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**Use of deep learning in combination with FT-NIR spectroscopy
for the analysis of extra virgin olive oil**

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Preface

The research presented in this thesis, conducted over three years, aimed to evaluate the application of neural networks to the analysis of oils. The study focused on the quality and compositional changes of oils obtained from olive drupes, with a particular emphasis on extra virgin olive oil (EVOO). The oils were analyzed using Fourier Transform Near-Infrared (FT-NIR) spectroscopy, as initially proposed; subsequently, the research was extended to include Visible (Vis) spectroscopy. The extraction of spectral information and the development of predictive models were performed using both classical and deep chemometric techniques. Partial Least Squares (PLS) was employed for classical chemometrics, while neural networks,

specifically Artificial Neural Networks (ANN) and Convolutional Neural Networks (CNN), were utilized for deep chemometrics. In each case, the approaches were compared and evaluated using common error metrics.

The manuscript begins by introducing the importance of the olive oil market and product quality, with a special focus on EVOO. The thesis then provides an overview of the current state-of-the-art regarding the use of FT-NIR and Vis spectroscopy in oil analysis and the application of neural networks for data analysis.

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Title of thesis

Use of deep learning in combination with FT-NIR spectroscopy for the analysis of extra virgin olive oil

Abstract

The increasing demand for olive oils, both in terms of quantity and economic value, has led industry to focus on maintaining product quality. Furthermore, consumers are increasingly focusing on the type of oil used, considering its chemical, physical, and nutritional characteristics. In Europe and Italy, extra virgin olive oil (EVOO) is an important product, considering its production methods, quality attributes, and health properties.

This PhD thesis utilized Visible (Vis) and Near-Infrared (NIR) spectrophotometric techniques for oil analysis. These methods are considered non-destructive, economical, rapid, and eco-friendly compared to traditional analytical techniques. Models development using spectral data was performed using two different chemometric approaches: i) classical, employing Partial Least Squares (PLS), and ii) deep learning, utilizing networks such as Artificial Neural Networks (ANNs) or Convolutional Neural Networks (CNNs). Classical chemometrics involved spectral pre-processing steps, including noise reduction, baseline correction, and scatter correction. Neural network models were evaluated both with and without this spectral pre-processing.

Over the three years, an initial study of the state-of-the-art regarding the combination of oil quality, spectroscopy, and deep learning was conducted. Thereafter, scientific activities focused on the development of predictive models using both Vis-NIR (380-900 nm) and FT-NIR (1 000-2 500 nm) spectroscopy. The comparison between classical and deep learning approaches applied to the spectral data aimed at developing models to predict: i) chemical parameters related to shelf-life monitoring of EVOO; ii) chemical parameters across different types of oil (mainly olive) supplied by a refinery; and iii) the presence and amount (quantification) of different types of seed oils used as adulterants in EVOO.

In the shelf-life study, CNN outperformed ANN and performed equally or, in some cases, showed superior results compared to PLS, even when PLS performance was enhanced using data augmentation. Data augmentation was applied to enable a fairer comparison between classical and deep learning models. In particular, the model obtained for peroxide content in EVOO showed very good results, especially when the CNN approach was combined with Vis-NIR spectroscopy, achieving an R^2 in prediction (R^2_P) greater than 0.99 and a root mean square

error in prediction (RMSEP) of 2.75 mEqO₂/Kg, considering that the peroxide reference values ranged from 7.50 to 163.90 mEqO₂/Kg.

In the study involving oils provided by the refinery with their chemical analyses, model development using the deep learning approach (CNN) generally yielded the best performance, regardless of the spectroscopic range studied. The exceptions were PLS models for predicting oleic and linoleic acid content using an augmented dataset. In this study, a robust model was developed to predict the free acidity value of vegetable oils, with an RMSEP of approximately 0.2 % (w/w). Notably, the mean free acidity value across all samples was 4.4 % (w/w).

In the prediction of adulterants (i.e., peanut, sunflower, corn, and soybean) oil content in EVOO, the predictive models performed differently depending on the type of adulterant, the pre-processing used, and the spectroscopic range considered. However, regardless of the specific spectroscopy or chemometric method employed, discriminating against low concentrations of adulterants (i.e., ≤ 1.5 %) proved challenging. A high degree of similarity was observed between EVOO and peanut oil, probably related to their fatty acid composition. This similarity led to the poorest predictions, especially for FT-NIR models without pre-processing. In PLS models, data augmentation generally improved robustness but not the accuracy of the models obtained. This part of the research also evaluated the effect of applying classical pre-processing methods prior to CNN analysis. The results demonstrated comparable performance for the FT-NIR models and superior performance when using Vis-NIR spectroscopy with pre-processing.

The outcomes demonstrate the potential of using FT-NIR and Vis-NIR spectroscopy in conjunction with deep learning for the analysis of oils. Convolutional neural networks show wide application potential, given their ability to process raw data, potentially reducing the need for extensive pre-processing.

Keywords

Olive oil, Vis spectroscopy, FT-NIR spectroscopy, Data Augmentation, Partial Least square, Convolutional Neural Network, Artificial Neural network

INTRODUCTION 1

1.1 OVERVIEW INTRODUCTION

The research presented in this thesis aims to contribute to the field of neural networks by evaluating their application in the food sector, specifically for the analysis of vegetable oils, with a particular focus on extra virgin olive oil (EVOO). While the application of FT-NIR and Vis spectroscopy in the olive oil sector has been widely studied, the application of these spectroscopic techniques in conjunction with deep chemometrics as an alternative to classical chemometrics needs to be further explored.

The combination of non-destructive analytical techniques and neural networks for data processing and predictive model development has considerable potential for innovation in the food sector, where there is an increasing need for rapid and accurate results that minimize human intervention.

1.2 PROPERTIES OF OILS

1.2.1 *Economy and product quality*

The market for edible vegetable oils is expanding due to their extensive use in food flavoring and cooking, both in domestic settings and in industrial food production (Rifna et al., 2022). This trend can be attributed, in part, to the global decline in the use of animal fats, driven by ethical considerations and economic factors (Casale & Simonetti, 2014).

Edible vegetable oils can be obtained from seeds as well as from oil-bearing trees (Rohman, 2017). Global consumption of these oils has exceeded 170 million tons per year, with China and India being among the largest consumers (Ng et al., 2015). In recent years, the oils that have dominated world trade include palm oil, soybean oil, and rapeseed oil (Tian et al., 2023). However, one of the most significant oils is that derived from the drupe of the olive tree: olive oil. From a commercial and legislative perspective, it is possible to identify eight categories of olive oil (EU, 2013): Extra virgin olive oil, Virgin olive oil, Lampante olive oil, Refined olive oil, Olive oil (composed of refined olive oils and virgin olive oils), Crude olive pomace oil, Refined olive-pomace oil, and Olive-pomace oil. European law, in accordance with international standards established by the CODEX Alimentarius (Codex stan. 33-1981), defines Extra Virgin olive oil as the only oil “obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alterations in the oil, it has not undergone any treatment other than washing, decantation, centrifugation, or filtration, to the exclusion of oils obtained using solvents or adjuvants having a chemical or biochemical

action, or by re-esterification process, and any mixture with oils of other kinds” (EU, 2013). Despite the common raw material (olives) and extraction technology, Extra Virgin olive oil differs significantly from Virgin and Lampante olive oils. In fact, EVOO is defined by “a maximum free acidity, expressed as oleic acid, of 0.8 g per 100 g” and other characteristics (EU, 2022) such as the absence of organoleptic defects, a median fruitiness greater than zero, and a peroxide value less than or equal to 20 meqO₂/kg. EVOO is widely recognized as one of the most valued oils from an organoleptic and nutritional point of view, which has led to an increase in commercial demand over the years. For the 2023-2024 period, global consumption for EVOO was projected to reach approximately 2 780 000 tons, with Europe contributing approximately 44 % (IOC, 2024).

