining the oxidative quality of a fat-rich product like baked goods and taralli. The complexity of formulated foods could partly explain this phenomenon, as the interaction between certain compounds, such as fats and antioxidants, might not be straightforward or immediate.

Frollini

Preliminary selection

After the first batches of production were obtained, a series of fracturability and breaking tests were made, followed by a sensorial analysis, in order to ensure recognizable differences between the control and the alternative fat formulations. Results for the penetrability test are briefly summarized in Table 4.18.

Sample	Height (mm)	Fracturability (g)	Consistency (g/s)
B0	11.1 ±0.4	2429.0 ±791.0	2052.0 ±1137.0
B1	14.0 ±0.6	3241.0 ±506.0	3237.0 ±1435.0
B2	11.0 ±1.1	152.0 ±124.0	1592.0 ±397.0
B3	11.1 ±0.7	317.0 ±198.0	2257.0 ±575.0
B4	15.9 ±0.9	3085.0 ±855.0	7623.0 ±1078.0
B5	11.8 ±0.7	918.0 ±596.0	4626.0 ±2864.0

Table 4.18. Parameters acquired from the penetrability test

The various formulations behaved differently during, not only, the analyses but also during the product formulation; for instance, the B4 biscuits proved to be stickier and more difficult to shape in contrast to the B0, B2 and B3 formulations, in which the quantity of saturated fats was higher, which led to a more consistent dough. Moreover, said differences had been further found and identified in the behaviour of the B4 formulation on the chew, resulting crisper and harder in contrast to the other formulations. To better comprehend and explain the results, more results obtained from the breaking test are reported in Table 4.19, and their graphical representations in Figure 4.59.

Sample	hardness (g)	Fracturability (g)	Deformation module (s)
B0	1754.0 ±228.0	5044.0 ±639.0	0.4±0.1
B1	1918.0 ±338.0	5550.0 ±509.0	0.4±0.03
B2	1082.0 ±58.0	3047.0 ±727.0	0.4±0.1
B3	1266.0 ±101.0	3505.0 ±477.0	0.4±0.02
B4	2486.0 ±489.0	6894.0 ±243.0	0.3±0.1
B5	1639.0 ±294.0	3729.0 ±861.0	0.4±0.1

Table 4.19. Parameters acquired from the breaking test

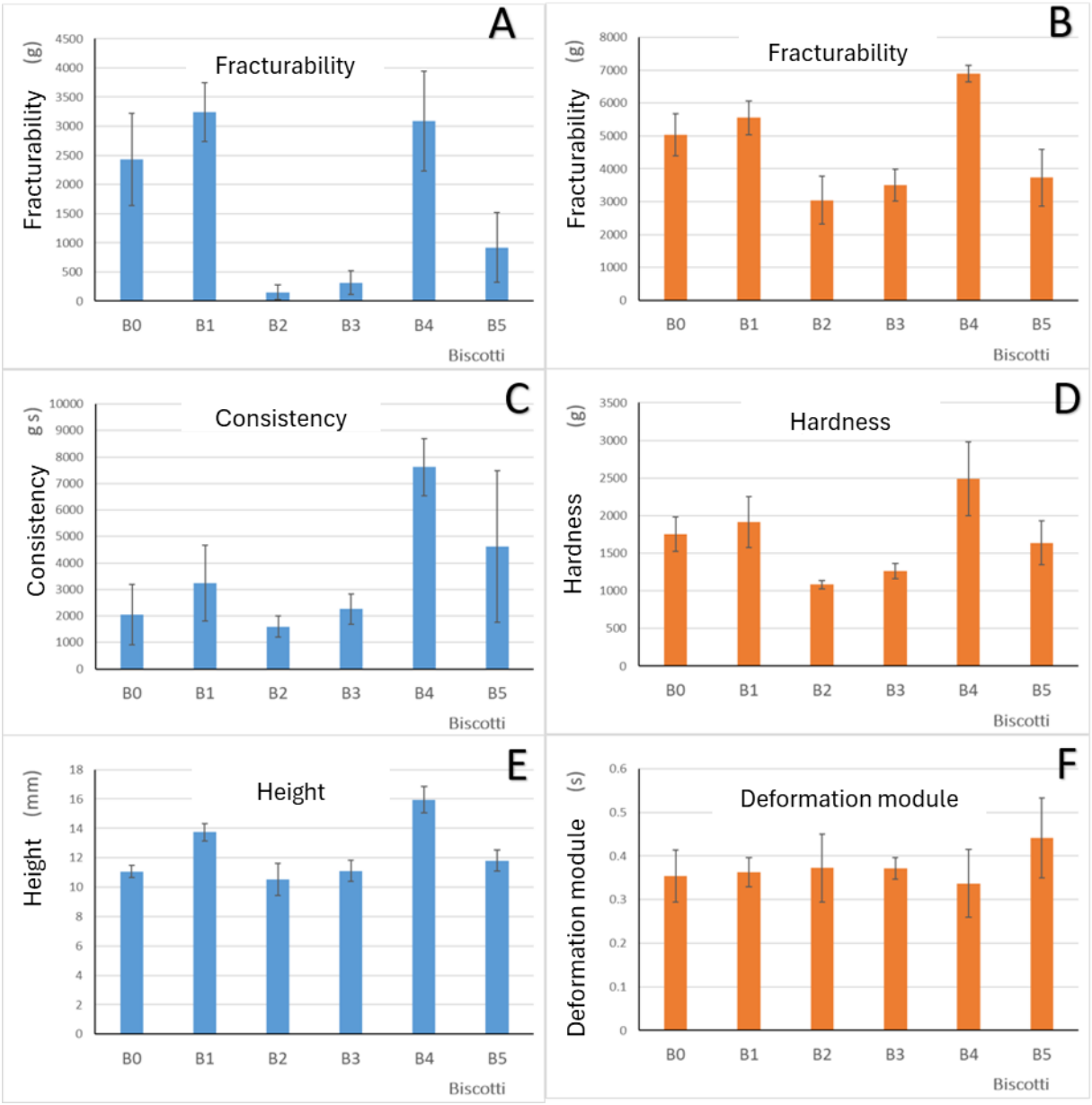

Figure 4.59. Results from the penetrability test (A,C,E) and the break test (B,D,F)

It is possible to observe how, consistency, hardness and both types of fracturability were higher in the samples obtained with unsaturated fats (B4), saturated fats, such as butter, palm oil, and coconut oil, are characterized by straight-chain fatty acids that pack tightly together, forming a stable crystalline structure, meanwhile unsaturated fats, such as sunflower oil, extra virgin olive oil, and other vegetable oils, contain one or more double bonds in their fatty acid chains, resulting in kinks that prevent tight packing and reduce crystallization, forming heterogeneous clusters which can lead to hardness increase. From these preliminary results, the formulation B0, as expected, resulted in the more fragrant and chewable out of the 5 tested, but in order to assess the quality of said control, a Sensorial analysis was made to ensure the quality of the products. The tasters were asked to provide a rating of liking/satisfaction/appreciation for each of the following attributes: appearance, shape, overall aroma, overall flavour, crunchiness, sweetness, and palatability. Using a structured scale, the tasters were instructed to mark a cross corresponding to the score they deemed appropriate. The results of the sensory evaluation of the biscuits are shown in Figure 4.60. The sensory perceptions for all the descriptors considered in the biscuits produced in this experiment revealed that the B5 biscuits, formulated with a blend consisting of coconut oil (87.5%) and sunflower oil (12.5%), were the most appreciated by the tasters. In contrast, the lowest overall liking score was assigned to the B4 biscuits, which were produced using only high-oleic sunflower oil. The B4 biscuits (high-oleic sunflower oil) were considered qualitatively inferior in terms of appearance, shape, flavor, and crunchiness compared to the other samples. The B0 (palm oil), B1 (butter), and B5 (coconut/sunflower) samples, as expected, did not exhibit any foreign tastes or aftertastes. Thirty percent of the tasters did not consider the poorly defined shape of the B1 biscuits a defect, while 100% deemed the absence of ridges on the surface of the B4 product to be a flaw.

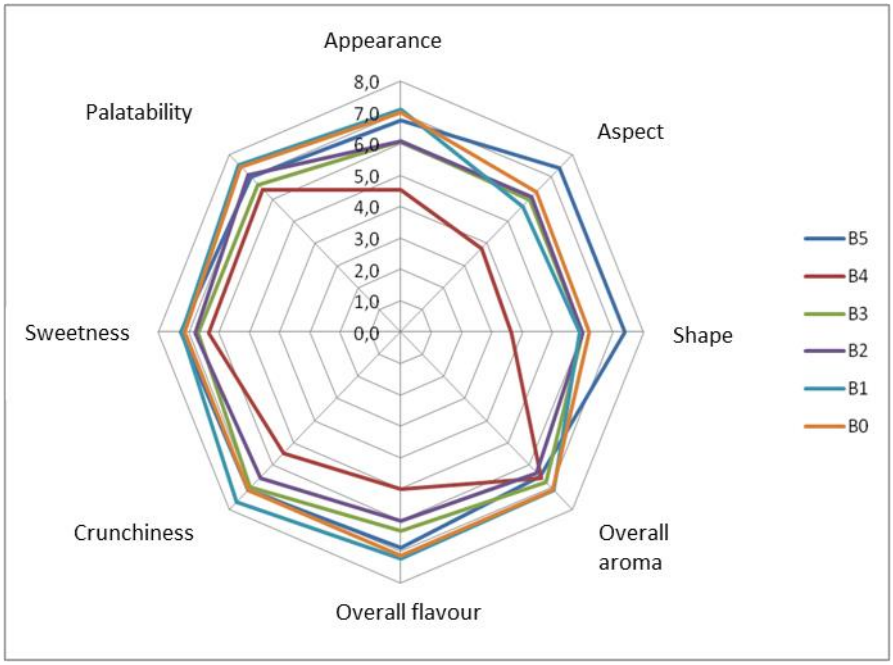

Figure 4.60. Spider plot describing the general appreciation, and overall performance of the 5 different biscuit formulations in sensorial analysis.

After preliminary sensorial and physical analyses, the work followed with the chemical characterization of 3 selected formulations, B0, as a control, B4, in virtue of being the only formulation with 100% of unsaturated fats in its formula, and B5, being one of the best performing formulations along the analyses. The said formulations were also preserved, as indicated in the "Shelf-life simulations" paragraph, and analysed.

Fatty acids analysis by FAST-GC-FID

Results regarding the FAME composition of the 3 types of Biscuits are shown in Table 4.20.

Sample	SFA	MUFA	PUFA
B0	46.7 ± 0.1	42.± 0.01	11.0 ± 0.1
B4	10.0 ± 0.1	78.4 ± 0.2	11.6 ± 0.05
B5	75.1 ± 0.1	13.1 ± 0.1	11.9 ± 0.04

Table 4.20. Average values of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids in the biscuit formulations, expressed in mg of FA/100 mg of FAME, along with their respective standard deviations.

As reported, the B0 and B5 samples composed entirely of palm oil (100%) and high-oleic sunflower oil (87.5%) plus coconut oil (12.5%), respectively shown a high SFA content. In contrast, the B4 sample, formulated solely with high-oleic sunflower oil (100%), was characterized by a predominance of MUFA. Moreover, the PUFA class is moderate in all three samples under analysis, and the differences among them are not

statistically significant. This trend naturally reflects the composition of the individual fatty acids within the samples. In the B0 and B5 samples, saturated fatty acids prevail due to the high content of palmitic acid (C16:0) and lauric acid (C12:0), respectively; on the other hand, B4 is composed primarily of monounsaturated fatty acids, particularly oleic acid, which constitutes over 77% of its total fatty acid profile. The Fatty acids analysis was conducted only at fresh state of the sample (t0), because the changes in the FA profile were deemed not detectable because of the high stability of the matrix.

Tocol analysis

In Table 4.23, the tocopherol and tocotrienol contents of the lipid blends characterizing the three selected biscuits are reported.

mg/100 g oil	B0	B4	B5
α-tocopherol	17.5 ± 1.6	90.1 ± 1.9	15.5 ± 0.4
α-tocotrienol	13.9 ± 2.0	n.d.	2.7 ± 0.02
β-tocopherol	1.1 ± 0.2	1.9 ± 0.6	1.7 ± 0.1
γ-tocopherol	3.7 ± 0.8	2.5 ± 0.6	2.1 ± 0.1
β-tocotrienol	4.9 ± 0.6	5.2 ± 1.1	5.4 ± 0.2
γ-tocotrienol	16.7 ± 1.9	n.d.	n.d.
δ-tocotrienol	5.6 ± 0.4	n.d.	n.d.
Total	**63.3 ± 7.4**	**99.8 ± 12.3**	**27.4 ± 0.9**

Table 4.21. Average tocol content for the three biscuit formulations (B0, B4, and B5), expressed in mg/100 g of oil. n.d. means non detectable.

Palm oil is known for its relatively high total tocol content and, importantly, for a broad spectrum of tocotrienols. This explains the presence of α-, β-, and γ-tocotrienols, as well as δ-tocotrienol, in addition to α-, β-, and γ-tocopherols. The rich tocotrienol profile reflects palm oil's distinctive composition and accounts for the substantial total tocol content observed in B0. High-oleic sunflower oil is predominantly rich in α-tocopherol, which is the main tocol present in sunflower seeds. This accounts for the high α-tocopherol value and the relatively simple tocol profile when compared to palm oil. Tocotrienols are typically scarce in sunflower oil, which explains their near absence in B4. Thus, the high total tocol content in B4 is largely attributable to α-tocopherol. Coconut oil, while valued for its stability, is generally low in tocols compared to oils derived from seeds such as palm or sunflower. The small proportion of high-oleic

sunflower oil in the blend provided some α-tocopherol, but the large coconut oil fraction lowered the overall tocol content. Hence, B5 presented moderate amounts of α- and β-, γ-tocopherols and fewer tocotrienols, resulting in a lower total tocol content than both B0 and B4.

Sterols analysis

Table 4.22 presents the sterol content in the analysed biscuit samples under study. Cholesterol was detected in all samples, attributable to the use of butter in samples B1, B2, and B3 and, additionally, to the inclusion of eggs in the formulations, which are known to contribute significant cholesterol concentrations (Aletor, 2017). Samples B1 and B2 exhibited the highest sterol contents, with 626 and 764 mg/100 g of oil, respectively, due to the elevated cholesterol levels (555 and 594 mg/100 g of oil, respectively), even though B1 was the sample with the lowest number of identified sterols. Subsequently, samples B3 and B4 demonstrated similar concentrations of total phytosterols, at 530 and 571 mg/100 g of oil, respectively. Notably, sample B4 had the lowest cholesterol concentration (394 mg/100 g of oil) but the highest β-sitosterol content (140 mg/100 g of oil), linked to the use of high-oleic sunflower oil in its lipid matrix. Furthermore, sample B4 was the only one to display the full spectrum of identified sterols. Finally, samples B0 and B5 reported the lowest sterol concentrations, at 307 and 508 mg/100 g of oil, respectively. In these samples, cholesterol was consistently the most abundant sterol (236 and 364 mg/100 g of oil), followed by β-sitosterol (47 and 95 mg/100 g of oil).

mg/100 g oil	B0	B4	B5
Cholesterol	236.4 ± 1.8	295.0 ± 0.8	364.6 ± 2.3
Campestanol	14.3 ± 0.5	30.9 ± 1.5	19.3 ± 0.6
Campesterol	3.2 ± 0.5	6.8 ± 0.1	3.9 ± 0.4
Stigmasterol	n.d.	16.7 ± 0.03	11.3 ± 1.4
β-sitosterol	47.4 ± 0.2	140.6 ± 1.0	95.3 ± 0.5
sitostanol	5.9 ± 0.8	11.5 ± 1.3	8.8 ± 0.9
avenasterol	n.d.	9.3 ± 0.7	5.0 ± 0.02
avenastanol	n.d.	47.4 ± 1.7	n.d.
Δ7-avenasterol	n.d.	13.0 ± 1.5	n.d.
Total	**307.3**	**571.2**	**508.2**

Table 4.22. Average sterol content for the three biscuit formulations (B0, B4, and B5), expressed in mg/100 g of oil. n.d. means non detectable.

Determination of the Peroxide value by spectrophotometric detection

As previously mentioned, peroxide quantification is one of the most used oxidation indices for rapidly assessing the oxidative state of a food product. Peroxides are primary products of lipid oxidation; they are odourless and tasteless and therefore not organoleptically detectable. They serve as precursors for volatile secondary oxidation products and can undergo further degradation if provided with sufficient energy (heat, light, metallic catalysts). The decomposition of peroxides leads to the formation of secondary products such as alcohols, aldehydes, and ketones, which are responsible for rancid odours and flavours (Manzocco *et al.,* 2020). The results obtained from the peroxide value (PV) analysis are expressed in milliequivalents of oxygen per kilogram of fat (meqO_2/kg of oil). The results are shown in Table 4.23.