The organoleptic and nutritional properties of olive oil are primarily attributable to the characteristic compounds obtained from the olive. Approximately 98 % of these compounds (Jimenez-Lopez et al., 2020) are represented by saturated and unsaturated fatty acids (e.g., oleic, linoleic, and linolenic), often esterified in triglycerides. The remaining 2 % of the extract, termed the unsaponifiable fraction, consists of phenolic compounds, tocopherols (α , β , γ , δ), carotenoids, squalene, simple triterpenes, volatile compounds, oleuropein, ligstroside, and hydroxytyrosol (Li & Wang, 2018; Rohman, 2017). Overall, these constituents are important for flavor characterization, antioxidant product protection, and health properties. Indeed, the health benefits of EVOO have been demonstrated to include anti-inflammatory, antihypertensive, chemopreventive, and tumor-inhibitory properties (Baccouri et al., 2008; Gómez-Caravaca et al., 2016). A diet rich in olive oil has been shown to reduce the risk of cardiovascular disease; lower plasma triacylglycerol levels; decrease blood pressure and the risk of hypertension; and increase HDL cholesterol levels by improving postprandial lipoprotein metabolism (García-González et al., 2008). Finally, as previously mentioned, the antioxidants present in olive oil, such as carotenoids, polyphenols, secoiridoids, and tyrosols, contribute to the product's preservation and have beneficial effects on human health (Rohman, 2017).

1.2.2 Product adulteration

The high nutritional and organoleptic benefits of EVOO increase its economic value, which makes it susceptible to adulteration with oils of lower quality and price. Adulterants may be derived from the same product (olives) or from different products (seeds). Adulteration can involve physically mixing oils of different origins, as well as mislabeling lower-quality oils (Hashempour-baltork et al., 2024). This practice leads to economic damage and also potential harm to consumer health, such as the “toxic oil syndrome” (Aldridge & Connors, 1982). The

adulteration process is often difficult to detect and distinguish in vegetable oils due to their high similarities, especially in terms of fatty acids, which represent most of the product. Consequently, as the compound profiles of oils often overlap, it becomes difficult to distinguish oils that have been blended (Vanstone et al., 2018).

1.2.3 Factors affecting the quality of EVOO

The quality characteristics of EVOO depend on several variables (Gómez-Caravaca et al., 2016; Lolis et al., 2020), including agronomic factors, technological processing, and storage and distribution conditions (time, temperature, light, packaging).

The agronomic characteristics include all factors that influence the quality of the olives during cultivation, harvest, and storage before transformation. These characteristics are linked to environmental conditions, cultivation techniques, plant cultivar, fruit ripening, harvesting methods, and storage practices. These elements can have a significant impact on the initial quality of the olive oil.

The initial quality of the oil is also strongly influenced by the production techniques related to the type of pressing, centrifugation, phase separation (e.g., two-phase or three-phase systems), and malaxation. Malaxation temperature and duration have been observed to affect the initial content of volatile compounds and polyphenols, which are known to correlate with the oxidative stability of the oil (Angerosa et al., 2001).

Storage conditions affect the quality retention of EVOO. Specifically, the presence of oxygen in the headspace, contact with light, and high temperatures promote the loss of compounds important for maintaining quality (Pristouri et al., 2010). One of the main threats to the degradation of molecules in oil during storage comes from oxidation reactions. These reactions lead to the formation of undesirable and sometimes toxic compounds, which cause a change in the quality category of the product and a consequent loss of economic value (Castelo-Branco et al., 2016). During storage, conditions such as elevated temperatures, exposure to light, contact with oxygen, and the use of inappropriate packaging materials can promote more rapid degradation and a reduction in shelf life (Iqdiam et al., 2020; Karami et al., 2020).

The selection of appropriate packaging during storage and distribution influences the quality of olive oil by protecting the product from both oxygen and light (Kanavouras et al., 2004).

Two types of oxidation can be distinguished: autoxidation and photooxidation (Lolis et al., 2020). Autoxidation, often considered the predominant process in product degradation, is initiated by the presence of triplet oxygen (Choe & Min, 2006). Generally, the reaction involves fatty acids that are degraded to hydroperoxides (primary oxidation). The products of primary

oxidation are subsequently degraded through further oxidative processes (secondary oxidation) that lead to the production of various compounds (e.g., aldehydes, ketones, alcohols, esters, hydrocarbons) that can be detrimental to health and cause off-flavors. The photooxidation reaction, initiated by the presence of light, involves photosensitizers (i.e., chlorophyll) that produce singlet oxygen from triplet oxygen. This highly reactive molecule reacts with unsaturated fatty acids to form free radicals, which then react in the primary and secondary oxidation reactions previously described for autooxidation. High temperatures accelerate both autooxidation and photooxidation. Another type of degradative reaction that leads to an increase in free fatty acids, tyrosol, and hydroxytyrosol content is caused by triglyceride and secoiridoid hydrolysis (Brenes et al., 2001; Chen et al., 2011).

1.3 ANALYTICAL TECHNIQUES

1.3.1 Official analytical methods for EVOO

Current regulations define the parameters that an olive oil must meet to be classified into a certain category. Specifically, Extra Virgin, Virgin, and Lampante olive oils (EU, 2013) are subject to the most restrictive values for the quality parameters considered. At the European level, the parameters to be analyzed in these oils are defined by the European Commission (EU, 2022) (**Tab. 1**). The determination of these parameters involves physicochemical and sensory laboratory analyses. These analyses follow well-defined protocols and typically involve measuring the content of specific compounds, such as free fatty acids, peroxides, and phenolic content, to assess the oil's quality and degradation state. For instance, the peroxide value indicates the state of primary oxidation, while the anisidine value and the K_{270} value suggest the state of secondary oxidation (Lolis et al., 2020). Organoleptic analysis, complementing the physicochemical analysis, is essential to classify a virgin olive oil as Extra Virgin and requires a trained panel following a specific tasting protocol (IOC, 2018). A single analysis is not sufficient to define the category; it is crucial that all specified parameters are met.

Traditional analyses for olive oil include titrations (e.g., acidity, peroxide value), spectrophotometric readings (extinction coefficients K), gas chromatography, and high-performance liquid chromatography (CEE, 1991). However, these methods have several drawbacks: they are expensive and time-consuming, require extensive sample preparation, and use chemical reagents that are harmful to human health and the environment (Abu-Khalaf & Hmidat, 2020; Gómez-Caravaca et al., 2016).

Table 1. Chemical parameters and limits (EU,2022) used to differentiate between Extra Virgin Olive Oil (EVOO), Virgin Olive Oil (VOO), and Lampante Olive Oil (LOO).

Chemical Parameters	EVOO	VOO	LOO
Acidity (%)	≤ 0 .8	≤ 2	> 2
Peroxide value (mEq O ₂ /kg)	≤ 20	≤ 20	—
K ₂₃₂	≤ 2 .5	≤ 2 .6	—
K ₂₆₈ or K ₂₇₀	≤ 0 .22	≤ 0 .25	—
ΔK	≤ 0 .01	≤ 0 .01	—
Organoleptic characteristics: defect	Md = 0	Md ≤ 3 .5	Md > 3 .5
Organoleptic characteristics: fruity	Mf > 0	Mf > 0	—
Fatty acid ethyl esters (mg/Kg)	≤ 35	—	—
Myristic acid	≤ 0,03	≤ 0,03	≤ 0,03
Linolenic acid	≤ 1	≤ 1	≤ 1
Arachidic acid	≤ 0 .6	≤ 0 .6	≤ 0 .6
Eicosenoic acid	≤ 0 .5	≤ 0 .5	≤ 0 .5
Behenic acid	≤ 0 .2	≤ 0 .2	≤ 0 .2
Lignoceric acid	≤ 0 .2	≤ 0 .2	≤ 0 .2
Total transoleic isomers	≤ 0 .05	≤ 0.05	0 .1
Total translinoleic + trans-linolenic isomers	≤ 0 .05	≤ 0 .05	0 .1
Stigmastadienes (mg/Kg)	≤ 0 .05	≤ 0 .05	0 .5
ΔECN42	≤ 0 .20	≤ 0 .20	≤ 0.30
2-glyceryl monopalmitate	≤ 0 .9 if total palmitic acid % ≤ 14 .00%		
	≤ 1 .0 if total palmitic acid % > 14 .00%		
Cholesterol (%)	≤ 0 .5	≤ 0 .5	≤ 0 .5
Brassicasterol (%)	≤ 0 .1	≤ 0 .1	≤ 0 .1
Campesterol (%)	≤ 4	≤ 4	≤ 4
Stigmasterol (%)	< Camp.	< Camp.	—
Apparent β-sitosterol(%)	≥ 93	≥ 93	≥ 93
Δ-7-stigmastenol (%)	≤ 0 .5	≤ 0 .5	≤ 0 .5
Total sterols (mg/Kg)	≥ 1 000	≥ 1 000	≥ 1 000
Erythrodiol and uvaol (%)	≤ 4 .5	≤ 4 .5	≤ 4 .5
Waxes (mg/kg)	≤ 150	≤ 150	≤ 150

Md, Median of defect; Mf, Fruity median; -, parameter uncalculated;

1.3.2 FT-NIR and Vis spectroscopy in food analysis

The difficulties associated with conventional analytical methods have led to the consideration of alternative analytical techniques that are non-destructive, economical, time-saving, and environmentally friendly (Karami et al., 2020). Over the years, techniques such as spectroscopy, electronic nosing, DNA-based methods, thermogravimetric analysis, and differential scanning calorimetry have been studied for oil analysis (Hashempour-baltork et al.,

2024). Among these, spectroscopy has been widely employed in the olive oil sector due to its versatility and ease of application.