Shelf-life (Days)	B0	B4	B5
0	1.7±0.2	3.0±0.4	3.1±0.1
28	1.3±0.02	2.8±0.02	0.5±0.03
50	1.7±0.06	1.7±0.06	11.7±0.1
90	2.5±0.2	4.2±0.2	1.2±0.1
120	0.7±0.06	1.6±0.1	2.4±0.2
175	0.6±0.05	0.3±0.04	1.1±0.03
240	1.0±0.1	1.2±0.2	0.4±0.02
300	1.2±0.01	2.7±0.03	0.9±0.15
355	4.6±0.2	0.9±0.1	5.4±0.02

Table 4.23. Peroxide content of biscuit samples stored at 20 °C, expressed as meqO2/kg of oil. B0 (100% palm oil), B4 (100% high-oleic sunflower oil), and B5 (87.5% coconut oil and 12.5% high-oleic sunflower oil).

In Figure 4.61 we can observe the trend of the peroxide content in the B0 sample stored at T=20 °C over different shelf-life periods. The peroxide concentration exhibits a linear pattern up to 50 days of storage, with values ranging between 1.65 and 1.72 meqO2/kg of fat and no significant differences. At 90 days, it rises significantly ($p \leq 0.05$) to reach 2.52 *meq*O_2/kg of oil, then decreases again after 120 days of storage, ultimately falling below the initial value (0.74 *meq*O_2/kg of oil). This relatively steady trend continues until 300 days, with values fluctuating without significant differences between 0.74 and 1.24 *meq*O_2/kg of oil. After this period, there is another significant increase at 355 days, where a peak value of 4.60 *meq*O_2/kg of oil is recorded, marking the highest level throughout the entire storage period.

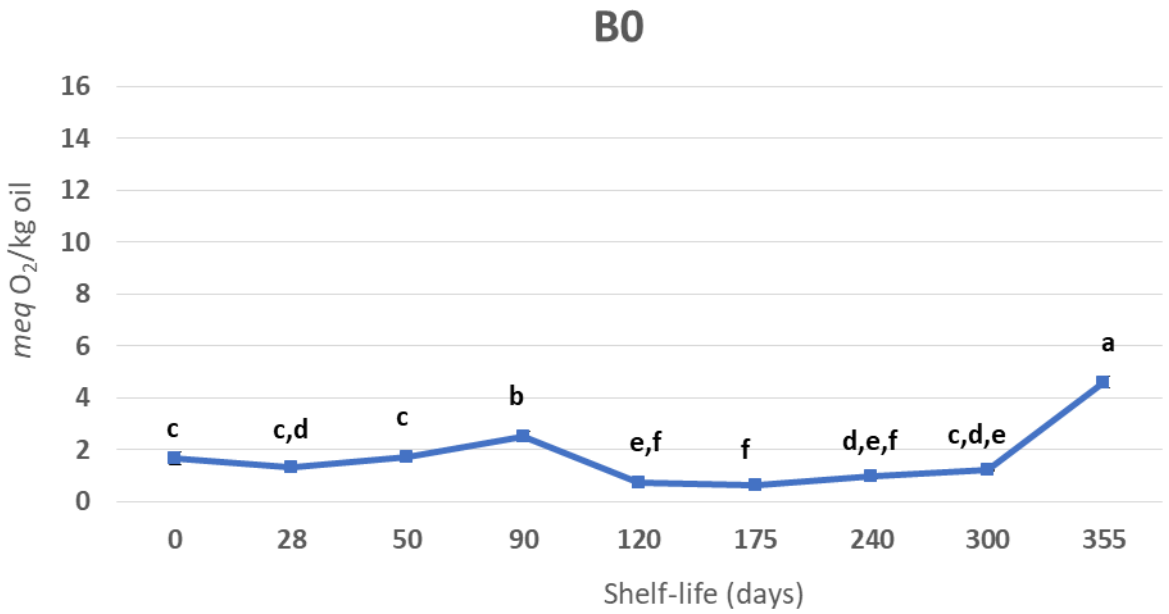

Figure 4.61. Trend of the peroxide number over time for the B0 sample stored at 20°C. Values are expressed as meqO_2/kg of oil. Values in the graph bearing different letters for the various shelf-life times are significantly different ($p \leq 0.05$).

In Figure 4.62, the same results for the B4 sample stored at 20°C can be observed. In this case, up to 28 days, the value remains unchanged at just under 3 *meq*O_2/kg of oil and then shows a decline at 50 days, reaching 1.66 *meq*O_2/kg of oil. Like the B0 sample, after 90 days of shelf life, the value rises again, reaching a peak of 3.90 *meq*O_2/kg of oil, before dropping again after 120 days to a level not significantly different ($p \leq 0.05$) from that recorded in the first 50 days of storage. This trend persists until 300 days, at which point another increase occurs (2.68 *meq*O_2/kg of oil), significantly similar to the values observed at the initial storage times. Unlike the previous sample, however, by the end of the storage period the PV tends to decrease toward levels recorded at earlier times (0.92 *meq*O_2/kg of oil).

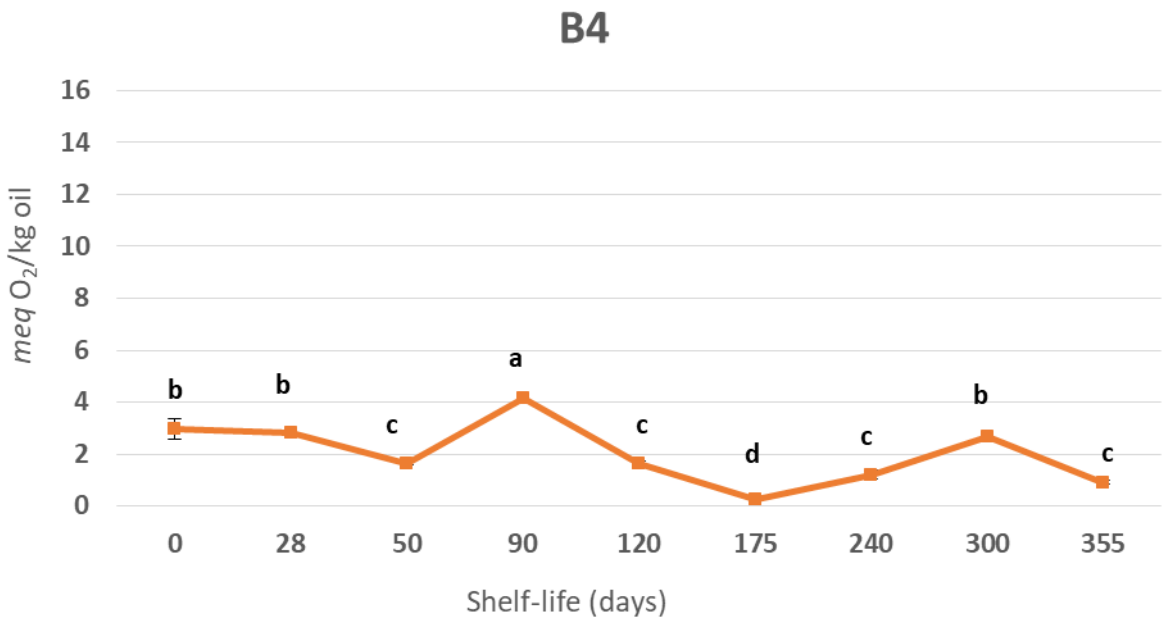

Figure 4.62. Trend of the peroxide number over time for the B4 sample stored at 20°C. Values are expressed as meqO_2/kg of oil. Values in the graph bearing different letters for the various shelf-life times are significantly different ($p \leq 0.05$).

As illustrated by Figure 4.63, the B5 sample exhibited a rather unusual trend. From day 0 to day 28, the peroxide content decreases significantly ($p \leq 0.05$), reaching a minimum of 0.51 *meq*O_2/kg of oil, only to rise dramatically at 50 days to a value substantially higher than even the peaks observed for the B0 and B4 samples, reaching 11.71 *meq*O_2/kg of oil. From day 90 to day 300, the content remains considerably lower, near the initial values, fluctuating between 0.42 and 2.35 *meq*O_2/kg of oil. Finally, at the end of the shelf life, the value increases again, reaching 5.44 *meq*O_2/kg of oil. Regarding the value recorded at 90 days, it can be considered an outlier, not attributable to measurement errors (as multiple tests were carried out) but likely due to a storage issue, such as incomplete sealing of the bag or a placement within the thermostat that might have caused localized overheating of the product.

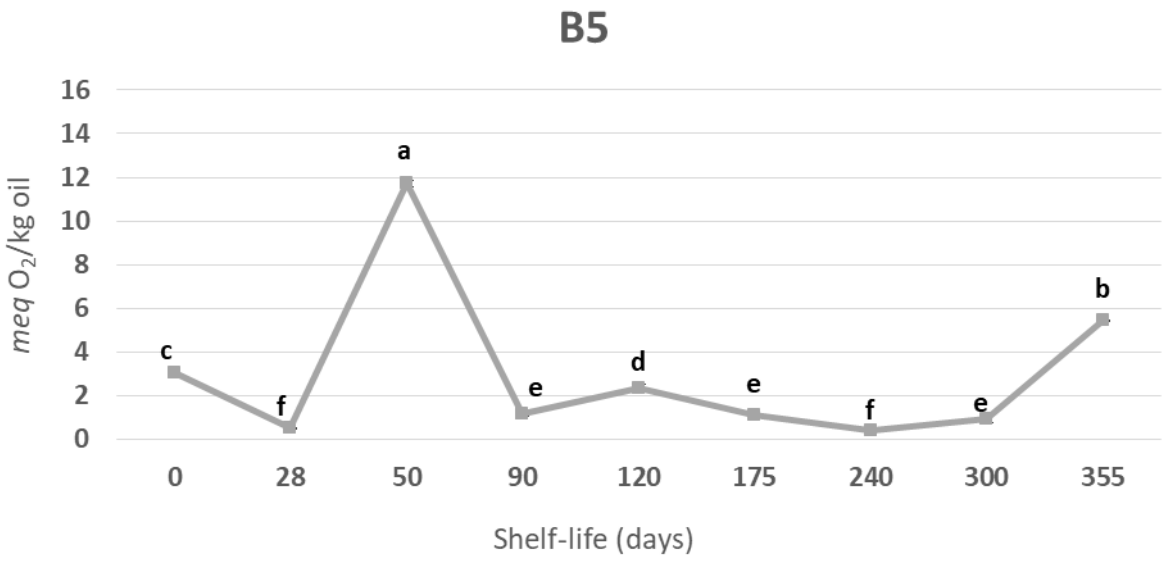

Figure 4.63. Trend of the peroxide number over time for the B5 sample stored at 20 °C. Values are expressed as meqO_2/kg of oil. Values in the graph bearing different letters for the various shelf-life times are significantly different ($p \leq 0.05$).

In Figure 4.64, the histograms highlight the differences in peroxide content among the three biscuit samples under study over various shelf-life times at 20 °C. The first observation is that, despite some fluctuations in values for all three biscuit types, for all the tested shelf-life times these remain below 6 *meq*O_2/kg of oil with the exception of the B5 sample at 50 days which, as previously explained, represents an outlier. These are decidedly low values, confirming that biscuits are a very oxidation-stable matrix. For the B0 sample, the PV is always lower or not significantly different ($p \leq 0.05$) from the value observed for the B4 sample. The only exception is at the end of storage, when B4 is the only sample to show a decrease rather than an increase. This is an unusual situation, as the peroxide value typically follows a cyclical trend during storage: peaks occur due to the formation of peroxides as primary oxidation products, while subsequent declines result from their transformation into secondary products, after which new oxidative phenomena can once again generate peroxides. The B5 sample sometimes shows significantly lower and at other times significantly higher values compared to the other two biscuits; similar to B0, B5 also shows a renewed increase at 355 days, reaching its highest final storage value, which is significantly greater than both B0 and B4. These results are favourable when compared with a shelf-life study conducted by Calligaris *et al.* (2007) on biscuits formulated with 20% fat (w/w), whose fatty acid composition was proportionally similar to that of the B0 sample. Under their 20 °C storage conditions, the reported PV ranged between 10 and 20 *meq*O_2/kg of oil between 60 and 140 days of storage significantly higher than the values obtained for

all three biscuit types in this project. Furthermore, the researchers observed that the PV at which 50% of consumers would reject the product (at 20°C) is between 10.6 and 15.4 *meq*O_2/kg of oil. Thus, it can be stated that all three samples in the present study achieved satisfactory results regarding primary oxidation.

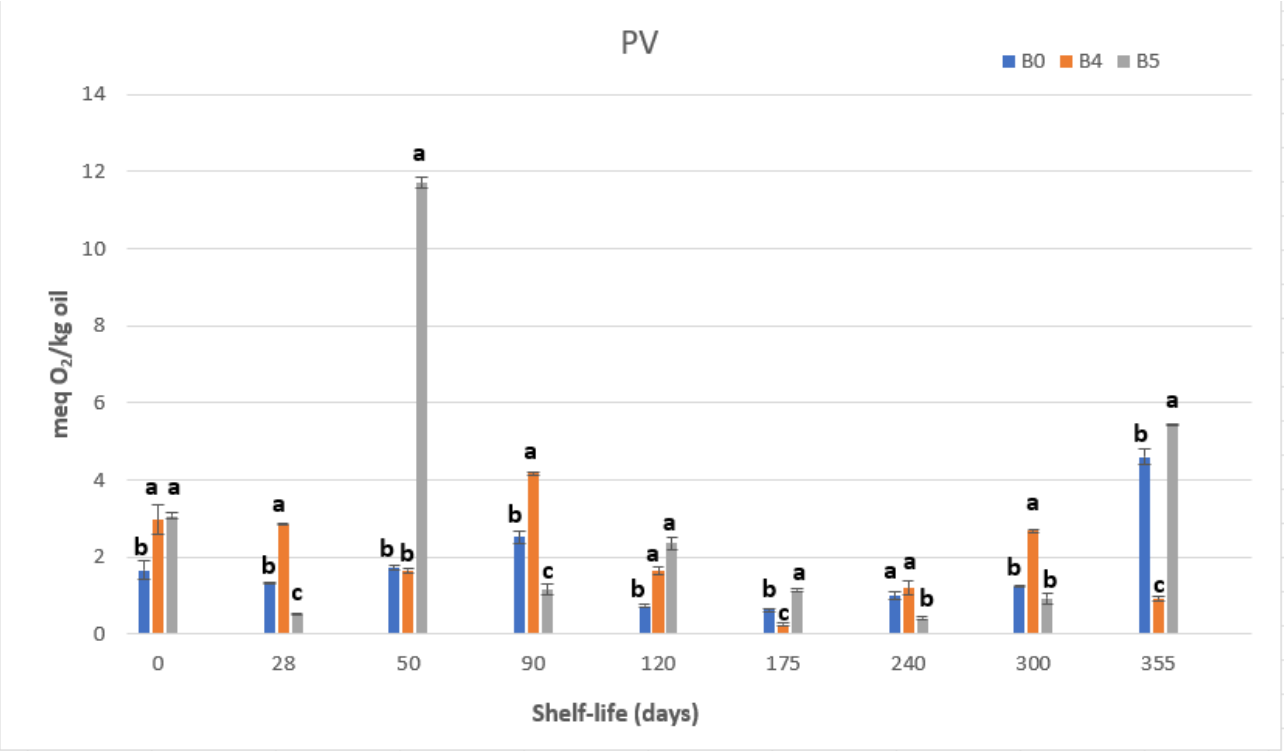

Figure 4.64. Comparison of the PV for all three samples (B0, B4, and B5) at 20°C, at equivalent shelf-life times, expressed as meq O_2/kg of oil. Bars marked with different letters within each shelf-life interval indicate significantly different ($p \leq 0.05$) oxidized fatty acid values among the various biscuits.

Determination by gas chromatographic analysis of oxidized fatty acids (OFA)

The determination of oxidized fatty acids (OFA) is still rarely applied in studies examining the oxidative state of fat-rich matrices, and for this reason there are not many literature references available for direct comparison. In this research project, this determination was chosen in order to gain additional insight into the oxidative progression of the samples. Since it was not possible to identify the OFA individually, a comprehensive quantification was performed by summing the areas of all associated peaks in the chromatograms of the lipid extracts from each of the three types of formulations. The values obtained are reported in Table 4.24.