Spectroscopy is based on the interaction between matter and electromagnetic radiation, which can be absorbed, reflected, or transmitted. The resulting electromagnetic spectrum typically plots absorbance or transmittance values against the corresponding wavelengths (or frequencies/wavenumbers). The values measured by the spectrophotometer also depend on the interaction between each wavelength and the analyzed product's physicochemical structure, which determines absorption and scattering (Nicolai et al., 2007). Light hitting the sample causes an energy transition from a ground state to an excited state. Optical radiation is subdivided into various wavelength regions according to the associated energy levels. The ultraviolet (UV) and visible (Vis) regions correspond to higher energy values, while the near (NIR), mid (MIR), and far infrared (FIR) regions correspond to lower energy values. The UV-Vis region, ranging from approximately 100 to 780 nanometers, is associated with electronic transitions in molecules containing features like peptide bonds and conjugated double bonds (e.g., carotenoids, chlorophyll). The infrared region is characterized by its wide range and can be divided into three sub-regions: i) NIR from 780 to 2 500 nm; ii) MIR from 2 500 to 25 000 nm; and iii) FIR from 25 000 to 1 000 000 nm. Radiation in the infrared region is generally related to vibrations involving chemical bonds within functional groups of the analyzed compounds (Porep et al., 2015). Specifically, in the NIR region, absorbed radiation excites molecules from their ground vibrational state to higher energy levels, causing an increase in molecular vibrations. The molecular vibrations characteristic of bonds that absorb in the NIR region typically involve carbon-hydrogen (C-H), nitrogen-hydrogen (N-H), oxygen-hydrogen (O-H), and sulfur-hydrogen (S-H) bonds (Blanco & Villarroya, 2002). Molecular vibrations include bond stretching (changes in bond length) and bending (changes in bond angle). The transition from the ground state to vibrationally excited states generates overtones (usually corresponding to the first, second, or third vibrational energy level transitions above the fundamental) that are mainly caused by stretching vibrations. Alternatively, combination bands may arise from the simultaneous excitation of multiple fundamental vibrations (e.g., stretching and bending) (Workman & Weyer, 2012). Finally, to enhance the quality of the NIR signal, modern NIR instruments often replace the traditional monochromator with an interferometer. The resulting signal (interferogram) is then processed using a Fourier transform (FT) algorithm to obtain the spectrum (Nicolai et al., 2007). These spectrophotometers, called FT-NIR, provide high and constant optical resolution along with an improved signal-to-noise ratio. Spectra can be acquired in transmittance, where light passes through the sample before detection, or in

reflectance, measuring light reflected from the sample surface. Alternatively, in the transreflectance mode, light passes through the sample, reflects off a surface behind it, passes through the sample again, and is then detected on the same side as the source. Thus, in the transreflectance mode, the radiation passes through the sample twice. A further method for acquiring spectra, often employed with fiber optics, especially for solid or bulk samples, is interactance, which involves analyzing light that enters the sample and is scattered back towards the detector from the irradiated surface (Alander et al., 2013).

FT-NIR and Vis spectroscopy have been widely applied in the EVOO sector, to characterize varietal and geographical origin, chemical profile and the presence of adulterants. **Table 2** shows several studies that evaluated the use of visible and infrared spectroscopy, separately or in combination, for the analysis of olive oils (Gómez-Caravaca et al. 2016).

Table 2. Major applications of Vis and NIR spectroscopy in the olive oil sector

<i>Application</i>	<i>Spectral range</i>	<i>Study topic</i>	<i>References</i>
Chemical profile	800-2500 nm	Classification of EVOO based on sensory attributes.	(Sinelli et al. 2010b)
	800-2500 nm	Predictive models for fatty acid profiles, volatile and phenolic compounds, and sensory attributes.	(Inarejos-García et al. 2013)
	900–1700 nm & 1350–2150	Predictive models for the phenolic profile in VOO.	(Li et al. 2025)
	833- 2500 nm	Quantification of sterol content and major fatty acids in EVOO.	(Özdemir et al. 2018)
	350-1000 nm & 1000-1800 nm	Prediction of saturated, monounsaturated, and polyunsaturated fatty acid content in olive oil.	(Cayuela-Sánchez et al. 2019)
	740-1070 nm	Correlation between the quality of the raw material (olives) and the sensory quality of the oil.	(Violino et al. 2022)
	200-800 nm	Evaluation and monitoring of the auto-oxidation process during EVOO storage.	(Gonçalves et al. 2018)
	390 – 720 nm	Prediction of carotenoid (lutein and β -carotene) and chlorophyll content (pheophytin-a and pheophytin-b).	(Domenici et al. 2014)
Origin	800- 2200 nm	Classification of EVOO monovarietal samples (Casaliva, Leccino and Frantoio).	(Sinelli et al. 2010a)
	1000-2200 nm	Classification of Tunisian virgin olive oils compared to Maghrebian (Algerian and Moroccan) and French olive oils.	(Laroussi-Mezghani et al. 2015)
	870 – 2500 nm	Classification of olive oils of different origins based on their fatty acid and triacylglycerol content.	(Gertz et al. 2019)
	300-700 nm	Classification of olive oil samples by variety and harvest year and prediction of chlorophyll content.	(Khani et al. 2024)
Adulteration	833- 2500 nm	Quantification of EVOO adulteration with corn, sunflower, soybean, and canola oils.	(Vanstone et al. 2018)
	908-1676 nm	Identify and quantify the adulteration of EVOO with soybean, sunflower, corn and rapeseed oils.	(Borghi et al. 2020)
	400-2500 nm	Determinations of olive oil adulteration with different concentrations of soybean oil.	(Meng et al. 2023)
	200-800 nm	Classifying and quantifying the adulteration of olive oil by oil from the previous year.	(Uncu and Ozen 2019)
	234-800 nm	Adulteration detection based on differences in the maximum absorption peaks between different oils (chlorophylls, conjugated diene and triene and carotenoids).	(Ok 2017)

1.4 CHEMOMETRICS AND MODEL DEVELOPMENTS

1.4.1 *Classic chemometrics*

Spectral data are often affected by the physical state of the product analyzed, scattering effects of radiation, instrumental noise, and environmental factors. These issues can obscure the correlation between wavelength and chemical composition in a spectrum. In fact, spectral data require multivariate analysis involving complex mathematical transformations to extract meaningful information. Chemometrics, as defined by the International Chemometrics Society, is a branch of chemistry that utilizes mathematical, statistical, and logical methods to design, select, and optimize procedures and experiments. It employs these methods to extract maximum information from chemical data, including spectral data, and often involves visualizing this information graphically (Grigoletto et al., 2025). Over the years, this discipline has found numerous applications in various fields, including quality control, monitoring, process control, food traceability, genomics, proteomics, metabolomics, and image analysis, among other applications in industrial and environmental fields. Chemometrics is also applied in the olive oil sector at different stages of production, from evaluating the raw material, monitoring the extraction stage, to ensuring the quality control of the finished product (Gómez-Caravaca et al., 2016). Chemometrics typically involves a phase of data preprocessing, essential for removing distortions that could hinder interpretation, and a subsequent model development phase. The selection and application of preprocessing techniques and the choice of appropriate modeling techniques often rely on the operator's domain knowledge and experience.

1.4.2 *Data preprocessing*

The raw spectra obtained from sample analysis usually require pre-processing. The objective of preprocessing techniques is to minimize the contribution of irrelevant information, which is often caused by factors like background interference, variations in light scatter, and spectral noise. Common spectral preprocessing methods include baseline correction, noise reduction, resolution enhancement, and normalization (Porep et al., 2015).

The initial step often involves baseline and scatter correction to reduce the effects of low-frequency noise, baseline shift, and variations in path length or scattering. This correction can be applied using mathematical filters such as 1st and 2nd derivatives. The 2nd derivative is often used as it allows the visualization of weak peaks obscured by broad background features in the original spectrum. However, using higher order derivatives increases the complexity of the spectra, and the signal-to-noise ratio decreases. Alternatively, a multiplicative scatter correction (MSC) filter can be employed to correct for baseline shifts and scaling variations due to light scatter (additive and multiplicative effects). This technique aims to remove scatter effects by

adjusting each spectrum relative to an ideal spectrum, which is the average spectrum of the dataset or the spectrum of a specific reference sample (MacDougall et al., 1985; Nicolai et al., 2007). An alternative method is the Standard Normal Variate (SNV) transformation, which processes each spectrum individually. SNV centers the spectrum around zero by subtracting its mean and then divides it by its standard deviation, effectively scaling each spectrum to unit variance.

Subsequently, noise reduction techniques may be performed. The objective of these techniques is to remove high-frequency noise and enhance the signal-to-noise ratio. The Savitzky-Golay algorithm, which is commonly used for this purpose, allows adjustment of both the order of the polynomial fit and the width of the smoothing window. It fits a low-degree polynomial to points within the chosen window and replaces the central point with the value calculated from the polynomial fit (Savitzky & Golay, 1964). Using too wide a smoothing window risks the loss of useful spectral features. The Savitzky-Golay algorithm can also be combined with derivatives to simultaneously perform smoothing and differentiation, potentially enhancing the effective resolution by separating overlapping bands and highlighting hidden features.

Normalization is another important preprocessing step applied to correct for intensity variations between samples that might occur during sample preparation or measurement. Mean centering (MC) is a common normalization method that highlights variations relative to the average. MC subtracts the mean spectrum (average of all spectra in the dataset) from each individual spectrum. Alternatively, autoscaling (AS) can be used to give equal weight to all variables (i.e., wavelengths). It involves mean-centering followed by dividing each variable by its standard deviation across all samples. However, AS risks amplifying noise, especially for variables with low variance, potentially obscuring important information (Boysworth and Booksh, 2008).

In conclusion, spectral preprocessing helps reduce unwanted spectral variation by removing irrelevant signals (Nicolai et al., 2007) and can potentially improve the accuracy and precision of the subsequently developed predictive models. Preprocessing techniques, however, should always be used with caution, as inappropriate application could introduce systematic errors or remove relevant information.

1.4.3 Development of predictive models

After data preprocessing, model development can be performed using either unsupervised chemometric techniques (e.g., Principal Component Analysis (PCA), cluster analysis) or supervised techniques. Unsupervised methods are typically used for exploratory data analysis

or clustering, whereas supervised techniques learn relationships from labeled data for developing both classification and quantification models. The difference between these two approaches is that supervised techniques utilize 'known' reference values or classes (labels), while unsupervised techniques do not use labels and explore inherent structures or patterns within the data. In classification models, a sample is assigned to a predefined class. In quantification models, the goal is typically to predict a numerical value (Kuhn & Johnson, 2013). Model performance is evaluated using appropriate metrics. For classification, these usually include accuracy, sensitivity, and specificity. For quantification, common metrics are Root Mean Squared Error (RMSE), BIAS (systematic error) and R^2 (coefficient of determination).

In quantification modeling, common chemometric regression techniques such as stepwise multiple linear regression (SMLR), principal component regression (PCR), partial least squares (PLS), and artificial neural networks (ANN) are employed (Cen & He, 2007). These techniques aim to establish a mathematical relationship between variations in the spectra of samples and variations in the measured parameter (López et al., 2013). Spectroscopic data are subject to collinearity, which makes traditional multivariate regression unstable. Partial Least Squares (PLS) regression is a technique particularly appropriate for such data. Unlike PCR, which focuses primarily on explaining variance in the spectral data (X), PLS also considers the response variable (Y) (e.g., a chemical parameter), looking for correlation between X and Y.

Specifically, PLS computes new variables, called latent variables (LVs), which are linear combinations of the original spectral variables. These LVs are orthogonal (uncorrelated) to each other and are derived to maximize the covariance between the X matrix (e.g., spectral data) and the response vector Y (e.g., a chemical parameter). Latent variables are ordered according to their relevance for the prediction of the Y variable, with the aim of using the fewest LVs possible while capturing the most relevant predictive information in the development of predictive models. The selection of the optimal number of LVs is critical to reduce the dimensionality of the spectral data without compromising important information. This selection helps manage spectral collinearity (Nicolai et al., 2007) and is crucial to avoid under- or over-fitting problems, which would affect the robustness and generalizability of the model.

In model development, the dataset is commonly split (Wold et al., 2001) into calibration and validation/test sets to train and independently evaluate the model, respectively. The split percentage between the two datasets is influenced by the size and variability of the data. Typically, around 60-70 % of the data is used for calibration, while the remaining percentage is reserved for validation (testing). The selection of algorithms for splitting datasets involves

numerous options. Two commonly utilized methods for achieving a representative split are based on Kennard-Stone and Onion algorithms. These methods differ in their approach to ensuring representativeness in both the calibration and validation datasets (Gallagher & O’Sullivan, 2012). The development of a calibration model requires a substantial amount of data.

To build a robust model and optimize its performance, cross-validation is usually performed. This step is typically performed on the calibration set to tune model parameters (e.g., the optimal number of LVs) and estimate performance before final evaluation on the independent validation/test set (Mathworks, 2025). Cross-validation splits the calibration dataset into multiple subsets (folds). In each iteration, some subsets are utilized for training the model, while the remaining subsets are used for internal validation. This process is repeated multiple times, and the average cross-validation error is utilized as a guide for selecting model hyperparameters (e.g., the number of LVs). The method used to generate subsets depends on the specific type of cross-validation employed (e.g., k-fold, leave-one-out, etc.). The selection of the appropriate number of LVs is necessary to minimize the risk of under- and overfitting, defined as the risk that the model is either too simple (underfit) or too complex and tailored specifically to the calibration data (overfit), leading to poor generalization performance on new data (Stafford et al., 2020). Finally, the performance of the chosen model is evaluated on the independent validation/test set using appropriate metrics.

1.4.4 Data Augmentation

To enhance the performance of a model and identify relationships within the data, large datasets are often necessary. However, acquiring sufficient experimental data can be challenging due to economic and temporal limitations. Data augmentation (DA) is a technique that artificially increases the size of a dataset using mathematical operations, without requiring additional experimental measurements. DA techniques aim to create realistic variations of the existing data (Georgouli et al., 2018). The application of data augmentation has been widely adopted in a variety of sectors, including image processing, video analysis, biometric analysis, text analysis, and food science. The use of DA aims to enhance model accuracy, robustness, and generalization ability. DA techniques have been demonstrated to be particularly effective for spectral data analysis, which is commonly characterized by high collinearity between variables and frequently suffers from limited sample sizes. Data augmentation increases the number of samples in the calibration dataset by applying transformations that simulate plausible variations in the data (Bjerrum et al., 2017). These transformations can simulate potential changes that might occur in the spectra due to instrumental fluctuations or variations in

measurement conditions (e.g., minor temperature shifts during acquisition, slight changes in optical path length, etc.). In addition, DA can consist in transformations that introduce controlled noise or modifications into the spectral data, such as the addition of additive noise, multiplicative effects, baseline slope variations, and spectral offsets (Sáiz-Abajo et al., 2005). This approach generates new calibration samples with the objective of minimizing the risk of overfitting and enhancing the predictive performance of the models. In a recent study (Xue et al., 2023), DA has been demonstrated to enhance the robustness of models by reducing their sensitivity to specific noise patterns or artifacts present in the original calibration data, leading to the development of predictors that are more likely to generalize well to new data.