Shelf-life (Days)	B0	B4	B5
0	7.6±0.7	3.7±0.4	3.2±0.4
50	3.3±0.4	3.1±0.4	2.9±0.5
120	2.7±0.1	3.6±0.2	3.6±0.3
240	2.4±0.3	4.7±0.3	2.4±0.1
355	2.6±0.4	2.6±0.4	3.0±0.5

Table 4.24. Registered values for the oxidated fatty acids content over different periods of storage. The results are expressed as mcg of OFA/mg of fat.

As shown in Figure 4.65, the B0 sample exhibited a significant decrease in OFA content from 0 to 50 days, after which it remained constant throughout the entire storage period, with values ranging from 3.3 to 2.4 mcg/mg. A plausible hypothesis could be that at t0 the biscuits had been subjected to a baking process that may have initiated and increased the oxidative degradation of the lipid matrix. The OFAs themselves were unstable compounds that could evolve into further oxidation products. Under these storage conditions at room temperature and protected from light, the biscuit produced solely with palm oil appeared to maintain particularly stable values throughout its shelf life.

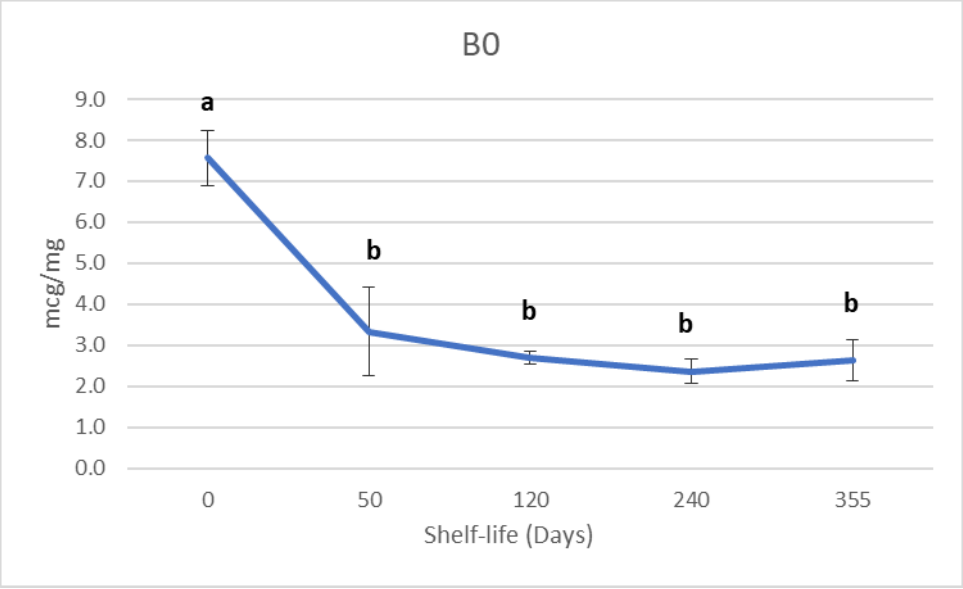

Figure 4.65. Trend of OFA content over time for the B0 sample at 20°C. The data are expressed as mcg/mg of fat. Values in the graph bearing different letters for the various shelf-life times were significantly different ($p \leq 0.05$).

The B4 sample, formulated entirely with high-oleic sunflower oil, exhibited a stable trend over the 355-day storage period, with values fluctuating between 2.6 and 4.7 mcg/mg.

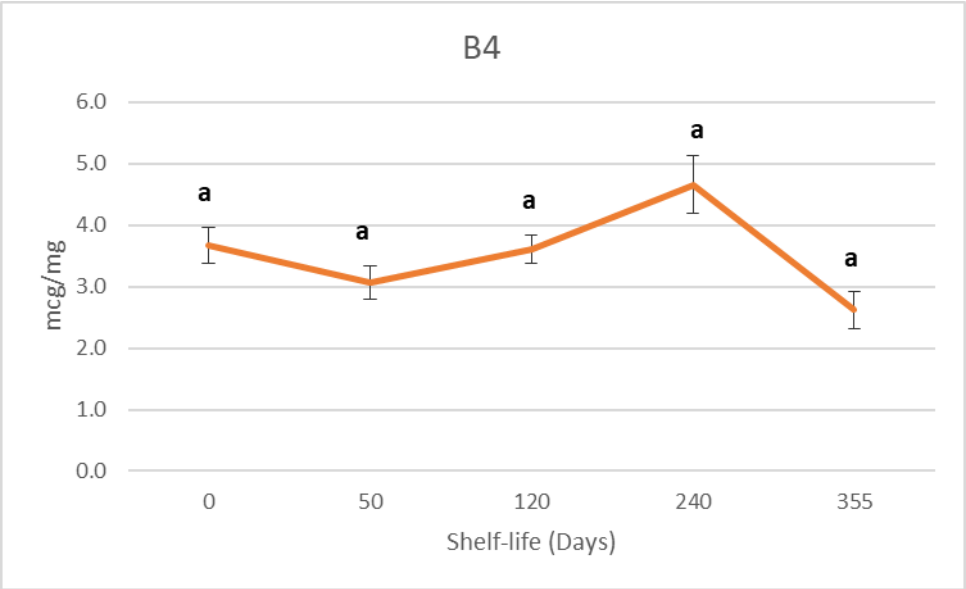

Figure 4.66. Trend of OFA content over time for the B4 sample at 20°C. The data are expressed as mcg/mg of fat. Values in the graph bearing different letters for the various shelf-life times were significantly different ($p \leq 0.05$).

Figure 4.67 shows the OFA content trend for the B5 sample, which, as in the previous cases, displayed entirely stable values that did not differ significantly ($p \leq 0.05$) from one another. The values recorded for this sample, obtained with a blend of high-oleic sunflower oil (12.5%) and coconut oil (87.5%), ranged between 2.4 and 3.6 mcg/mg.

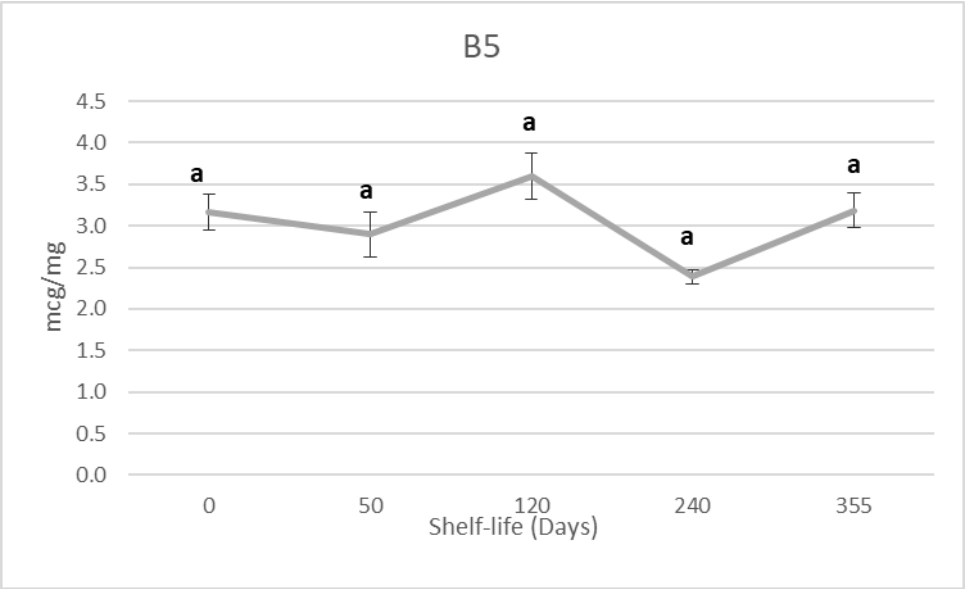

Figure 4.67. Trend of OFA content over time for the B5 sample at 20°C. The data are expressed as mcg/mg of fat. Values in the graph bearing different letters for the various shelf-life times were significantly different ($p \leq 0.05$).

As shown in Figure 4.68, the trends of OFA content in the three biscuit types analysed were compared. Except at time zero (t0), where B0 exhibited a significantly higher

value, and at 240 days, where B4 had a significantly ($p \leq 0.05$) greater OFA content than the other two samples, at all other shelf-life times the values remained significantly similar. This provided further confirmation that all three formulations demonstrated comparable and satisfactory oxidative stability.

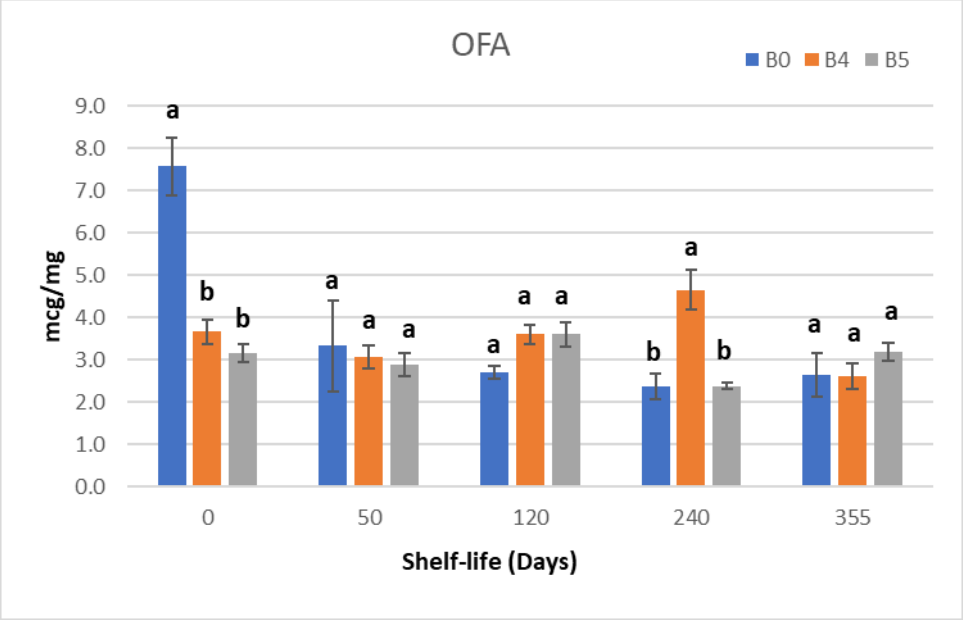

Figure 4.68. Comparison of OFA content for all three samples (B0, B4, and B5) at 20°C, at equivalent shelf-life times, expressed as mcg/mg of fat. Bars marked with different letters within each shelf-life interval indicate significantly different ($p \leq 0.05$) oxidized fatty acid values among the various biscuits.

Volatile compounds determination by SPME-GC-MS

In Table 4.25 the results of the Hexanal content all over the 3 samples are reported.

Shelf-life (Days)	B0	B4	B5
0	4.2± 0.2	3.1± 0.2	4.5± 0.1
28	2.6± 0.1	3.1± 0.4	2.8± 0.3
50	1.2±0.01	1.3±0.4	1.9± 0.2
90	0.8±0.8	1.0±0.2	0.7± 0.2
120	2.9±0.5	1.3±0.4	0.3± 0.2
175	8.3± 1.1	5.1±1.6	11.1± 1.7
240	9.8± 0.4	9.3±1.0	17.3± 1.1
300	13.8±0.3	14.8± 3.5	13.4± 1.3
355	4.0±0.1	10.6± 0.5	14.1± 1.0

Table 4.25. Average hexanal content for the three biscuit formulations (B0, B4, and B5), expressed in mcg/g of product.

The Sample B0 is characteristically rich in saturated and monounsaturated fatty acids and also contains a complex profile of tocopherols and tocotrienols, which provide antioxidant activity. At the beginning of storage (0 days), B0 exhibited moderate hexanal values around 4.22 mcg/g. This initial presence may have stemmed from the high-temperature baking process, which can initiate some degree of lipid oxidation and produce volatile compounds. Over the first 50 days, hexanal levels in B0 decreased significantly, dropping to as low as 1.18 mcg/g. As storage progressed (90 to 120 days), hexanal levels remained relatively low, reflecting the inherent oxidative stability of palm oil. However, at 175 and 240 days, the values began to increase markedly, reaching 8.32 mcg/g and 9.75 mcg/g, respectively. These mid-to-late surges suggested that, over time, some oxidative processes had advanced likely due to gradual depletion or reduced efficacy of antioxidants and the formation of secondary oxidation products. By 300 days, hexanal values in B0 reached approximately 13.80 mcg/g, representing a substantial oxidative impact despite the stable nature of the base oil. Interestingly, at the end of the shelf life (355 days), the hexanal level fell back to around 3.98 mcg/g, indicating that the system had undergone further transformations. Compounds like hexanal can degrade or react with other ingredients, illustrating the non-linear, dynamic nature of oxidation pathways over extended storage.

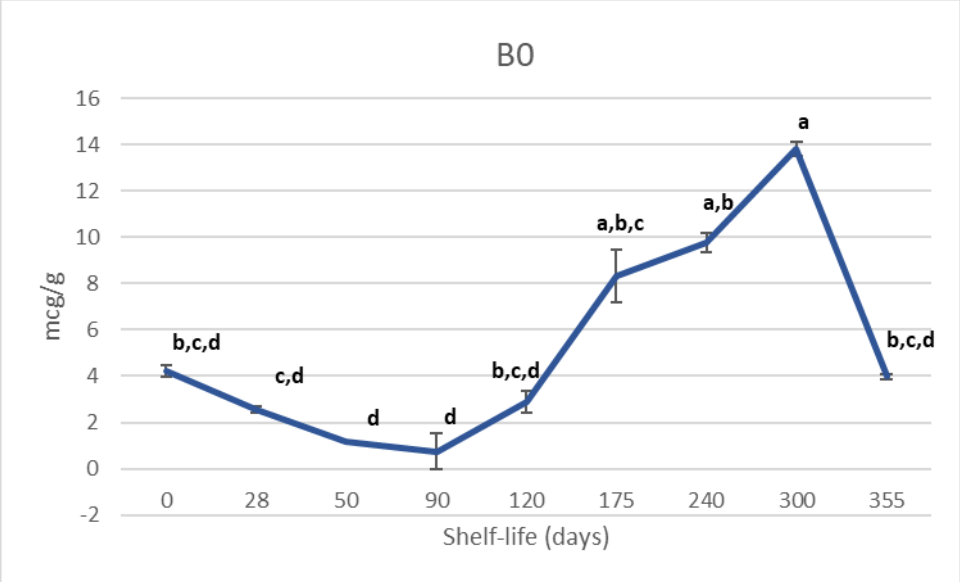

Figure 4.69. Trend of hexanal content over time for the B0 sample at 20°C. The values are expressed as mcg of hexanal/g of product. Values in the graph bearing different letters for the various shelf-life times were significantly different ($p \leq 0.05$).

Initially, B4 recorded relatively moderate hexanal values, starting at about 3.13 mcg/g. Over the first 50 days, hexanal in B4 fluctuated only slightly, suggesting a stable oxidative state. The presence of a high proportion of monounsaturated fatty acids and α-tocopherol appeared to slow the early formation of secondary oxidation products.

From 90 to 120 days, hexanal levels in B4 remained low (around 1.03 to 1.30 mcg/g), demonstrating that high-oleic sunflower oil did not readily generate large amounts of hexanal during this earlier to mid-shelf-life period. However, as the storage period lengthened, hexanal values increased significantly at 175 and 240 days 5.05 mcg/g and 9.27 mcg/g, respectively indicating that prolonged storage eventually overcame the initial antioxidant protections. By 300 days, B4 reached even higher values (14.77 mcg/g), aligning with the cyclical nature of oxidation. Near the end of the shelf life (355 days), B4 still displayed a relatively high hexanal level (10.60 mcg/g).