Data augmentation is considered essential when training complex models like neural networks (NNs), but several applications have also been studied in the context of classical methods like PLS (Mevik et al., 2004; Sáiz-Abajo et al., 2005). In NNs, DA helps prevent overfitting by exposing the network to a wider range of input variations during training. This allows the network to learn more robust and generalizable features, promoting better predictive performance on independent datasets.

1.4.5 Deep Chemometrics

The more recent techniques in modern chemometrics utilize artificial intelligence (AI) for data processing and model development. Artificial intelligence involves the study and development of machines and software that can reason, learn, acquire knowledge, communicate, manipulate objects, and perceive their environment (Pannu & Student, 2008). Some of the AI techniques used for chemometrics include neural networks. NNs are inspired by the structure of neurons in the human brain: each neuron is connected to other neurons, and information is transferred only when a signal threshold is crossed (McCulloch & Pitts, 1943).

Machine learning (ML) is a subset of AI that focuses on developing algorithms that enable computers to learn from data and perform specific tasks, such as classification and regression, without being explicitly programmed for each case (Stafford et al., 2020). In ML, algorithms are trained to recognize patterns in data, based on the instructions provided by the developers. The knowledge acquired during training allows the algorithm to make predictions or decisions on new instances without the need of a retraining (Krohling & Krohling, 2023;Taye, 2023). Human effort is limited to the design of the training system and to the data handling, but it remains important for the success of the ML algorithm. Machine learning can be supervised or unsupervised, depending on whether the training dataset includes labeled outputs or not (Almalaq & Edwards, 2017). The supervised approach enables the extraction, learning, and correlation of features with properties of interest (Mishra & Passos, 2021). Unsupervised

machine learning does not involve the labeling of features. In these cases, the technique is employed to learn information about the structure or patterns within the dataset.

Deep learning (DL), a subset of ML characterized by NNs with multiple layers (deep architectures), finds increasing application in the extraction of chemical information from data and the development of prediction models. The branch of chemometrics that utilizes deep learning is sometimes referred to as deep chemometrics. Deep learning models usually excel when operating on large, complex datasets and require high computing power for training. Indeed, the integration of DL in chemometrics was accelerated by technological advancements, particularly the availability of graphics processing units (GPUs), which can perform the parallel computations required by neural networks much more efficiently than traditional CPUs (Taye, 2023). The application of deep learning in the scientific domain has been observed in various fields, including computer vision, spectral modeling, hyperspectral image processing, data clustering, molecular generative modeling, soft sensor modeling, and peak identification in chromatographic data (Mishra & Passos, 2021). The employment of deep neural networks (DNNs) involves the use of multiple interconnected layers (i.e., deep layers), and a self-learning process that enables the correction of errors, the adaptation to novel circumstances, and the performance of new solutions. Notably, the advantage of DNNs over traditional ML techniques is their ability to automatically learn relevant features from raw data, reducing the need for feature engineering by humans. Specifically, the depth of the network enables the initial layers to process the input data or learn simple features; their output is then fed to subsequent deeper layers, which are responsible for learning complex features. The basic unit within a network is conceptualized as an artificial neuron, node or perceptron. Input signals arrive at the artificial neuron, are processed, and an output signal is generated. This transformation typically involves an activation function (e.g., Rectified Linear Unit, Linear, Sigmoid, Tanh, SoftMax) (Taye, 2023), which introduces non-linearity, allowing the model to potentially discover complex patterns. Each input connection to a neuron has an associated weight, which is used to determine the influence of the input on the neuron's output. A single neuron cannot process multiple inputs but processes multiple weighted inputs to produce a single output.

There are different types of neural network architectures, each differing in its structure and suited for different tasks or data types. The simplest structure refers to artificial neural networks (ANNs) and includes at least three layers, each with one or more neurons (Krohling & Krohling, 2023): an input layer, one or more hidden layers, and an output layer. In general, the input layer receives the raw or preprocessed data features, while the output layer is the final layer and uses an activation function selected for a specific task (Acquarelli et al., 2017). The hidden layers

are located between the input and output layers and perform the intermediate computations. While conceptually similar, DNNs differ from ANNs due to a significantly larger number of hidden layers, neurons and parameters. The greater depth of the network number of layers increases its complexity, providing a higher level of abstraction that can detect more complex features in the input data and improve performance (Pan et al., 2022; Taye, 2023). There are several types of DNNs (e.g., Recurrent Neural Network, Autoencoder, Long Short-Term Memory networks, Restricted Boltzman Machines), but Convolutional Neural Networks (CNNs) are among those most commonly applied to one-dimensional (1D) spectral data (Almalaq & Edwards, 2017). Convolutional layers automatically perform hierarchical feature extraction, reducing the need for manual preprocessing of the data (Krohling & Krohling, 2023; Mishra & Passos, 2021), which is common in classical chemometrics. Training NNs, including traditional ANNs, for spectrophotometric data analysis can present challenges related to selecting the optimal network structure and tuning hyperparameters. Without careful design and validation, these factors can increase the risk of overfitting. CNNs help mitigate these issues, partly through parameter sharing in convolutional layers and by exploiting the local structure typical of spectral data (e.g., correlations between adjacent wavelengths). The structure of CNNs, along with regularization techniques employed during training, contributes to their ability to learn robust and abstract features, and propagate them through the deeper layers (Albawi et al., 2017; Pan et al., 2022). The fundamental distinction between CNNs and standard fully connected ANNs can be attributed to the incorporation of convolution and pooling layers (Krohling & Krohling, 2023). Typically, CNN architecture alternates convolution layers with pooling layers. Specifically, convolution layers are employed for feature extraction. They apply filters (kernels) to the input data (e.g., spectrum) using convolution operation to detect local patterns (Krohling & Krohling, 2023). Subsequently, an activation function is typically applied to introduce nonlinearity into the model. The Rectified Linear Unit (ReLU) is a popular activation function in CNNs due also to its capability in reducing the dimensionality of data while preserving the essential information. The presence of pooling layers then reduces the dimensionality of features, thereby minimizing information redundancy and preserving the principal features (Krohling & Krohling, 2023; Wu et al., 2022). The most common types of pooling are Max Pooling and Average Pooling. Max Pooling selects the maximum value within a local region (capturing the strongest presence of a feature), while Average Pooling calculates the average value (providing a more smoother representation) (Swapna et al., 2020). The output features from the final convolution/pooling blocks are usually fed into fully connected layers where high-level combinations of features are learned and used for the final classification or

regression output. Usually, the fully connected layers consist of a multilayer perceptron (MLP) that is a feed-forward network in which information is propagated in one direction, from input to output.

During training, the entire CNN, including the MLP section, aims to minimize a loss function, which computes the difference between the predicted output and the true output. The network typically uses the backpropagation algorithm (Krohling & Krohling, 2023; Taye, 2023), whereby the loss function is computed and propagated backwards through the network to adjust the weights. Through this iterative process, the network's weights are adapted to the desired outputs. In addition, it is also possible to regularize weights to avoid overfitting. One common technique is Dropout, which randomly sets to zero a fraction of neuron outputs during each training iteration. This allows the network to learn more robust and distributed representations, enhancing its generalization ability (Nallan Chakravartula et al., 2022). The specific architecture, choice of activation functions, pooling methods, regularization techniques, and training parameters represent just a few of the many design choices to create a deep neural network.

In conclusion, CNNs offer a powerful approach for chemometric analysis of spectra. Their architecture allows for automatic feature extraction, identification of relevant spectral patterns, and, when combined with appropriate training and regularization strategies, this architecture can help reduce overfitting and improve generalization compared to some traditional methods or simpler network architectures. As in classical chemometrics, the development of ML and DL models requires the splitting of the dataset in calibration and prediction sets.

An increasing number of studies are combining deep learning with spectroscopic techniques to assess olive oil quality characteristics. In particular, several research projects have focused on detecting possible contaminants or adulterants in the oil. Such contamination or adulteration may occur accidentally during transport, storage, and production (Mendes et al., 2024; Wu, Du, et al., 2024; Zhu et al., 2025) or due to fraudulent practices (Liang et al., 2025; Wu et al., 2022; Wu, Zhang, et al., 2024; Zeng et al., 2023). The application of DL in the olive oil sector also concerns the study of its physicochemical composition and monitoring reactions that affect product quality (Michelucci & Venturini, 2024).