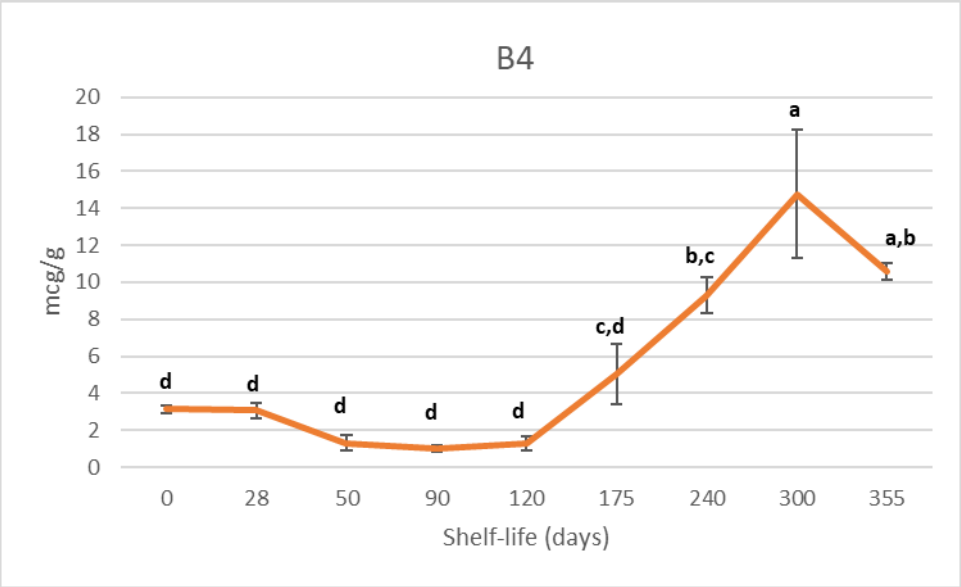

Figure 4.70. Trend of hexanal content over time for the B4 sample at 20°C. The values are expressed as mcg of hexanal/g of product. Values in the graph bearing different letters for the various shelf-life times were significantly different ($p \leq 0.05$).

Initially, B5 exhibited a hexanal content of about 4.52 mcg/g, comparable to B0 and slightly higher than B4. In the early stages (up to 50 days), hexanal levels in B5 dropped to values as low as 1.91 mcg/g, possibly due to the relative oxidative stability offered by coconut oil and the small input of monounsaturated fatty acids from the sunflower oil fraction. From 90 to 120 days, B5's hexanal content remained low (0.68 mcg/g to 0.27 mcg/g), suggesting that the combined matrix effectively resisted oxidative deterioration during this period. However, at 175 days, B5 experienced a significant jump to 11.11 mcg/g more than the other two formulations at the same time. This spike indicated that once certain oxidative thresholds were crossed, the system could experience substantial generation of secondary oxidation compounds. By 240 days, the hexanal level surged even further, reaching 17.30 mcg/g, the highest among the three biscuit types. by 300 days, B5's hexanal value returned to approximately 13.38 mcg/g, and at 355 days it remained high at 14.11 mcg/g.

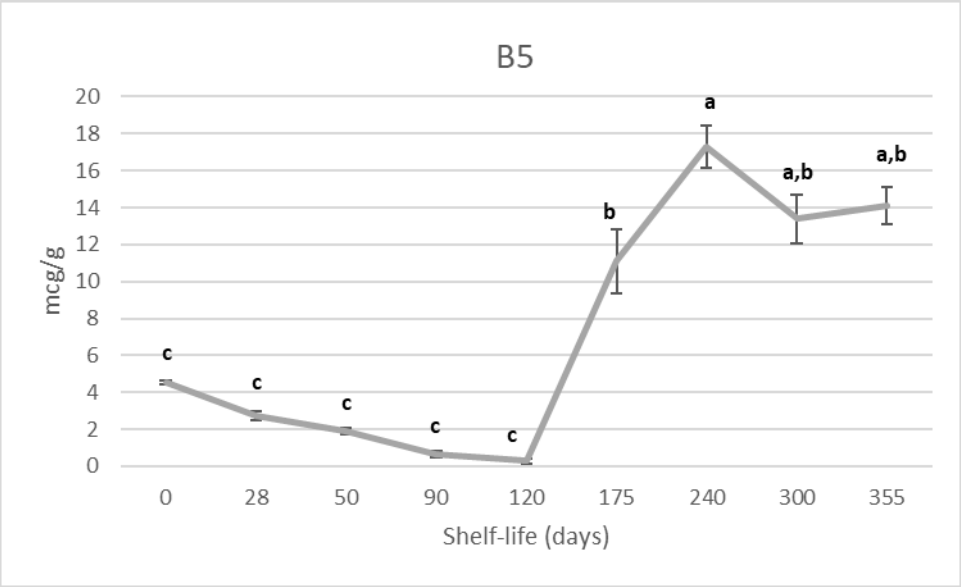

Figure 4.71. Trend of hexanal content over time for the B5 sample at 20°C. The values are expressed as mcg of hexanal/g of product. Values in the graph bearing different letters for the various shelf-life times were significantly different ($p \leq 0.05$).

By taking a look at the various results compared, as shown in Figure 4.72, it is possible to observe how among all of the samples B0 exhibited the lowest concentration of hexanal among the formulations, the reason is probably correlated with the high saturation of the fatty acids present in the oil use, which leads to a minor presence of oxidation phenomena; moreover, the B4 formulation with the latest development of hexanal, in fact in this formulation critical levels of hexanal were not reached up until the 240 days of conservation, meanwhile, the other samples started to develop high amounts at 175 days of conservation; the high presence of α-tocopherol, and its relative high anti-oxidative properties, are probably the main factor which contributed to the phenomenon. Finally, the B5 formulation showed intermediate values between the B0 and B4 formulations, exhibiting an initial trend comparable to the B0 formulation, probably attuned to the high presence of coconut oil, and a final evolution of the parameter with the highest spike in the hexanal trend, relatable to the oxidation of the sunflower oil present.

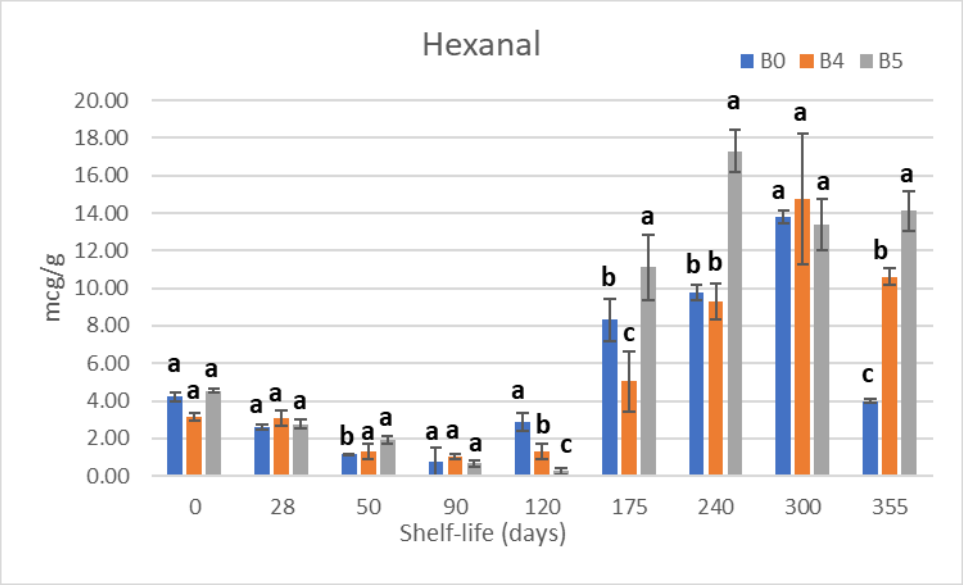

Figure 4.72. Comparison of the hexanal content for all three samples (B0, B4, and B5) at 20°C, at equivalent shelf-life times, expressed as mcg/g of product.

Determination of the general oxidative stability – OXITEST

To gain a more comprehensive understanding of the oxidative state of the biscuits under study, the samples were subjected to an accelerated oxidation test using the OXITEST instrument. As described in Materials and Methods, the results of this analysis are expressed in terms of an induction period (IP), the threshold time during which the sample can withstand specific forced oxidation conditions (T=100°C and oxygen pressure=6 bar) before undergoing qualitative deterioration. The results are thus expressed in minutes and obtained using a two-tangent method. For all the samples analysed, only a subset of the shelf-life points was selected, compared to the other analyses, specifically: 0, 50, 120, 240, and 355 days.

Shelf-life (Days)	**B0**		**B4**		**B5**	
	IP (minutes)					
0	3897.5± 275.1	A, a	2733± 91.9	A, b	2421.6± 148.4	A, b
50	3811.5± 335.9	A, a	3146± 367.7	A, ab	2440.5± 122.3	A, b
120	3836.5± 709.2	A, a	3209± 110.3	A, a	2804.2± 28.8	A, a
240	4969± 558.6	A, a	3011± 346.5	A, b	2397± 222	A, b
355	4361± 66.5	A, a	3415± 132.9	A, b	2545± 260.2	A, c

Table 4.26. IP values registered for the 3 different formulations (B0, B4 and B5). Letters in uppercase show significant differences (p≤0.05) in the same sample among different days of shelf-life; letters in lowercase showcase differences in different samples and same days of shelf-life.

In sample B0, although the absolute values appeared to show a slight increase in the biscuit's resistance to forced oxidation, the statistical analysis revealed that these recorded data did not exhibit any significant differences ($p \leq 0.05$). This finding underscored how the biscuit formulated with 100% palm oil remained highly stable throughout its entire shelf life. Consequently, this fat provided the product with excellent long-term stability, maintaining not only consistent but also high resistance values under forced oxidation conditions (approximately 60 to 80 hours). The B4 biscuit formulation also demonstrated excellent resistance to forced oxidation over time, with values that remained significantly similar from day 0 to day 355, ranging from 2,733 to 3,415 minutes. Finally, the B5 sample also presented a situation similar to the previous samples, with forced oxidation resistance remaining stable and unchanged as storage time progressed. Figure 90 compares the IP values of the various samples at the different tested storage times. Despite the pronounced stability of all three formulations over time noted above, it can be observed here that the B0 sample, formulated with 100% palm oil, exhibited significantly higher IP values than the other samples at all the shelf-life times examined, except for 120 days, when all three biscuits showed an IP that did not differ significantly from one another. This result aligns with the previously observed PV values and may indicate that the palm oil control remained the biscuit most resistant to the onset of oxidative processes. B4 and B5 showed IP values similar to each other, except at the final storage time point, when the B5 sample appeared to have a significantly reduced resistance to forced oxidation compared to the B4 biscuit. This result also seems to mirror the findings for both peroxide content and, especially, hexanal levels in this sample, which reached significantly higher final values than those of the other two formulations.

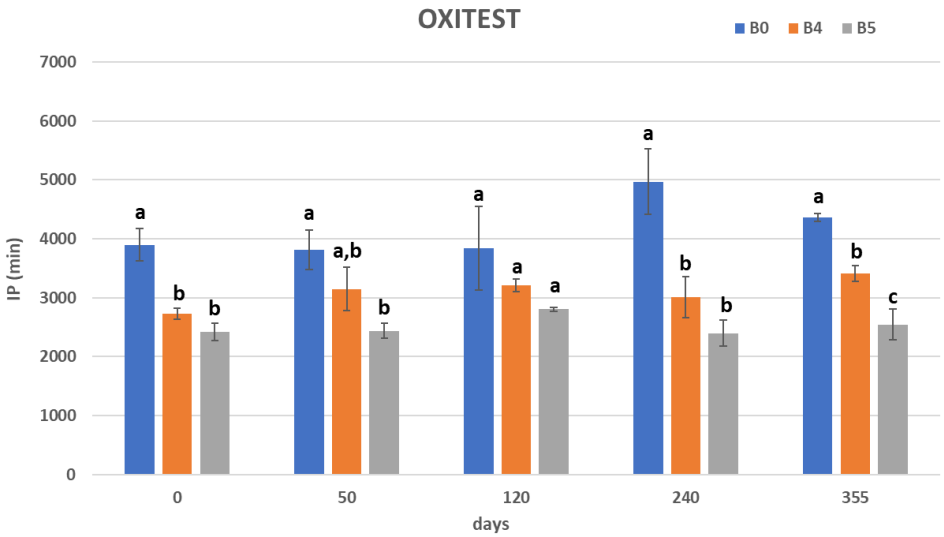

Figure 4.73. Comparison of IP values among B0, B4, and B5 obtained by performing the analysis at T = 90°C and a pressure of 6 bar. Bars with different letters within each category are significantly different ($p \leq 0.05$).

Sensory analysis

The sensory analysis was carried out by a panel of ten tasters, recruited from among the technical staff and researchers of the Department of Agriculture, Environment, and Food at the University of Molise. Each panellist was asked to provide an individual rating of liking/satisfaction/appreciation for the following attributes: Appearance, Shape, Overall aroma, Overall flavour, Crunchiness, Sweetness, Palatability. The results of the sensory test are presented using spider charts for the biscuits (Figure 4.74). In spider chart representations, the more the points connected by the continuous line overlap, the greater the similarity among the products. By using a spider chart, one can visually compare all product descriptors, numerically evaluating the characteristics/attributes under consideration. The primary purpose of this graphic representation is to highlight the level of perceived intensity for the product's attributes of interest, enabling a direct comparison among multiple products and underscoring the dominant features. The vertices of the spider chart represent the maximum perceived intensity values of the descriptors.

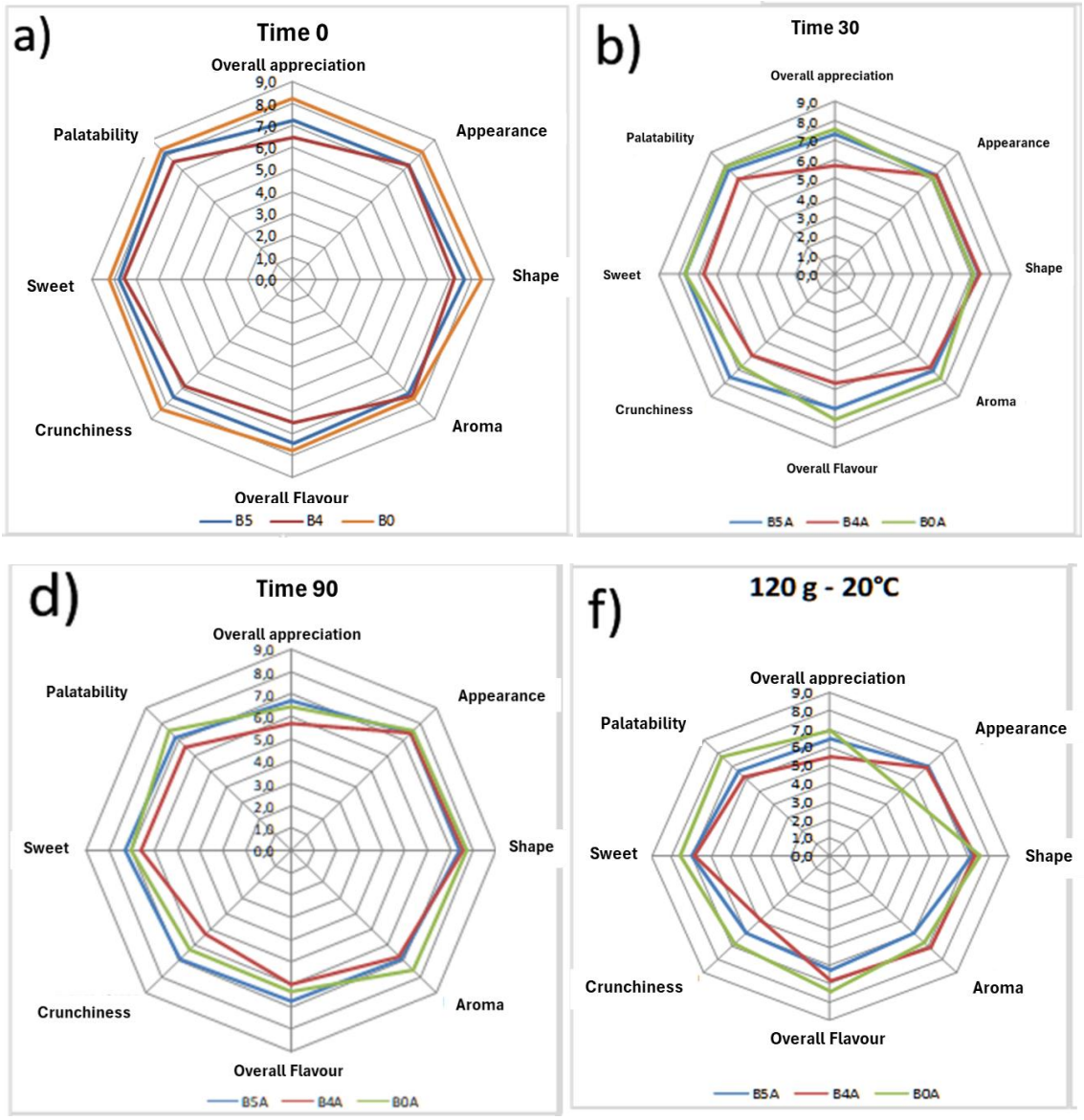

Figure 4.74. Results of the sensorial analysis for the three formulations (B0, B4 and B5) conducted at different days of shelf-life.