1.4.6 Predictive model performance evaluation

Models developed with classical or deep chemometrics are evaluated with commonly used performance metrics (Nicolai et al., 2007) such as root mean square error (RMSE), BIAS, and coefficient of determination (R^2). The overall prediction error is often assessed using RMSE, which expresses the discrepancy between the measured values and the values predicted by the

model (eq. 1). Because RMSE squares the errors before averaging, outliers (if present) can significantly affect this metric and thus the apparent performance of the model. Their identification and potential removal is often considered to obtain a more robust model evaluation. The Bias represents the systematic error of the model, indicating its average tendency to over- or under-predict the reference values (eq. 2). A low Bias suggests that, on average, the predictions are centered around the true values. The coefficient of determination (R^2) is a common metric for evaluating regression model fit. R^2 measures the proportion of the variance in the measured values (y) that is explained by the model (eq.3). Values closer to 1 indicate a better fit, meaning the model explains a larger portion of variance.

$$RMSE = \sqrt{\frac{\sum_{i=1}^m (\hat{y}_i - y_i)^2}{m}} \quad (\text{eq. 1})$$

$$BIAS = \frac{\sum_{i=1}^n (\hat{y}_i - y_i)}{m} \quad (\text{eq. 2})$$

$$R^2 = 1 - \frac{\sum_{i=1}^m (\hat{y}_i - y_i)^2}{\sum_{i=1}^m (y_i - \bar{y})^2} \quad (\text{eq. 3})$$

m is the number of observations, y_i the measured values, $\bar{y}$ the mean value, $\hat{y}_i$ the values predicted by the model.

1.5 RESEARCH MOTIVATION

This study evaluated the implementation of FT-NIR and Vis spectroscopy, in conjunction with chemometric algorithms, to predict quantitative and qualitative aspects of olive oil. The application of spectroscopy in the olive oil sector has been widely studied because it is a rapid, economical, and environmentally friendly technique compared to traditional analysis.

The extraction of information from spectral data and the development of predictive and classification models using classical chemometric techniques have presented problems mainly related to feature engineering. Indeed, the successful extraction and processing of spectral data to obtain the final result have depended on the expertise of specialists in this field. Their role was also crucial in the removal of spectral distortions caused by the physical state of the sample and the environmental conditions in which the analysis was carried out. Data preprocessing, as well as the selection of latent variables in techniques such as Partial Least Squares (PLS), represented a critical step where the operator's subjective choices risked introducing systematic errors and removing relevant information in case of erroneous evaluations.

The objective of this thesis was to compare classical and deep chemometrics algorithms by assigning the feature engineering phase to neural networks, thereby excluding human intervention. In particular, the thesis focused on Convolutional Neural Networks (CNNs), due to the increasing number of studies and applications in the food sector owing to their promising

performance in the processing of raw spectral data. The use of different levels of neural networks and the high computing power of GPUs made it possible to develop qualitative and quantitative predictive models for olive oil using deep chemometrics. These models were then compared with those obtained using classical chemometrics, specifically PLS.

1.6 RESEARCH OUTLINE

This research investigated and compared the effectiveness of deep chemometrics, specifically Artificial Neural Networks (ANNs) and Convolutional Neural Networks (CNNs, against classical chemometrics, i.e. Partial Least Squares (PLS) regression, when combined with FT-NIR (1 000-2 500 nm) and Vis-NIR (380-900 nm) spectroscopy for the qualitative and quantitative analysis of olive oil characteristics. The spectral data obtained were processed using these classical and deep chemometric approaches to develop predictive models.

The activity comprised three studies focused on olive oil quality and composition, primarily Extra Virgin Olive Oil (EVOO):

- i) monitor changes in EVOO during eight months of storage under different conditions (shelf-life assessment);
- ii) quantify key chemical parameters in olive oil samples provided by a refinery;
- iii) determine the percentage of adulteration using common seed oils (peanut, sunflower, corn, and soybean) in EVOO.

The results obtained are being disseminated within the scientific community through peer-reviewed articles submitted to journals indexed in major databases such as Scopus and Web of Science.

Finally, the work also included a literature review, performed during the first year of doctorate, examining the effect of process parameters on oil quality, with a particular focus on the impact of the malaxation stage on the physicochemical and organoleptic characteristics of EVOO.

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EVOO PROCESS AND QUALITY 2

FOOD CHEMISTRY ADVANCES

IMPACT OF TRADITIONAL AND INNOVATIVE MALAXATION TECHNIQUES AND TECHNOLOGIES ON NUTRITIONAL AND SENSORY QUALITY OF VIRGIN OLIVE OIL — A REVIEW

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Keywords: VOO; phenolic compounds; volatile compounds; malaxation temperature; pulsed electric field; ultrasound.

Abstract

Virgin olive oil (VOO) nutritional and sensory quality depends on several aspects, including mechanical extraction technology: in this regard, malaxation conditions play a pivotal role since the major transformations linked to phenolic compounds (PC) and volatile compounds (VC) development and degradation take place during this step. Most of the reported studies state that malaxation should be carried out at temperature ranging 18-27 °C for 30-40 min: this allows PC solubilization in the oil phase, the production of secoiridoid derivatives, and the development of aroma-active aldehydes, alcohols, and esters, limiting both PC losses

and the generation of undesired VC. Reducing oxygen concentration in the headspace up to 5 % helps to preserve the phenolic fraction but slightly impairs VC generation. The use of pectolytic enzyme preparations and the application of innovative technologies like pulsed electric fields and ultrasound promote cell damage, and thereby the release of enzymes and micro-components. This review prioritizes the importance of further research aimed at defining specific optimization charts for each olive cultivar and maturity level, due to the relevant impact of genetics and agronomical factors. Furthermore, kneading conditions may be defined also depending on the desired quality of VOO, e.g., antioxidant power and/or bitterness level.

List of acronyms:

VOO: Virgin Olive Oil	POD: Peroxidases
PC: Phenolic compounds	LOX: Lipoxygenases
VC: Volatile compounds	HPL: Hydroperoxide lyases
OP: Olive paste	ADH: alcohol dehydrogenases
PEF: Pulsed electric fields	TPC: Total phenolic content
US: Ultrasound	MT: Malaxation temperature
β -glu: β -glucosidases	Mt: Malaxation time
PPO: Polyphenol oxidases	CC: Calcium carbonate

INTRODUCTION

Global olive oil production has tripled in the last 60 years: estimates for the 2021/22 crop year put production at 3 098 500 tons, as reported by the International Olive Council. Countries belonging to the Mediterranean region are characterized by a considerable use of virgin olive oil (VOO) and have become the largest consumers and producers: Spain, Italy, Tunisia, and Greece cover about 67 % of the global production (Olive Oil Council, 2022) The nutritional and biological activities as well as the unique flavor of VOO are attributed to its concentration of minor compounds, chiefly phenolic and volatile compounds (PC and VC, respectively) (Clodoveo et al., 2014; G. Fregapane & Salvador, 2013).

It is well known that PC show outstanding biological properties (Parkinson & Cicerale, 2016; Servili et al., 2014), playing a major role in the protection against cardiovascular (Ditano-Vázquez et al., 2019; Hohmann et al., 2015) and neurodegenerative (Rodríguez-Morató et al., 2015) diseases, as well as exhibiting antioxidant (Oliveras-López et al., 2014), anti-inflammatory (de Souza et al., 2017), and anti-proliferative (De Stefanis et al., 2019) activities. They are also found to be involved in the prevention and management of type-2 diabetes (Santangelo et al., 2016), postmenopausal osteoporosis (Keiler et al., 2014), and metabolic

syndrome (Bulotta et al., 2014). In the light of this, a health claim on olive oil polyphenols was approved by the European Food Safety Authority through Commission Regulation (EU) 432/2012: “VOO polyphenols contribute to the protection of blood lipids from oxidative stress”. The claim may be used only for VOOs, containing at least 5 mg of hydroxytyrosol and its derivatives per 20 g of product (López-Huertas et al., 2021).