At time 0, the B0 biscuit received the highest score for all the attributes considered. The B5 biscuit's evaluation was very close to that of the control, while B4 differed due to lower scores for crunchiness and flavour, which reduced its overall acceptability. After 30 days of storage at 20 °C, there was a decline in the overall liking of the control sample (B0) and B4, whereas the overall liking of B5 remained virtually unchanged. Tasters detected the appearance of unpleasant off-flavours in the B4 biscuit. After 90 days of storage the main attribute that reduced acceptability for all biscuit types was crunchiness. At 90 days at 20 °C, some tasters began to perceive an unpleasant "old" flavour in the B4 biscuit. After 120 days of storage, overall acceptability of biscuits stored was similar to that observed at 90 days, at 20 °C, the B5 biscuit was described as having an "old" flavour, meanwhile the B0 biscuit presented a "bitter and pungent"

aftertaste. Further sensorial analyses on samples at time 175, 240 and 355 were not conducted due to the already satisfactory results obtained.

Hardness measurement

The results of the texture analyses conducted are shown in Table 4.27.

28 gg	**B0**	**B4**	**B5**
Fracturability (g)	2425.7 ± 256.4^{a}	1939.9 ± 548.0^{b}	2546.3 ± 763.7^{a}
Consistency (g·s)	1233.2 ± 281.7^{a}	1118.1 ± 687.5^{a}	901.4 ± 72.8^{a}
Hardness (g)	1823.5 ± 350.0^{a}	1795.5 ± 301.3^{a}	846.9 ± 156.1^{b}
Fracturabiity (-)	9063.7 ± 822.3^{a}	9338.85 ± 883.8^{a}	5988.0 ± 784.7^{b}
90 gg	**B0**	**B4**	**B5**
Fracturability (g)	2560 ± 400.8^{a}	2537.3 ± 403.7^{a}	2904.3 ± 119.3^{b}
Consistency (g·s)	1020.7 ± 232.7^{a}	1638.3 ± 346.8^{b}	1394.7 ± 261.2^{ab}
Hardness (g)	2216.3 ± 214.0^{a}	1696.1 ± 439.5^{b}	2577.0 ± 199.9^{c}
Fracturabiity (-)	8830.3 ± 146.5^{a}	8257.7 ± 480.5^{a}	11704.0 ± 526.7^{b}
120 gg	**B0**	**B4**	**B5**
Fracturability (g)	1972.6 ± 94.2^{a}	2594 ± 251.8^{b}	1999.7 ± 365.2^{a}
Consistency (g·s)	1443.7 ± 327.8^{a}	5870.0 ± 636.9^{b}	1384.1 ± 354.1^{a}
Hardness (g)	2983.0 ± 536.9^{a}	1789.0 ± 470.2^{b}	2170.5 ± 281.6^{bc}
Fracturabiity (-)	8101.3 ± 117.6^{a}	12553.7 ± 285.3^{b}	9824.0 ± 411.9^{c}

Table 4.27. Resume of the textural parameters analysed for the three different formulations of biscuit at different storage periods.

Analysis of the results revealed that the B0 sample remained unchanged in its response to the penetration test during storage. However, its hardness increased over the course of storage, showing higher values than those measured initially after 120 days. The B4 biscuit similarly showed no significant differences in penetration test results, while in contrast, its hardness decreased under storage conditions, and at the same time the force required to break the biscuit also decreased. The consistency of B5 remained unchanged at 20°C. Likewise, the hardness of this biscuit did not vary substantially, except after the first month of storage, after which it returned to values comparable to those observed at the start.

c. Third goal: non-destructive analysis of refined vegetable oils, building and modelling of calibration curves for analytical parameters of quality in vegetable oils.

Analytical parameters

All the samples analysed, recorded values regarding the already cited parameters (Free acidity, red and yellow colour), in line for what are described as good indexes by various disciplinaries. Values for free acidity ranged from 0.02 to 0.08 g of oleic acid/kg of oil, values considered under the limits proposed by international organisations (*SECTION 2. Codex Standards for Fats and Oils from Vegetable Sources*, no date). Red and yellow colours always assumed values ranging from 0.1 to 1.8 and 2.0 to 20 Lovibond units for all the samples considered, it is important to note how, especially the yellow parameter is strongly dependant on the concentration of various pigments and fatty acids present in the sample, of which will be highlighted a further discussion in the following subparagraph.

NIR readings and data elaboration

By looking at the signal presented by the various samples analysed it is possible to assess 3 regions regarded as "of interest", specifically, Region 1 (1100÷1260 nm), region 2 (1340÷1480 nm) and region 3 (1700≥ nm). These regions of the spectra have been characterized, over the course of the years, with the capability of interacting with C=C, C=O, C-N and C-OH bonds, which are, as reported in various studies, imputable to the lipidic fraction of these kind of samples. Refined vegetable oils are characterized by high contents of lipidic molecules, mostly Triglycerides (TG), with a smaller content of Di and Monoglycerides, Free Fatty acids (FFA) and waxes.

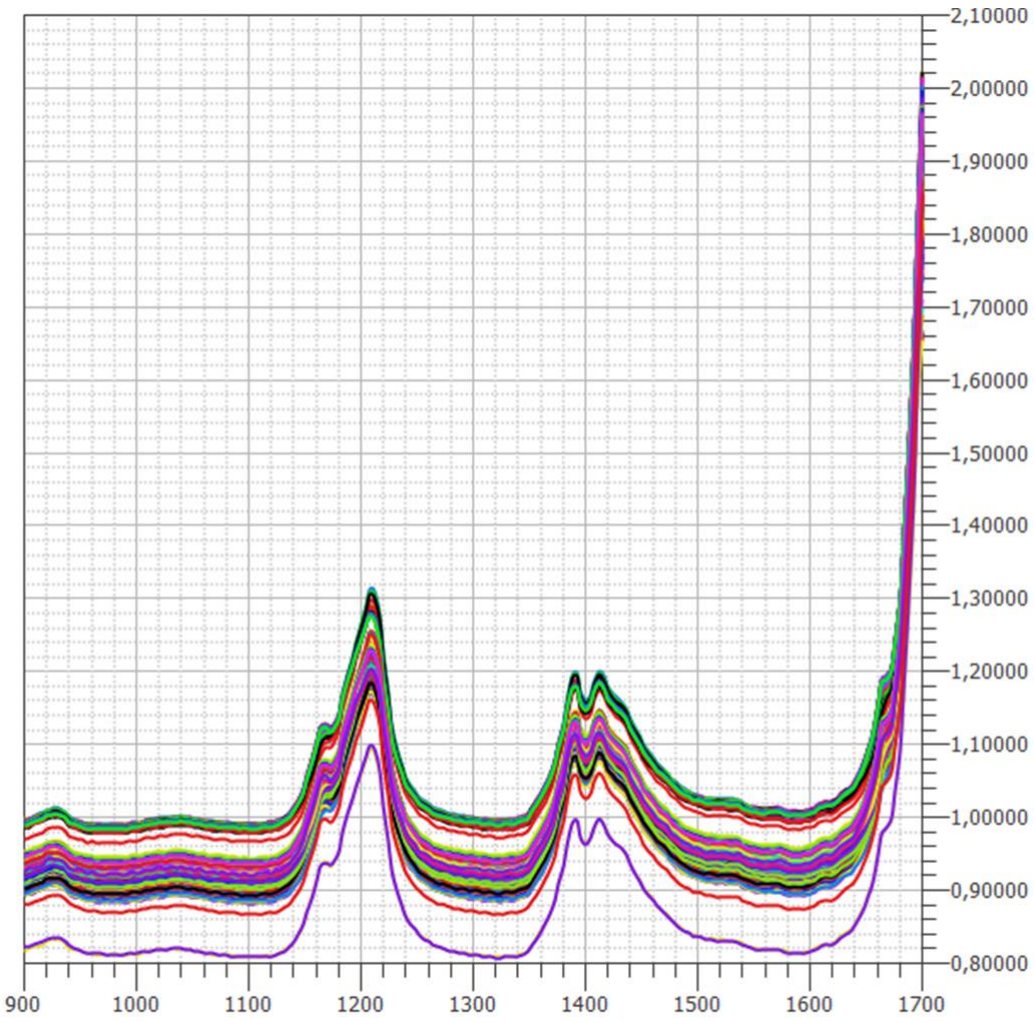

Figure 4.75. Raw signal of all the samples analysed, it is possible to see how the regions between 1140÷1260, 1360÷1480 and over 1700 nm tend to give same behaviour all over the different kinds of samples considered.

The signals obtained couldn't discriminate peculiar differences between the different samples, so in order to better highlight them a second derivative transformation has been made. This mathematical technique highlights subtle changes in the spectra by emphasizing peak curvature and reducing overlapping signals, making it easier to differentiate closely spaced absorption bands. Additionally, the second derivative suppresses broad baseline variations caused by light scattering, instrument drift, or sample heterogeneity, which can obscure meaningful spectral information (Rinnan, Berg and Engelsen, 2009; Sohn *et al.,* 2021). The reflectance was treated with a standard second derivative, which, In the context of NIR spectroscopy, could be approximated numerically using finite difference methods. For a set of spectral data points $f(x_i)$ at equally spaced intervals (Δx), the second derivative at a point x_i is:

$$f''(x_i) \approx \frac{f(x_{i-1}) - 2f(x_i) + f(x_{i+1})}{(\Delta x)^2}$$

Once transformed, the data were then loaded into the software and used to build up calibration curves with the aid of some "anchor" results obtained from the analytical analyses, specifically, a Partial Least Squares (PLS) model was used: PLS projects the high-dimensional spectral data into a smaller set of latent variables (factors) that maximize the covariance between the spectral data (predictors) and the reference property values (responses), then a regression model is constructed using the latent variables as predictors and the reference values as responses. The model coefficients describe the relationship between the spectral data and the target property (Nicolaï *et al.,* 2007; Alander *et al.,* 2013). For the sake of this study, the measurements obtained through conventional analyses were used in a ratio of 70:30 to compute the calibration curve with the first 70% of the data, and then cross-validate the results of the latter 30% using the model built. Results showed Determination coefficients (R^2) and standard errors (Standard error of Calibration, SEC; Standard error of cross validation, SECV) as shown in Table 4.28, the table shows also the ranges of prediction of the single models.

Parameter	Calibration R^2	SEC	Cross-validation R^2	SECV	Range
Free acidity	0.950	0.0025	0.923	0.0031	0.02-0.1%
Red	0.997	0.0159	0.996	0.0177	0.1-1.8
Yellow	0.999	0.0758	0.998	0.0965	1.0-20.0
C16:0	0.992	0.1817	0.989	0.2050	4-12%
C18:0	0.953	0.1189	0.940	0.1347	1.7-4.5%
C18:1	0.999	0.6245	0.999	0.6749	24-84%
C18:2	0.999	0.7265	0.999	0.8000	2.0-60.0%
C18:3	0.990	0.1577	0.987	0.1798	0.01-6.0%
C20:0	0.989	0.0258	0.986	0.0288	0.01-1.2%
C20:1	0.994	0.0293	0.993	0.0324	0.1-2.0%
C22:0	0.987	0.0614	0.984	0.0692	0.1-2.9%
C22:1	0.990	0.0337	0.988	0.0371	0.01-1.8%

Table 4.28. Statistical parameters for the analytical values predicted using the NIR instrumentations

The results obtained figure how it is possible to implement and develop practical models, useful in the industrial analysis of vegetable oils, however, the only sight at the numbers obtained may fail in the interpretation of the results; in fact it is possible to observe, in the Figures 5.76 and 5.77, how the models work and how the values registered spread evenly all along the regression models; however, in figures 5.78 and 5.79 the situation is quite different, as it is possible to observe how the values are spread evenly among the regression; this fact is imputable to the high heterogeneity of specific parameters, such as linolenic acid (C18:3) for the soy oil, which tends to be naturally

higher than in its other counterparts (RIF). Same consideration could be made for the eicosenoic and beenic acids (C20:1, C22:0), which tend to be naturally higher in peanut oil. This situation led to the creation of models which have regressions characterized by an abundance of points in the latter part of the ranges of predictions, and a relative scarcity of points in the upper range.

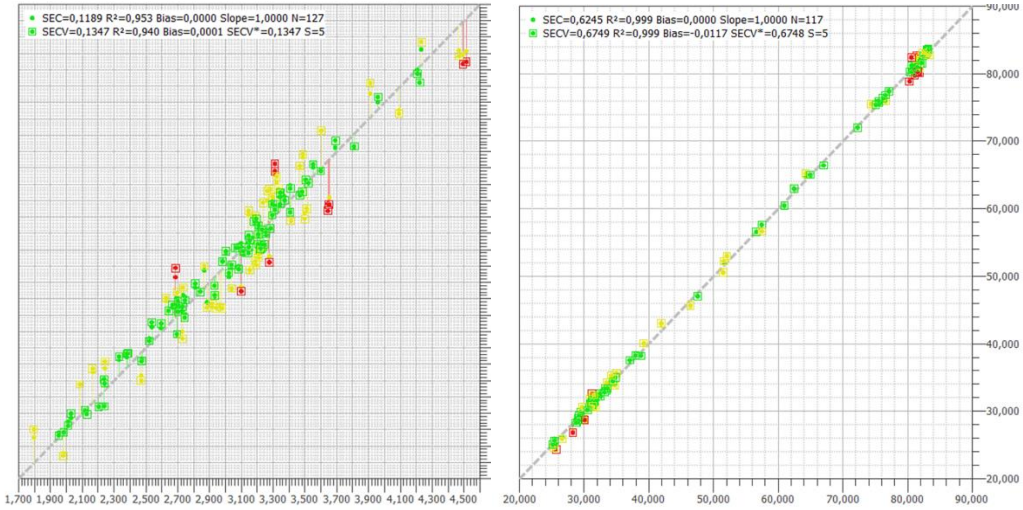

Figure 4.76 and 5.77. Regression models for the stearic and oleic acids, it is possible to observe the almost even distribution of points along the model.

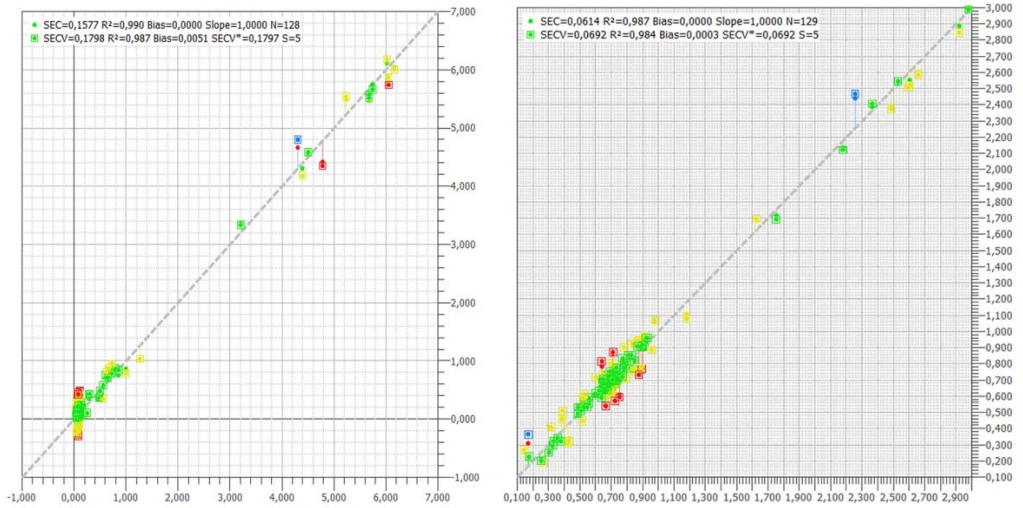

Figure 4.78 and 5.79. Regression models for the linolenic and beenic acids, it is clearly observable how the models may result in high, theoretical, descriptive accuracy, however the missing of intermediate points along the regression model leads to high standard errors, which impair the accuracy of the prediction.

Similar observations could be made for the curve regarding the free acidity parameter, as shown in Figure 4.80 the results tend to be clustered on specific values (0.04, 0.06 and 0.08 %). This is imputable to a quantification limit imposed by the reference analysis; in fact, the method not only uses the expertise and eye of a trained operator

to be made, that ultimately leaves open possibilities of human error, but also has limitation in regards of the type and detection limit of the glassware used, which in turn leads to approximations to the quantity of sodium hydroxide used. This ultimately leads to a falsed building of the calibration curve, in which the reference values cannot distinguish differences in the acidity values, that in turn, could be detected by the VisNIR instrument.

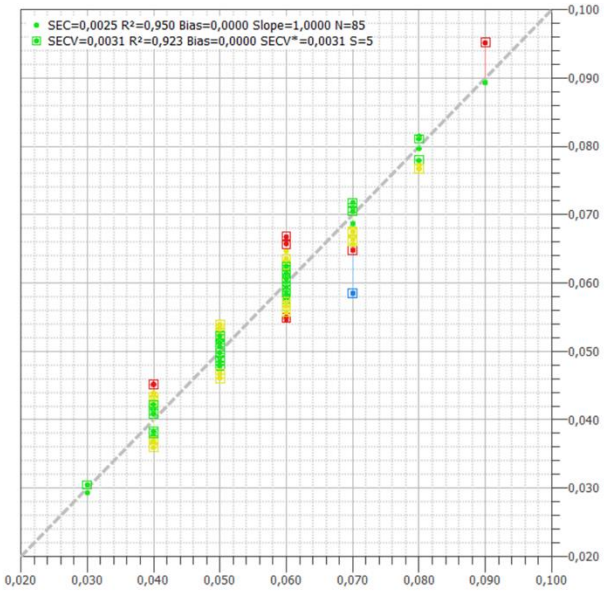

Figure 4.80. Regression model for free acidity, it is clearly observable how the models may result in high, theoretical, descriptive accuracy, however the clusterization of multiple values along the regression model leads to high standard errors, which impair the accuracy of the prediction.

Conclusions

The study leads to two major conclusions: in first place, it is possible to develop calibration curves regarding the quantification of quality parameters of refined vegetable oils, the adoption of a VisNIR instrument, in contrast to a NIR only apparatus, creates the possibility of conjunct analysis of physical parameters with chemical ones. However, the application and building of said curves cannot be made in an approximate way. By taking a look at the reality of big refining industries it is to consider that the matrices and the product treated inside the production implants vary based on the multiple agronomical campaigns taking place all over the world, this often leads to a multitude of oils extracted and refined; and, if it is possible to group the different kinds of vegetable oils for common parameters, such as yellow colour, concentration of stearic acid or peroxide value; however, it is more difficult to obtain similar results for minor components, as shown in the previous paragraph. The natural differences between the oils make up for their unique characteristics desired by the consumers but prove to be difficult matter when creating estimation models. The

solution proposed, in that sense, is to make up for those differences by creating artifact samples. By taking the route proposed it is possible to not only “dilute” the chemical differences of specific oils, such as peanut oils, but also to corroborate the prediction accuracies of the other chemical parameters considered. In turn this leads to an extensive planning process of making the singular blends on a laboratory scale, that ought to take place all over the year, in order to furnish the instrument with ever adjourned and reliable results. In second place, the study demonstrated the feasibility and application of the flux chamber prototype furnished, the study did not concentrate itself on the confront between regular equipment and innovative one; however, the flux chamber not only reduced the analysis times by reduction of time required between the loading and unloading of the single samples, but also reduced the quantity of solvents needed in the cleaning of the technical equipment. In the modern context of sustainability it is important to remark and recognize that the industrial processes are not the only factor in place in the green revolution proposed, but also the adoption of small laboratory applications and practices can lead to denotable improvements in the overall quality of the work and for the operator.

5.Conclusions

This thesis explored innovative pathways for enhancing the sustainability, technological performance, and nutritional value of food lipids through the valorisation of by-products from various agro-industrial processes. By addressing three specific goals, the research has provided comprehensive insights into the chemical, sensory, and analytical dimensions of these eco-friendly lipid formulations.

Summary of Objectives and Key Findings

1. **Chemical Characterization of Vegetable Oils Under Stress**: the first objective delved deeply into understanding the oxidative stability and degradation pathways of various vegetable oils when subjected to UV light and mild temperature stresses. The research highlighted that the chemical composition of these oils, particularly their fatty acid profiles and levels of natural antioxidants like tocopherols, significantly influenced their oxidative resilience. Sunflower oil, rich in polyunsaturated fatty acids, exhibited rapid peroxide formation, a precursor to rancidity, while oils with higher monounsaturated fatty acid content demonstrated comparatively greater stability. Techniques such as FAST-GC-FID and SPME-GC-MS were instrumental in providing detailed analyses of oxidation products, including volatile compounds like aldehydes and ketones. These findings have profound implications for industries aiming to optimize formulations and extend the shelf life of oils used in food production (Frankel, 2012). Additionally, understanding the kinetics of oxidation under different stressors enables manufacturers to tailor storage and processing conditions, reducing waste and ensuring product quality.
2. **Formulation and Shelf-Life Evaluation of Baked Goods**: the second objective aimed to incorporate eco-friendly lipids into "tarallini" and "frollini" biscuits to improve their sensory qualities and oxidative stability. By utilizing by-products such as rice germ and grape seed oils, rich in bioactive compounds like tocopherols, sterols, and γ-oryzanol, the formulations achieved enhanced shelf-life performance without compromising sensory attributes. Analytical methods, including OXITEST, quantified the lipid oxidative stability over time, demonstrating a marked reduction in peroxide and anisidine values compared to conventional formulations. Furthermore, sensory analysis revealed that these formulations retained desirable textural properties and flavor profiles, even after prolonged storage. The integration of these lipid sources not only contributes to waste reduction in agro-industrial chains but also provides a healthier lipid profile with potential cardiovascular benefits linked to their unsaturated fatty acid content (Nyam and Ken, 2014). This approach aligns with contemporary

consumer preferences for sustainable and functional foods, positioning the baking industry to adopt innovative yet practical solutions for product development.

3. **Non-Destructive Analysis for Quality Assessment**: the third objective introduced and validated advanced non-destructive techniques for evaluating the quality of refined vegetable oils. Near-Infrared (NIR) spectroscopy emerged as a cutting-edge tool, providing rapid, reagent-free assessments of crucial parameters such as free acidity, peroxide value, and oxidative stability. By developing robust calibration models, this research demonstrated the high predictive accuracy of NIR for real-time quality control in industrial settings. This advancement represents a significant step toward reducing the reliance on traditional wet chemistry methods, which are often time-consuming, labor-intensive, and environmentally taxing due to solvent usage. The applicability of NIR spectroscopy extends beyond laboratory settings, offering potential integration into automated production lines for continuous monitoring. Such innovation aligns with the food industry's broader goals of enhancing sustainability and efficiency while maintaining stringent quality standards (Rohman *et al.,* 2020).

Contributions to Science and Industry

This research bridges critical gaps in the food science domain by combining sustainability with technological and sensory performance. The valorisation of agro-industrial by-products not only aligns with global goals for waste reduction but also creates value-added products with improved functionality. Furthermore, the adoption of non-destructive techniques represents a paradigm shift in food quality assessment, reducing environmental impact and operational costs.

Future Perspectives

Building on the foundations laid by this work, future research could explore:

- **Broader Applications**: testing these lipid formulations in other food matrices such as spreads, sauces, and ready-to-eat meals.
- **Advanced Extraction Methods**: implementing supercritical fluid extraction to further enhance the yield and purity of bioactive compounds.
- **Consumer Perception Studies**: understanding consumer acceptance and willingness to pay for products derived from sustainable lipid sources.

- **Scaling Up Analytical Technologies**: expanding the scope of NIR spectroscopy for in-line quality control during industrial processing.

By integrating these avenues, the food industry can achieve a more sustainable, resilient, and health-conscious future.

6.Bibliography

Abdelhedi, O. *et al.* (2016) 'Combined biocatalytic conversion of smooth hound viscera: Protein hydrolysates elaboration and assessment of their antioxidant, anti-ACE and antibacterial activities', *Food Research International*, 86, pp. 9–23. Available at: https://doi.org/10.1016/j.foodres.2016.05.013.

Aguchem, R.N. *et al.* (2024) 'Role of CETP, PCSK-9, and CYP7-alpha in cholesterol metabolism: Potential targets for natural products in managing hypercholesterolemia', *Life Sciences*, 351. Available at: https://doi.org/10.1016/j.lfs.2024.122823.

Alander, J.T. *et al.* (2013) 'A Review of Optical Nondestructive Visual and Near-Infrared Methods for Food Quality and Safety', *International Journal of Spectroscopy*, 2013(1), p. 341402. Available at: https://doi.org/10.1155/2013/341402.

Aletor, O. (2017) 'Vitamins, Amino Acids, Lipids and Sterols of Eggs from Three Different Birds Genotypes', *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 11(03), pp. 41–47. Available at: https://doi.org/10.9790/2402-1103014147.

Ali, M.A. *et al.* (2013) 'Effect of heating at frying temperature on the quality characteristics of regular and high-oleic acid sunflower oils', *Acta Scientiarum Polonorum Technologia Alimentaria*, 12(2), pp. 159–167.

Aryana, K. j *et al.* (2003) 'Functionality of Palm Oil as a Stabilizer in Peanut Butter', *Journal of Food Science*, 68(4), pp. 1301–1307. Available at: https://doi.org/10.1111/j.1365-2621.2003.tb09643.x.

Ashton, J.S. *et al.* (2021) 'The effects of medium chain triglyceride (MCT) supplementation using a C8:C10 ratio of 30:70 on cognitive performance in healthy young adults', *Physiology & Behavior*, 229, p. 113252. Available at: https://doi.org/10.1016/j.physbeh.2020.113252.

Asokapandian, S., Sreelakshmi, S. and Rajamanickam, G. (2021) 'Lipids and Oils: An Overview', in *Food Biopolymers: Structural, Functional and Nutraceutical Properties*, pp. 389–411. Available at: https://doi.org/10.1007/978-3-030-27061-2_16.

Bai, G., Ma, C. and Chen, X. (2021) 'Phytosterols in edible oil: Distribution, analysis and variation during processing', *Grain & Oil Science and Technology*, 4(1), pp. 33–44. Available at: https://doi.org/10.1016/j.gaost.2020.12.003.

Bail, S. *et al.* (2008) 'Characterisation of various grape seed oils by volatile compounds, triacylglycerol composition, total phenols and antioxidant capacity', *Food Chemistry*, 108(3), pp. 1122–1132. Available at: https://doi.org/10.1016/j.foodchem.2007.11.063.

Barbieri, S. *et al.* (2018) 'Sensory and instrumental study of Taralli, a typical Italian bakery product', *European Food Research and Technology*, 244(1), pp. 73–82. Available at: https://doi.org/10.1007/s00217-017-2937-8.

Barden, L. and Decker, E.A. (2016) 'Lipid Oxidation in Low-moisture Food: A Review', *Critical Reviews in Food Science and Nutrition*, 56(15), pp. 2467–2482. Available at: https://doi.org/10.1080/10408398.2013.848833.

Butinar, B. (2008) 'Chemical changes in extra virgin olive oils from Slovenian Istra after thermal treatment', *Food Chemistry* [Preprint]. Available at: https://www.academia.edu/22729184/Chemical_changes_in_extra_virgin_olive_oils_from_Slovenian_Istra_after_thermal_treatment (Accessed: 27 November 2023).

Cabras, P. and Martelli, A. (2004) *Chimica degli alimenti*. Piccin-Nuova Libraria.

Cai, Z. *et al.* (2025) 'Comparison of extraction and refinement techniques for volatile compound analysis in camellia oil', *Food Chemistry*, 469. Available at: https://doi.org/10.1016/j.foodchem.2024.142501.

Calligaris, S. *et al.* (2008) 'Shelf life prediction of bread sticks using oxidation indices: a validation study', *Journal of Food Science*, 73(2), pp. E51-56. Available at: https://doi.org/10.1111/j.1750-3841.2007.00624.x.

Capella, P. (1997) *Manuale degli oli e dei grassi*. Tecniche Nuove.

Caponio, F. *et al.* (2009) 'Influence of Processing and Storage Time on the Lipidic Fraction of Taralli', *Journal of Food Science*, 74(9), pp. C701–C706. Available at: https://doi.org/10.1111/j.1750-3841.2009.01357.x.

Caponio, F. *et al.* (2011) 'Influence of the Different Oils Used in Dough Formulation on the Lipid Fraction of Taralli', *Journal of Food Science*, 76(4), pp. C549–C554. Available at: https://doi.org/10.1111/j.1750-3841.2011.02113.x.

Cardenia, V. *et al.* (2012) 'Analysis of cholesterol oxidation products by Fast gas chromatography/mass spectrometry', *Journal of Separation Science*, 35(3), pp. 424–430. Available at: https://doi.org/10.1002/jssc.201100660.

Carrai, B. (2010) *Arte bianca. Materie prime, processi e controlli*. Edagricole-New Business Media.

Cauvain, S. and Young, L.S. (2007) 'Baked Products: Science, Technology and Practice', *Baked Products: Science, Technology and Practice*, pp. 1–228. Available at: https://doi.org/10.1002/9780470995907.

CDC - NIOSH Pocket Guide to Chemical Hazards - Methylene chloride (no date). Available at: https://www.cdc.gov/niosh/npg/npgd0414.html (Accessed: 25 November 2024).

Chaiyasit, W. *et al.* (2007) 'Role of physical structures in bulk oils on lipid oxidation', *Critical Reviews in Food Science and Nutrition*, 47(3), pp. 299–317. Available at: https://doi.org/10.1080/10408390600754248.

Chandrasekaran, I. *et al.* (2019) 'Potential of Near-Infrared (NIR) Spectroscopy and Hyperspectral Imaging for Quality and Safety Assessment of Fruits: an Overview', *Food Analytical Methods*, 12(11), pp. 2438–2458. Available at: https://doi.org/10.1007/s12161-019-01609-1.

Chang, X. *et al.* (2025) 'Profiling the aroma compounds of sunflower seed oils subjected to different roasting conditions based on GC–Orbitrap-MS and sensory molecular network', *Journal of Food Composition and Analysis*, 139. Available at: https://doi.org/10.1016/j.jfca.2024.107124.

Cheng, Q. *et al.* (2022) 'Study on the antioxidant activity of β-sitosterol and stigmasterol from Sacha Inchi oil and *Prinsepia* oil added to walnut oil', *Food Science and Technology*, 42, p. e69522. Available at: https://doi.org/10.1590/fst.69522.

Chevallier, S. *et al.* (2000) 'Physicochemical behaviors of sugars, lipids, and gluten in short dough and biscuit', *Journal of Agricultural and Food Chemistry*, 48(4), pp. 1322–1326. Available at: https://doi.org/10.1021/jf990435+.

Chowdhury, K. *et al.* (2007) 'Studies on the Fatty Acid Composition of Edible Oil', *Bangladesh Journal of Scientific and Industrial Research*, 42(3), pp. 311–316. Available at: https://doi.org/10.3329/bjsir.v42i3.669.

Comandini, P. *et al.* (2009) 'Accelerated oxidation: Comparative study of a new reactor with oxidation stability instrument', *European Journal of Lipid Science and Technology*, 111(9), pp. 933–940. Available at: https://doi.org/10.1002/ejlt.200900009.

Engelsen, M.M. and Hansen, Å. (2009) 'Tocopherol and Tocotrienol Content in Commercial Wheat Mill Streams', *Cereal Chemistry*, 86(5), pp. 499–502. Available at: https://doi.org/10.1094/CCHEM-86-5-0499.

Esposto, S. *et al.* (2017) 'Effect of light exposure on the quality of extra virgin olive oils according to their chemical composition', *Food Chemistry*, 229, pp. 726–733. Available at: https://doi.org/10.1016/j.foodchem.2017.02.151.

'Evaluation of Chemical and Physical Changes in Different Commercial Oils during Heating' (no date) *Olive Wellness Institute*. Available at: https://olivewellnessinstitute.org/article/evaluation-of-chemical-and-physical-changes-in-different-commercial-oils-during-heating/ (Accessed: 2 December 2024).

Ferrari, M., Mottola, L. and Quaresima, V. (2004) 'Principles, Techniques, and Limitations of Near Infrared Spectroscopy', *Canadian Journal of Applied Physiology*, 29(4), pp. 463–487. Available at: https://doi.org/10.1139/h04-031.

Firestone, D. (1994) 'Gas chromatographic determination of mono- and diglycerides in fats and oils: summary of collaborative study', *Journal of AOAC International*, 77(3), pp. 677–680.

Fitzherbert, E.B. *et al.* (2008) 'How will oil palm expansion affect biodiversity?', *Trends in Ecology & Evolution*, 23(10), pp. 538–545. Available at: https://doi.org/10.1016/j.tree.2008.06.012.

Fodor, M. *et al.* (2024) 'The Role of Near-Infrared Spectroscopy in Food Quality Assurance: A Review of the Past Two Decades', *Foods*, 13(21), p. 3501. Available at: https://doi.org/10.3390/foods13213501.