Most importantly, PC were found to confer the characteristic bitter and pungent notes to VOO (Dierkes et al., 2012; Vulcano et al., 2015) and, together with a high monounsaturated-to-polyunsaturated fatty acid ratio, significantly contribute to the stability towards lipid oxidation (Aparicio & Harwood, 2013). Whereas the characteristic aroma of VOO is mainly due to low molecular weight VC that contribute to balanced flavor by providing green and fruity notes (da Silva et al., 2012). Moreover, these compounds, mainly aldehydes and alcohols, but also esters, ketones, and others, are responsible for the stimulation of the olfactory epithelium and specific receptors during olive oil tasting. This aspect of VC within an evaluated VOO corroborates with the wide variety of sensations perceived by the consumer, and thereby the sensory quality and acceptability (Campestre et al., 2017). The negative sensory notes that are generally related to lipid oxidation or exogenous enzyme activity are also linked to VC composition (da Silva et al., 2012; Kalua et al., 2007).

The concentration of PC and VC in a VOO is heavily influenced by a broad variety of agronomical and technological factors (Angerosa et al., 2004; da Silva et al., 2012; Gmez-Romero et al., 2012; Servili et al., 2004, 2014). Specifically, among agronomical aspects the most relevant ones are olive fruit maturity stage (Cherfaoui et al., 2018; Deiana et al., 2019; El Riachy et al., 2011; Romero et al., 2016) and harvesting method (Famiani et al., 2020), while examining the technological factors the importance of fruit storage duration prior to transformation (Famiani et al., 2020), malaxation conditions (Angerosa et al., 2001; Diamantakos et al., 2020) and eventual oil filtration (Brkić Bubola et al., 2012; Lozano-Sánchez et al., 2010) should be emphasized. In addition, genetics (i.e., cultivar) (Cherfaoui et al., 2018; Deiana et al., 2019; Miho et al., 2021; Pouliarekou et al., 2011; Romero et al., 2016), geographical origin (El Riachy et al., 2011; Pouliarekou et al., 2011; Romero et al., 2016), water availability (El Riachy et al., 2011) and, in general, interannual variability (Miho et al., 2021) play a major role in defining the VOO’s phenolic and volatile profiles.

Within the olive oil mechanical extraction process it was demonstrated that olive crushing and, in particular, olive paste (OP) malaxation are the most critical steps concerning PC and VC development, transformation and degradation (Aparicio & Harwood, 2013). During the malaxation process the OP is continuously kneaded at low speed (typically 20–30 rpm) for

variable times (generally 30–45 min) (Clodoveo, 2012). This step takes place in the malaxer, a semi-cylindrical tank equipped with a shaft, rotating arms, blades and a heating jacket for temperature regulation (Figure 1). Depending on the rotation axis, malaxers are classified as horizontal, more commonly used, and vertical types (Clodoveo, 2012). Nevertheless, innovative plants have also been described in the literature, with design modifications aimed at improving oil yield and quality (Difonzo et al., 2021; Leone et al., 2014). Particularly, the adoption of a low-speed mixing is favored in case of metal hammer crushing as it promotes the formation of more stable emulsions (Boskou, 2006).

The malaxation process principally allows for the breakup of the aforementioned emulsions in order to ensure contact among oil droplets so that they can merge into larger drops (Figure 1), thus facilitating oil separation and enhancing oil yield (Aparicio & Harwood, 2013; Boskou, 2006; Clodoveo, 2012; Kalogianni, Georgiou, & Hasanov, 2019; Tamborrino, 2014). The percentage of drops with a diameter higher than 30 microns, which corresponds to the minimum size to achieve an effective separation, increases from 45 % after crushing to around 80 % after kneading (Boskou, 2006). In addition, slow stirring of OP promotes the disruption of uncrushed cells (Aparicio & Harwood, 2013; Clodoveo, 2012) leading to further release of oil and oil-soluble components that are made available for further chemical and biochemical transformation (Aparicio & Harwood, 2013). Malaxation should not be intended just as a mechanical operation but rather as a series of complex physical, physico-chemical, chemical, and biochemical modifications that profoundly affect the VOO chemical composition and, as a result, its nutritional and sensory quality. Among these phenomena, the most relevant ones are oil droplets coalescence, rheological changes, diffusion, heat exchange, chemical oxidation, and triggering of endogenous enzymes (Clodoveo et al., 2014; Kalogianni, Georgiou, & Exarhopoulos, 2019; Milos, 2017; Tamborrino, 2014).

To the best of our knowledge there are a few reviews concerning VOO processing relevance to its nutritional and sensory quality (Clodoveo et al., 2014; Frankel et al., 2013) and about the impact of malaxation parameters on VOO yield and overall quality (Clodoveo, 2012), but there are no reviews examining the impact of traditional malaxation parameters and innovative malaxation technologies on VOO sensory and nutritional aspects. Therefore, the objective of this review is to summarize the main findings regarding the selection of proper malaxation parameters and technologies to obtain VOOs with tailored nutritional and sensory properties, i.e., PC and VC qualitative and quantitative composition. Data on the impact of malaxation conditions on VOO quality were collected from studies published in the 2016-2022 period,

whereas all existing literature was used as a complementary source of knowledge. Among the examined scientific papers, the most relevant ones on the issue of this review were included.

The review is organized in three sections: the first two sections regard the main mechanisms and parameters linked to traditional malaxation, while the third one introduces possible innovations aimed at improving process efficiency and VOO quality. In particular, [i] the first section concerns the mechanisms involved in PC and VC development, transformation and degradation during malaxation, as affected by endogenous enzymes activity, since it is vital to understand the processes involved in such phenomena prior to investigate malaxation conditions; [ii] the second one examines the main critical parameters to modulate in a traditional malaxation operation, i.e. temperature, time and oxygen concentration in the headspace; [iii] the third section concerns the possibility to assist malaxation by physical adjuvants addition or by combining it with non-thermal technologies, such as pulsed electric field (PEF) and ultrasound (US).

I: PC AND VC OCCURRENCE, DEVELOPMENT, AND TRANSFORMATION DURING MALAXATION

PC and VC composition in VOO is strongly influenced by the different phenomena taking place during malaxation, such as micro-components diffusion, chemical oxidation, and enzymatic activity as introduced previously (Clodoveo et al., 2014; Frankel et al., 2013; Milos, 2017). In this section, these phenomena will be discussed with particular attention to action of endogenous enzymes to understand whether it is possible to promote or hinder specific transformations that would result in different qualitative and quantitative PC and VC compositions.

Phenolic compounds

Phenolic compounds (PC) exert various physiological roles in the olive fruit like protection against pathogens, ultraviolet rays, and majorly contribute to plant growth as well as to the VOO's nutritional and sensory profiles (Figure 2). Olive PC are grouped into four main classes: phenolic acids, phenolic alcohols, flavonoids and secoiridoids of which the most representative were found to be caffeic acid, vanillic acid (phenolic acids), tyrosol, hydroxytyrosol (phenolic alcohols), luteolin-7-glucoside, rutin (flavonoids and derivatives), oleuropein and ligstroside (secoiridoids) (Milos, 2017; Yorulmaz et al., 2012). Other relevant PC groups are lignans ((+)-pinoresinol, 1-acetoxypinoresinol), hydroxy-isochromans, and phenylpropanoids (verbascoside and its hydroxylated derivatives like β -OH acteoside isomers) (Bianco et al., 2002; López-Biedma et al., 2016; Xie et al., 2021). PC composition is significantly affected by agronomical factors like ripening index as well as their partitioning during processing (Artajo et al., 2006).

Furthermore, some of these compounds undergo complex modifications during OP malaxation due to enzymes release and activation (Gmez-Romero et al., 2012). Among VOO PC groups, secoiridoids are the most representative and studied since their derivatives have paramount involvement in both biological and sensorial properties of VOOs (Lozano-Castellón et al., 2020; Mateos et al., 2004). Regarding the sensory perception, secoiridoids show high intensity of bitterness attribute, while other compounds like flavones, do not significantly affect VOO bitterness (Mateos et al., 2004). An interesting research paper confirmed that oleacein and oleocanthal strongly activate the two most relevant TAS2Rs receptors, leading to VOO bitter perception (Cui et al., 2021). Unlike other PC classes, secoiridoids are characteristic of the fruit of plants belonging to Oleaceae family with the two most representative compounds being oleuropein and ligstroside that are esters of elenolic acid with hydroxytyrosol and tyrosol, respectively (Gmez-Romero et al., 2012).