Frankel, E.N. (2012) 'Chapter 4 - Hydroperoxide decomposition', in E.N. Frankel (ed.) *Lipid Oxidation (Second Edition)*. Woodhead Publishing (Oily Press Lipid Library Series), pp. 67–98. Available at: https://doi.org/10.1533/9780857097927.67.

Ghotra, B.S., Dyal, S.D. and Narine, S.S. (2002) 'Lipid shortenings: a review', *Food Research International*, 35(10), pp. 1015–1048. Available at: https://doi.org/10.1016/S0963-9969(02)00163-1.

Gibon, V., De Greyt, W. and Kellens, M. (2007) 'Palm oil refining', *European Journal of Lipid Science and Technology*, 109(4), pp. 315–335. Available at: https://doi.org/10.1002/ejlt.200600307.

Giuffrè, A.M. *et al.* (2018) 'EFFECT OF HEATING ON CHEMICAL PARAMETERS OF EXTRA VIRGIN OLIVE OIL, POMACE OLIVE OIL, SOYBEAN OIL AND PALM OIL', *Italian Journal of Food Science*, 30(4). Available at: https://doi.org/10.14674/IJFS-1269.

González Belo, R. *et al.* (2017) 'Dynamics of oil and tocopherol accumulation in sunflower grains and its impact on final oil quality', *European Journal of Agronomy*, 89, pp. 124–130. Available at: https://doi.org/10.1016/j.eja.2017.06.003.

Gotor, A.A. and Rhazi, L. (2016) 'Effects of refining process on sunflower oil minor components: a review', *OCL*, 23(2), p. D207. Available at: https://doi.org/10.1051/ocl/2016007.

Habeeb, F. *et al.* (2024) 'An overview of lipids from plant sources', in *Handbook of Plant-Based Food and Drinks Design*, pp. 59–66. Available at: https://doi.org/10.1016/B978-0-443-16017-2.00008-5.

ben Hammouda, I. *et al.* (2017) 'Comparison and characterization of volatile compounds as markers of oils stability during frying by HS–SPME-GC/MS and Chemometric analysis', *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 1068–1069, pp. 322–334. Available at: https://doi.org/10.1016/j.jchromb.2017.10.063.

Hansen, S.B. *et al.* (2015) 'Trends in global palm oil sustainability research', *Journal of Cleaner Production*, 100, pp. 140–149. Available at: https://doi.org/10.1016/j.jclepro.2015.03.051.

Hashemi, S.M.B. *et al.* (2017) 'Heating, microwave and UV irradiation effects on oxidative stability of Sardasht red grape (Vitis vinifera cultiv. Sardasht) seed oil', *International Journal of Food Science & Technology*, 52(6), pp. 1341–1347. Available at: https://doi.org/10.1111/ijfs.13421.

Ichihara, K. *et al.* (2021) 'Simultaneous Determination of Free Fatty Acids and Esterified Fatty Acids in Rice Oil by Gas Chromatography', *Journal of the American Oil Chemists' Society*, 98(2), pp. 149–155. Available at: https://doi.org/10.1002/aocs.12442.

Imoisi, O.B. *et al.* (2015) 'Palm oil, its nutritional and health implications (Review)', *Journal of Applied Sciences and Environmental Management*, 19(1), pp. 127–133. Available at: https://doi.org/10.4314/jasem.v19i1.17.

ISO 6885:2016(en), Animal and vegetable fats and oils — Determination of anisidine value (no date a). Available at: https://www.iso.org/obp/ui/en/#iso:std:iso:6885:ed-4:v1:en (Accessed: 7 January 2025).

ISO 6885:2016(en), Animal and vegetable fats and oils — Determination of anisidine value (no date b). Available at: https://www.iso.org/obp/ui/en/#iso:std:iso:6885:ed-4:v1:en (Accessed: 2 July 2024).

Issever, H. *et al.* (2002) 'Impairment of colour vision in patients with n-hexane exposure-dependent toxic polyneuropathy', *Occupational Medicine (Oxford, England)*, 52(4), pp. 183–186. Available at: https://doi.org/10.1093/occmed/52.4.183.

Jr, Workman, J. and Weyer, L. (2007) 'History of Near-Infrared (NIR) Applications', in *Practical Guide to Interpretive Near-Infrared Spectroscopy*. CRC Press.

Jung, T.-D. *et al.* (2017) 'Comparative Analysis of γ-Oryzanol, β-Glucan, Total Phenolic Content and Antioxidant Activity in Fermented Rice Bran of Different Varieties', *Nutrients*, 9(6), p. 571. Available at: https://doi.org/10.3390/nu9060571.

Kadandale, S., Marten, R. and Smith, R. (2019) 'The palm oil industry and noncommunicable diseases', *Bulletin of the World Health Organization*, 97(2), pp. 118–128. Available at: https://doi.org/10.2471/BLT.18.220434.

Kalua, C.M. *et al.* (2006) 'Discrimination of storage conditions and freshness in virgin olive oil', *Journal of Agricultural and Food Chemistry*, 54(19), pp. 7144–7151. Available at: https://doi.org/10.1021/jf061038j.

Kiczorowska, B. *et al.* (2019) 'Comparative analysis of selected bioactive components (fatty acids, tocopherols, xanthophyll, lycopene, phenols) and basic nutrients in raw and thermally processed camelina, sunflower, and flax seeds (Camelina sativa L. Crantz, Helianthus L., and Linum L.)', *Journal of Food Science and Technology*, 56(9), pp. 4296–4310. Available at: https://doi.org/10.1007/s13197-019-03899-z.

Kiralan, M. *et al.* (2019) 'Stability and volatile oxidation compounds of grape seed, flax seed and black cumin seed cold-pressed oils as affected by thermal oxidation', *Grasas y Aceites*, 70(1), pp. e295–e295. Available at: https://doi.org/10.3989/gya.0570181.

Koski, A. *et al.* (2002) 'Oxidative stability and minor constituents of virgin olive oil and cold-pressed rapeseed oil', *European Food Research and Technology*, 214(4), pp. 294–298. Available at: https://doi.org/10.1007/s00217-001-0479-5.

Koutoulis, K. (2019) 'Effects of Supplementing Various Linoleic to α-linolenic Acid Ratios and Vitamin A on Production Performance and Egg Characteristics of Laying Hens during Summer Months', *Journal of Zoological Research* [Preprint]. Available at: https://www.academia.edu/42838945/Effects_of_Supplementing_Various_Linoleic_to_%CE%B1_linolenic_Acid_Ratios_and_Vitamin_A_on_Production_Performance_and_Egg_Characteristics_of_Laying_Hens_during_Summer_Months (Accessed: 2 December 2024).

Lajnef, H.B. *et al.* (2017) 'Lipid characterization of Eryngium maritimum seeds grown in Tunisia', *Industrial Crops and Products*, 105, pp. 47–52. Available at: https://doi.org/10.1016/j.indcrop.2017.05.001.

Lanza, T. (2006) *Tecnologia e pratica per la produzione dei biscotti*. Chiriotti.

Latimer, G.W., Jr. and Latimer, G.W., Jr. (eds) (2023) 'Official Methods of Analysis: 22nd Edition (2023)', in *Official Methods of Analysis of AOAC INTERNATIONAL*. Oxford University Press, p. 0. Available at: https://doi.org/10.1093/9780197610145.002.001.

Li, L., Liu, G. and Guo, Z. (2024) 'Oleogels produced by direct methods using as gelator: Fatty acids (including 12-HSA), fatty alcohols, ceramides, lecithins, sterols, cellulose fibers, and fumed silica', in *Advances in Oleogel Development, Characterization, and Nutritional Aspects*, pp. 177–207. Available at: https://doi.org/10.1007/978-3-031-46831-5_8.

Liu, R. *et al.* (2020) 'Evaluation of the Antioxidant Properties of Micronutrients in Different Vegetable Oils', *European Journal of Lipid Science and Technology*, 122(2), p. 1900079. Available at: https://doi.org/10.1002/ejlt.201900079.

Loskutov, I.G. and Khlestkina, E.K. (2021) 'Wheat, Barley, and Oat Breeding for Health Benefit Components in Grain', *Plants (Basel, Switzerland)*, 10(1), p. 86. Available at: https://doi.org/10.3390/plants10010086.

Luna, G., Morales, M.T. and Aparicio, R. (2006) 'Changes Induced by UV Radiation during Virgin Olive Oil Storage', *Journal of Agricultural and Food Chemistry*, 54(13), pp. 4790–4794. Available at: https://doi.org/10.1021/jf0529262.

Madhujith, T. and Sivakanthan, S. (2019) 'Oxidative Stability of Edible Plant Oils', in J.-M. Mérillon and K.G. Ramawat (eds) *Bioactive Molecules in Food*. Cham: Springer International Publishing (Reference Series in Phytochemistry), pp. 529–551. Available at: https://doi.org/10.1007/978-3-319-78030-6_94.

Mandak, E. and Nyström, L. (2013) 'Influence of baking and *in vitro* digestion on steryl ferulates from wheat', *Journal of Cereal Science*, 57(3), pp. 356–361. Available at: https://doi.org/10.1016/j.jcs.2012.12.004.

Manzocco, L. *et al.* (2020) 'Modeling the Effect of the Oxidation Status of the Ingredient Oil on Stability and Shelf Life of Low-Moisture Bakery Products: The Case Study of Crackers', *Foods*, 9(6), p. 749. Available at: https://doi.org/10.3390/foods9060749.

Marzocchi, S. *et al.* (2018) 'Value-addition of Beef Meat By-products: Lipid Characterization by Chromatographic Techniques', *Journal of Oleo Science*, 67(2), pp. 143–150. Available at: https://doi.org/10.5650/jos.ess17139.

Massarolo, K.C. *et al.* (2017) 'The impact of Rhizopus oryzae cultivation on rice bran: Gamma-oryzanol recovery and its antioxidant properties', *Food Chemistry*, 228, pp. 43–49. Available at: https://doi.org/10.1016/j.foodchem.2017.01.127.

Matthäus, B. (2007) 'Use of palm oil for frying in comparison with other high-stability oils', *European Journal of Lipid Science and Technology*, 109(4), pp. 400–409. Available at: https://doi.org/10.1002/ejlt.200600294.

Meilgaard, M.C., Carr, B.T. and Civille, G.V. (2006) *Sensory Evaluation Techniques, Fourth Edition*. CRC Press.

Moreau, R.A. (2015) 'Composition of Plant Sterols and Stanols in Supplemented Food Products', *Journal of AOAC International*, 98(3), pp. 685–690. Available at: https://doi.org/10.5740/jaoacint.SGEMoreau.

Moulodi, F. *et al.* (2015) 'Effect of Fatty Acid Composition on Thermal Stability of Extra Virgin Olive Oil'.

Musakhanian, J., Rodier, J.-D. and Dave, M. (2022) 'Oxidative Stability in Lipid Formulations: a Review of the Mechanisms, Drivers, and Inhibitors of Oxidation', *AAPS PharmSciTech*, 23(5), p. 151. Available at: https://doi.org/10.1208/s12249-022-02282-0.

Nadimi, M. and Paliwal, J. (2024) 'Recent Applications of Near-Infrared Spectroscopy in Food Quality Analysis', *Foods*, 13(16), p. 2633. Available at: https://doi.org/10.3390/foods13162633.

Nicolaï, B.M. *et al.* (2007) 'Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: A review', *Postharvest Biology and Technology*, 46(2), pp. 99–118. Available at: https://doi.org/10.1016/j.postharvbio.2007.06.024.

Noshad, M., Behbahani, B.A. and Karabagias, I.K. (2023) 'Volatilomic with chemometrics: a toward authentication approach for food authenticity control', *European Food Research and Technology*, 249(9), pp. 2215–2226. Available at: https://doi.org/10.1007/s00217-023-04291-0.

Nyam, K. and Ken, K. (2014) 'A Comparative Study of Stability of Extra Virgin Olive Oil, Virgin Coconut Oil and Grape Seed Oil against Domestic Deep Frying', 4, pp. 71–81.

Orsavova, J. *et al.* (2015) 'Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids', *International Journal of Molecular Sciences*, 16(6), pp. 12871–12890. Available at: https://doi.org/10.3390/ijms160612871.

Panfili, G., Fratianni, A. and Irano, M. (2003) 'Normal phase high-performance liquid chromatography method for the determination of tocopherols and tocotrienols in cereals', *Journal of Agricultural and Food Chemistry*, 51(14), pp. 3940–3944. Available at: https://doi.org/10.1021/jf030009v.

Pasini, F. *et al.* (2024) 'Effect of replacing olive oil with oil blends on physicochemical and sensory properties of taralli', *International Journal of Food Science and Technology*, 59(4), pp. 2697–2706. Available at: https://doi.org/10.1111/ijfs.17019.

Pelillo, M. *et al.* (2003) 'Identification of plant sterols in hexaploid and tetraploid wheats using gas chromatography with mass spectrometry', *Rapid communications in mass spectrometry: RCM*, 17(20), pp. 2245–2252. Available at: https://doi.org/10.1002/rcm.1156.

Peyronel, F. (2019) 'Medium Chain Triacylglycerides', in L. Melton, F. Shahidi, and P. Varelis (eds) *Encyclopedia of Food Chemistry*. Oxford: Academic Press, pp. 132–137. Available at: https://doi.org/10.1016/B978-0-08-100596-5.21594-1.

Privett, O.S. and Blank, M.L. (1961) 'A new method for the analysis of component mono-, di-, and triglycerides *', *Journal of Lipid Research*, 2(1), pp. 37–44. Available at: https://doi.org/10.1016/S0022-2275(20)39036-2.

'Public Health Goals for Trihalomethanes in Drinking Water' (2020).

Rajhi, I. (2020) 'Phytosterols in Wheat: Composition, Contents and Role in Human Health', *Open Access Journal of Biogeneric Science and Research* [Preprint]. Available at: https://www.academia.edu/101388563/Phytosterols_in_Wheat_Composition_Contents_and_Role_in_Human_Health (Accessed: 16 December 2024).

Raza, S.A. (2009) 'Analytical Investigation of oxidative deterioration of Sunflower Oil Stored under Different Conditions'.

Riciputi, Y. and Caboni, M.F. (2017) 'Assessing oil oxidative stability in tarallini by OXITEST®', *Italian Journal of Food Science*, 29(1), pp. 63–73.

Rinnan, Å., Berg, F. van den and Engelsen, S.B. (2009) 'Review of the most common pre-processing techniques for near-infrared spectra', *TrAC Trends in Analytical Chemistry*, 28(10), pp. 1201–1222. Available at: https://doi.org/10.1016/j.trac.2009.07.007.

Sander, B.D. *et al.* (1989) 'Quantification of Cholesterol Oxidation Products in a Variety of Foods', *Journal of Food Protection*, 52(2), pp. 109–114. Available at: https://doi.org/10.4315/0362-028X-52.2.109.

Schwartz, H. *et al.* (2008) 'Tocopherol, tocotrienol and plant sterol contents of vegetable oils and industrial fats', *Journal of Food Composition and Analysis*, 21(2), pp. 152–161. Available at: https://doi.org/10.1016/j.jfca.2007.07.012.

SECTION 2. Codex Standards for Fats and Oils from Vegetable Sources (no date). Available at: https://www.fao.org/4/y2774e/y2774e04.htm (Accessed: 29 December 2024).

Shantha, N.C. and Decker, E.A. (1994) 'Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids', *Journal of AOAC International*, 77(2), pp. 421–424.

Smith, J.P. *et al.* (2004) 'Shelf life and safety concerns of bakery products--a review', *Critical Reviews in Food Science and Nutrition*, 44(1), pp. 19–55. Available at: https://doi.org/10.1080/10408690490263774.

Sohn, S.-I. *et al.* (2021) 'An Overview of Near Infrared Spectroscopy and Its Applications in the Detection of Genetically Modified Organisms', *International Journal of Molecular Sciences*, 22(18), p. 9940. Available at: https://doi.org/10.3390/ijms22189940.

Squeo, G. and Amigo, J.M. (2023) 'Successful Applications of NIR Spectroscopy and NIR Imaging in the Food Processing Chain', *Foods*, 12(16), p. 3041. Available at: https://doi.org/10.3390/foods12163041.

Sweeley, C.C. *et al.* (1963) 'Gas-Liquid Chromatography of Trimethylsilyl Derivatives of Sugars and Related Substances', *Journal of the American Chemical Society*, 85(16), pp. 2497–2507. Available at: https://doi.org/10.1021/ja00899a032.

Tekin, L., Aday, M.S. and Yilmaz, E. (2009) 'Physicochemical Changes in Hazelnut, Olive Pomace, Grapeseed and Sunflower Oils Heated at Frying Temperatures', *Food Science and Technology Research*, 15(5), pp. 519–524. Available at: https://doi.org/10.3136/fstr.15.519.

Traitler, H. and Dieffenbacher, A. (1985) 'Palm oil and palm kernel oil in food products', *Journal of the American Oil Chemists' Society*, 62(2), pp. 417–421. Available at: https://doi.org/10.1007/BF02541414.

Vaidya, B. and Eun, J.-B. (2013) 'Effect of temperature on oxidation kinetics of walnut and grape seed oil', *Food Science and Biotechnology*, 22(1), pp. 273–279. Available at: https://doi.org/10.1007/s10068-013-0077-x.

Vijay, V. *et al.* (2016) 'The Impacts of Oil Palm on Recent Deforestation and Biodiversity Loss', *PLOS ONE*, 11(7), p. e0159668. Available at: https://doi.org/10.1371/journal.pone.0159668.

Wen, Y. *et al.* (2020) 'Assessing the Impact of Oil Types and Grades on Tocopherol and Tocotrienol Contents in Vegetable Oils with Chemometric Methods', *Molecules*, 25(21), p. 5076. Available at: https://doi.org/10.3390/molecules25215076.

Williams, P. and Norris, K.H. (2001) *Near-infrared technology: in the agricultural and food industries*. 2nd ed. St. Paul, Minn.: American Association of Cereal Chemists.

Xagoraris, M. *et al.* (2021) 'Quality Evaluation of Winery By-Products from Ionian Islands Grape Varieties in the Concept of Circular Bioeconomy', *Sustainability*, 13(10), p. 5454. Available at: https://doi.org/10.3390/su13105454.

Yalcin, H. *et al.* (2017) 'Determination of Fatty Acid Composition, Volatile Components, Physico-Chemical and Bioactive Properties of Grape (Vitis vinifera) Seed and Seed Oil', *Journal of Food Processing and Preservation*, 41(2), p. e12854. Available at: https://doi.org/10.1111/jfpp.12854.

Yang, P. *et al.* (2019) 'Characterization of Key Aroma-Active Compounds in Black Garlic by Sensory-Directed Flavor Analysis', *Journal of Agricultural and Food Chemistry*, 67(28), pp. 7926–7934. Available at: https://doi.org/10.1021/acs.jafc.9b03269.

Zhang, L. *et al.* (2025) 'The volatile compounds changes of Malus asiatica Nakai under different drying methods', *Journal of Future Foods*, 5(3), pp. 276–282. Available at: https://doi.org/10.1016/j.jfutfo.2024.07.007.

Zhang, M. *et al.* (2021) ‘Data Statistics in Volatile Analysis by Accurate HS-SPME-GC/MS Measurement and Calculation’, in. *Journal of Physics: Conference Series*. Available at: https://doi.org/10.1088/1742-6596/2083/3/032002.

Zhang, W. *et al.* (2022) ‘A Review of Machine Learning for Near-Infrared Spectroscopy’, *Sensors*, 22(24), p. 9764. Available at: https://doi.org/10.3390/s22249764.

7.List of publications and research contributions

Poster contributions

▪ Ravagli Cesare*, Pasini Federica, Marzocchi Silvia, di Cori Cristina and Caboni Maria Fiorenza, "Technological, sensory, and nutritional assessment of eco-friendly food lipids." 26th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, 19-21 September 2022, Asti (Italy), ISBN 9788875902278, P. 163.

▪ Ravagli Cesare*, Pasini Federica, Marzocchi Silvia, Caboni Maria Fiorenza, "Volatile compounds evolution in vegetable oils subjected to mild thermal stress.", 7 th MS Food day, Florence (Italy), 5-7 October 2022, Firenze (Italy), ISBN 9788894952117, P. 131-132, https://www.spettrometriadimassa.it/Congressi/7MSFoodDay/program_7MSFoodDay.html

▪ Pasini Federica*, Marzocchi Silvia, Ravagli Cesare, Messia Maria Cristina and Caboni Maria Fiorenza, "Studio di shelf-life di biscotti con miscele lipidiche diverse.", 12° Convegno AISTEC "Cereali e Scienza: resilienza, sostenibilità e innovazione", 15-17 June 2022, Portici (Naples, Italy), P. 50, https://cris.unibo.it/handle/11585/901541.

▪ Pasini Federica*, Marzocchi Silvia, Ravagli Cesare, Messia Maria Cristina and Caboni Maria Fiorenza, "Studio Di Shelf-Life Di Taralli Con Miscele Lipidiche Diverse.", 12° Convegno AISTEC "Cereali e Scienza: resilienza, sostenibilità e innovazione", 15-17 June 2022, Portici (Naples, Italy), https://cris.unibo.it/handle/11585/901532.

▪ Marzocchi, Silvia*, Ravagli Cesare, Caboni Maria Fiorenza, and Pasini Federica, "Effect of Light on the Initiation of Oxidation in Selected Vegetable Oils.", XIII CONGRESSO NAZIONALE DI CHIMICA DEGLI ALIMENTI, 29-31 May 2023, Marsala (Italy), ISBN 9788894952377, P. 138, https://cris.unibo.it/handle/11585/941261.

▪ Marzocchi Silvia*, Ravagli Cesare, Caboni Maria Fiorenza, and Pasini Federica, "Study of the Oxidative Stability of Thermally Stressed Vegetable Oils.", XIII CONGRESSO N AZIONALE DI CHIMICA DEGLI ALIMENTI, 29-31 May 2023, Marsala (Italy), ISBN 9788894952377, P. 137, https://cris.unibo.it/handle/11585/958168.

▪ Ravagli Cesare*, Pasini Federica, Marzocchi Silvia, di Cori Cristina and Caboni Maria Fiorenza, "Technological, sensory, and nutritional assessment of eco-friendly food lipids." 27th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, 13-15 September 2023, Portici (Naples, Italy), P.364-365.

▪ Ravagli Cesare*, Pasini Federica,Marzocchi Silvia, and Caboni Maria Fiorenza, "Low Sodium Cheeses Lipidic Characterization, a Research to Better Understand the Kinetics of Cheese Aging.", FoodOmics 2024 7th International Conference, Cesena (Italy), 14-16 February 2024, ISBN 9788854971325, P. 159-161, https://cris.unibo.it/handle/11585/962404?mode=complete#.

▪ Pasini Federica*, Marzocchi Silvia, Ravagli Cesare, and Caboni Maria Fiorenza, "Problemi e sfide nella macinazione della farina integrale: il caso della pasta integrale.", Atti del 13° Convegno AISTEC FILIERE CEREALICOLE E RIGENERATIVE: Cambiamenti climatici e nuove esigenze qualitative e nutrizionali,19-21 June 2024, Turin (Italy)P. 133, https://cris.unibo.it/handle/11585/973479.

▪ Pasini Federica*, Marzocchi Silvia, Ravagli Cesare, Messia Maria Cristina, Caboni Maria Fiorenza, "Effect of replacing palm oil with different oil blends on lipid oxidation of biscuits.", 5th International Symposium on Lipid Oxidation and Antioxidants, 08-10 July 2024, Bologna (Italy), (LIP-009, p.89).

*= presenting author

▪ **Articles**

▪ Pasini, F., Marzocchi, S., Ravagli, C., Cuomo, F., Messia, M.C., Marconi, E. and Caboni, M.F. (2024), Effect of replacing olive oil with oil blends on physicochemical and sensory properties of taralli. Int J Food Sci Technol, 59, 2697-2706. https://doi.org/10.1111/ijfs.17019. (IF 3.1, Q2).

▪ **Oral presentations**

▪ Pasini Federica*, Marzocchi Silvia, Ravagli Cesare, Messia Maria Cristina, Caboni Maria Fiorenza, "Studio di shelf-life di biscotti con miscele lipidiche diverse.", 12° Convegno AISTEC CEREALI E SCIENZA: resilienza, sostenibilità e innovazione. 15-17 june, Portici (Naples, Italy), ISBN: 9788890668074, P.96.

▪ Ravagli Cesare*, di Cori Cristina and Caboni Maria Fiorenza, "Building of NIR calibration curves for different vegetable oil analyses using a flux chamber innovative prototype", 2° CONVEGNO Diagnostica nel settore Agroalimentare Indagini, non Distruttive, Normative, Esigenze, 16/17 May 2024, Parma (Italy), https://www.aipnd.it/img/uploads/file/aipnd-convegno-agroalimentare-2024-programma-finale.pdf.

▪ Ravagli Cesare*, Caboni Maria Fiorenza, "Sostituzione dei grassi alimentari, una sfida nel mutamento dell'uso di lipidi eco-sostenibili", La giornata green del dottorato | Acqua, resilienza ed oltre. EU Green Week, 30 May 2024 Bologna/Cesena (Italy). https://www.youtube.com/watch?v=JBMXHOauhQs

▪ Ravagli Cesare*, Toth Szimonetta, Solomonova Marya, Recseg Katalin and Caboni Maria Fiorenza, "Developing of NIR calibration curves using a flux chamber innovative prototype, applications on the food industry, vegetable oils", X Simposio nazionale NIR Italia, 26-28 June 2024, Turin (Italy), DOI 10.52810/zenodo.12658120 P.29-30.

▪ Ravagli Cesare*, Pasini Federica, Marzocchi Silvia, di Cori Cristina and Caboni Maria Fiorenza, "Technological, sensory, and nutritional assessment of eco-friendly food lipids." 28th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, 18-20 September 2024, Catania (Italy), P.364-365.

*= presenting speaker

8.Resume of the research activity

During the first year of this PhD project, as indicated in the Gantt chart several types of oil,s extracted from Italian by-products, were evaluated on the basis of their chemical composition and technological properties with both laboratory analyses (FAME quantification and accelerated oxidation tests using OXITEST) and bibliographic sources. The most promising results, obtained from a combination of: yields, unsaturated/saturated fatty acids composition, oxidation induction point and volatile compounds evolution during thermal stress were: Grapeseed oil, Rice oil and Wheat germ oil; these oils were then combined on a theoretical level to obtain fat blends using also normally used oils in industry like Sunflower seed oil, both high oleic and low oleic types. Due to the actual Ukrainian-Russian conflict, and the subsequent reduction of sunflower seeds supplies, the latter oils are on stand-by for next studies and formulations, because the risk of absent of said products on the market is not a distant prediction. To fill the gap left by the sunflower oil, coconut oil is also being evaluated (used in minor content than market formulations). Wheat germ oil production was also studied to find alternative, and economically sustainable methods of extraction prior to industrial solvent extraction. Enzymatic-aqueous extraction was evaluated and tested by using several bibliographic references and tests but the production yields of said process was not sustainable; so also, for wheat germ oil also industrial solvent extraction was proposed and is being evaluated in order to study and obtain specific refining conditions tailored for said oil. During the second year of this PhD project, and as indicated in the Gantt chart, the production of sample products, “frollini” biscuits and tarallini using pilot implants lended to us by the University of studies of Molise, was conducted. Said products were all produced using the same base formulation of flour, water, sugar and/or salt and different blends of fats in each one (5 different formulations for the “frollini”, and 4 different for the tarallini), all the formulations were compared with specific standard formulations (using fats normally implemented in the production of said products), specifically palm oil for “frollini” and

extra-virgin olive oil for tarallini. The products were then analyzed to assess the contents of primary and secondary oxidation products (by peroxide value analysis and volatile compounds analysis with SPME-GC-MS) and general oxidation resistance by OXItest®. The products were then stored for different periods of time in thermally regulated chambers at 20°C, in order to simulate and evaluate the progression of oxidation during storage time (for "frollini" biscuits the products were also stored at 45°C in order to assess changes in conditions of thermal abuse). The "frollini" biscuits were stored from 0 to 355 days, the tarallini from 0 to 75 days, given their lower shelf-life. At different sampling days all the previously mentioned analysis were again conducted and added with the analysis of the oxidated fatty acids content (OFA) by implementation of Fast GC-FID instrument. During the year the company period in BUNGE was also started with the involvement inside the company laboratories and the immediate quality check and development of the sunflower oil production. Over the third year of the PhD, the final part of the project was conducted. Inside the company's Italian headquarters (BUNGE, Porto Corsini, Ravenna (RA), Italy), we begun the development of quick analytic methodics for the analysis of refined vegetable oils, with a focus on the individuation of adulterations/accidental pollution of different kinds of vegetable oils, namely: sunflower oil, both In high and low oleic form, soy oil, corn oil, peanut oil, and various vegetable oil blends specific for the industry ("frying mix oils). The oils were collected and sampled along the production chain of the Bunge refining implant and counted over 180 samples, which were analysed, over the course of various months for chemical quality parameters such as: Red and Yellow colour, using a Lovibond® PFXi-195 system, general oil Acidity, measured by means of the ISO 660:2020 protocol and Fatty acid methyl esters (FAME) composition by means of GC-FID internal analysis. The collected results were used, in tandem, with a Vis-NIR PROXIMATE™ system provided by BUCHI, and equipped with a flux chamber prototype, in order to collect Near-Infrared spectras of the various oils. The spectras obtained were then used to create calibration curves useful in the estimation of the parameters previously described; the means of procedure to obtain the calibration curves were specifically the application of a Partial least squares (PLS) regression coupled with a simple second derivative pre-treatment of the data. Results shown the development of appreciable models, with determination coefficients ranging from 0.923 to 0.998, and the best results for specific parameters such as the Oleic acid (C18:1) concentration, which is a useful parameter to identify potential pollutions of the High oleic sunflower oil production. The results obtained during the company period were then implemented inside the PhD work and feasibility tests and studies were conducted, regarding all of the aspects of the project considered until now, with

a particular focus on the alternative formulations of the tarallini and the “frollini” biscuits.

9. Acknowledgments

This thesis was written with the assistance and support of the Professor Maria Fiorenza Caboni and her research group, and the staff of BUNGE Italy with a special thanks go to Lorenzo Ferrari of BUCHI Italy, for providing technical and theoretical support for the third goal of this thesis. This journey was a long one, I can't say that looking back 4 years ago I knew that today I was going to get a doctoral degree, and yet again here we are…can't say it was an easy journey nor a difficult one, sometimes were hard, and others were simpler. The research world was something that I ever pictured since I was little, and I could be amazed by the many wonders hidden behind a mere word. When I started, I wanted to explore wildly whatever I had under my nose, and that emotion didn't change, for this I will briefly thank my inner child, ever present. It was a journey in which I had to come down with solid method, in which I had to learn again how to be organized, cold and methodical, for this I have to thank 2 persons in my life that I always saw as impeccable, my mother Giovanna, for which I always had unwavering trust in how to run your life, and my "boss" Federica, a figure that I learnt to respect, and hate sometimes (for which I know she also see it back this way ahahahahaha), but in the end she was and is the main reason for which I wanted to continue this journey, a complete opposite in what I am, but that I respect from the bottom of my heart. Along the road I met many people, some from the past, such as my colleagues from the university, Federico, Marianna and Mara, that next year will probably be here in my place writing their thesis and for which I wish good luck; and some new, such as all of the other PhDs and researchers of the Cesena campus, the chill Gebre (3G for friends), the gentle Solidea, the energetic Beatrice, Giacomo and its continuous spontaneous bursts, the always smiling Francesca and Jasmine and many many others…to all of you I say thank you for making this journey always filled with experiences. Along everyone I worked hard, but always with a smile, because two persons teach me how to take life for whatever brings you with a laugh on my belly, and those are my father Michele, the

always child, that now that I'm mature, I see why we have to behave this way; and my professor Maria Fiorenza Caboni, that always reminded me why science is beautiful and full of wonders. These thanks will not be complete without my "second" family, all of you guys that always stayed beside me, in good and bad moments, all of you friends that I could always talk to: Ivan, Alessandro, Daniel, Valentina, Elisa, Mattia, Luca, Nicolò, Tommaso and many others. I ought a part of my life to you, you were ever present since I was maybe more "innocent" of life, and accompanied me through who I am now. I just hope that one day I can repay what you gave to me. Last but not least I have to thank my two sisters, Clarissa and Valentina, one, my sister for life, that always cared for me, and that multiple times I took for granted, out of pettiness and pride; and the other, Valentina, the friend that apparently is continuing to stay in my life, and that now I have to recognize also of part of who I am. I really care for both of you, and hope you will always be happy. Finally, I want to wish the best of luck to Silvia, my co-worker, and to the new beautiful life that will accompany her journey.

Thank you all,

Cesare.

"Yesterday is history, tomorrow is a mystery, but today is a gift. That is why it is called the present."

Master Oogway, 2008