The phenolic fraction undergoes different phenomena during OP malaxation of which PC solubilization and transfer between phases, hydrolysis, and oxidation due to endogenous enzymes action are the notable ones (Artajo et al., 2006; García-Rodríguez et al., 2011; Servili et al., 2004). Specifically, PC partitioning occurs after crushing when the oil droplets are released from the fruit making OP a complex multiphasic system eventually resulting in phase separation of PC depending on their relative polarities (Artajo et al., 2006). Moreover, the release of endogenous β -glucosidases (β -glu) showing optimal activity at 45 °C (Romero-Segura et al., 2009) triggers the biochemical transformation of the secoiridoid phenolic fraction to derivatives of oleuropein and ligstroside as a result of enzymatic hydrolysis, leading to the cleavage of the glycosidic bond and the formation of oleuropein and ligstroside aglyconic forms. Since elenolic acid undergoes keto-enolic tautomerism, involving ring opening, several isomers of these aglycons can be formed (Kanakakis et al., 2013). The most representative VOO secoiridoid derivatives are 3,4-dihydroxyphenyl-ethanol linked to dialdehydic form of elenolic acid (3,4-DHPEA-EDA), p-hydroxyphenyl-ethanol linked to dialdehydic form of elenolic acid (p-HPEA-EDA), known as oleacein and oleocanthal, respectively, and p-hydroxyphenyl-ethanol linked to elenolic acid (3,4-DHPEA-EA), an isomeric form of oleuropein aglycon (Artajo et al., 2006; García-Rodríguez et al., 2011; Servili et al., 2004). Oleacein and oleocanthal are the most studied VOO secoiridoids, due to their primary contribution to health properties and bitterness (Lozano-Castellón et al., 2020; Mateos et al., 2004). However, also other glycosilated secoiridoids are present at a significant concentration in VOOs, i.e., demethyloleuropein, formed from the activity of endogenous esterases on oleuropein, nuzhenide, present at high concentrations in olive seeds but also, at lower concentration, in OP,

where it is degraded during malaxation, and its derivative, nuzhenide 11-methyl-oleoside (Kanakakis et al., 2013). On the other hand, the activity of some enzymes like polyphenol oxidases (PPO) and peroxidases (POD) is implicated in PC losses during olive oil mechanical extraction. In particular, PPO (EC 1.10.3.1) are the main class of enzymes catalyzing phenols oxidation which includes two different reactions: hydroxylation of monophenols to orthodiphenols, and their subsequent oxidation to quinones (García-Rodríguez et al., 2011). Phenol oxidation by POD (EC 1.11.1.7) produces biphenols, which rapidly undergo polymerization (Rocha-Martin et al., 2014). The activity of both PPO and POD enzymes might be modulated by processing parameters (e.g., temperature and atmosphere composition) to promote the formation of secoiridoid derivatives and to minimize PC losses during malaxation. However, some discrepancies are reported in literature about the optimal conditions for the activity of these enzymes in terms of temperature and pH, depending on the evaluated olive variety (Taticchi et al., 2013). Another factor responsible for the reduction of phenolic content is PC complexation with polysaccharides during malaxation (Servili et al., 2004; Vierhuis et al., 2001).

Volatile compounds

The volatile compounds (VC) in VOO are characterized by low molecular weight, high volatility, limited hydro solubility, and ability to interact with specific gustative and olfactive receptors (Angerosa, 2002). The approx. 280 VC isolated from VOO belong to a plethora of chemical classes including aldehydes, the most relevant fraction responsible for green aroma notes; alcohols; ketones; esters, accounting for VOO sweet and fruity notes; ethers; saturated and unsaturated hydrocarbons; and carboxylic acids; as well as minor compounds such as thiophene derivatives, which positively affect VOO aroma even at a low concentration (Boskou, 2006; Cecchi et al., 2021). Only 67 of these compounds are usually present at concentrations higher than their odor threshold, thus affecting VOO aroma (Boskou, 2006). Table 1 resumes the main VC that determines VOO typical sensory notes. Most of these compounds are intermediates/products of the lipoxygenase (LOX) pathway, a complex series of reactions catalyzed by specific enzymes released during olive fruit crushing and activated by the subsequent malaxation step. In details, after fruit milling, triglycerides and phospholipids are hydrolyzed by acyl hydrolase, making fatty acids available for enzymatic transformation (da Silva et al., 2012). Lipoxygenases (LOX) mediate the oxygenation of linoleic and linolenic acids to produce their 9- and 13-hydroperoxides in varying proportions, depending on fruit fatty acid composition and enzyme affinity for substrates, which also varies depending on localization of the enzyme in tissues (Angerosa et al., 1999; Clodoveo et al., 2014). Only 13-hydroperoxides are further transformed through a series of enzymatic reactions wherein

hydroperoxide lyases (HPL) promote their cleavage, yielding C₆ aldehydes; unsaturated aldehydes might undergo isomerization to the more stable *trans*-2 form; alcohol dehydrogenases (ADH) reduce aldehydes to alcohols and eventually C₆ esters are produced because of alcohol acetyl transferases activity (Angerosa et al., 2004). The most relevant compounds produced through the LOX pathway are hexanal, which enhances sweet and banana notes; hexan-1-ol, giving rise to tomato flavor; *trans*-2-hexenal, promoting apple and lawn aroma; and *trans*-2-hexen-1-ol, positively linked to flower nuances (Angerosa et al., 1999, 2000, 2001). The formation of C₅ VC is attributed to an additional branch of the LOX pathway: after the hydroperoxides formation, LOX would also catalyze their cleavage giving rise to the generation of 1,3-pentene radicals, which would undergo non-enzymatic homolytic β -scission to form a 1,3-pentene allylic radical that is chemically dimerized yielding C₁₀ hydrocarbons (known as pentene dimers) or react with a hydroxyl radical to produce C₅ alcohols. Pentene dimers are positively correlated with VOO bitterness and pungency (Cerezo et al., 2021; Hachicha Hbaieb et al., 2016).

On the contrary, other pathways are implicated in the development of unpleasant VC in VOO, namely: fatty acid metabolism, which yields linear alcohols, acids, esters, and ketones; aminoacids conversion to C₄ and C₅ branched aldehydes, alcohols, and acids (fusty defect); and sugar fermentation in acetic acid, ethyl acetate and ethyl alcohol (winey-vinegary defect) (Angerosa, 2002). The aminoacid conversion pathway may also be activated due to the selection of unsuitable malaxation parameters, i.e., longer process and/or higher temperature than optimal, leading to increased 2- and 3-methyl butanal, which account for the typical sensory heated-oil notes (Angerosa, 2002). Such malaxation parameters might also trigger fatty acid oxidation. During the primary oxidation, lipid hydroperoxides are produced: they do not affect the sensory properties of VOO but, being particularly reactive species, they quickly react with other low molecular weight molecules to generate secondary products (aldehydes, ketones, alkanes, alkenes) that are responsible for the rancid defect, which is the most common in VOO. Some unsaturated C₇-C₁₂ aldehydes (2, 4-heptadienal, *trans*-2-nonenal and *trans*-2-octenal) are of main importance in this regard (Cecchi et al., 2021; Garcia-Oliveira et al., 2021).

Concluding remarks

In order to produce VOOs with superior nutritional and sensory quality, it is crucial to extensively clarify both beneficial and detrimental enzymes localization in olive tissues, their affinity for substrates, and optimal environmental conditions for catalysis, as well as the opportunity to modulate their activities through the addition of adjuvants and/or the adoption of novel technologies. Optimal processing parameters should be defined for each cultivar and

maturity stage since these are particularly significant factors for the enzymatic activities related with PC and VC development, transformation, and degradation (Kalua et al., 2005).

II: CRITICAL MALAXATION PARAMETERS

As already mentioned, malaxation parameters are crucial to VOO nutritional and sensory quality and therefore should be properly selected through tailored optimization charts. It is worth noting that the transformations taking place during malaxation depend on several variables, including genetic and agronomical aspects. Accordingly, it is tough to compare the results obtained in distinct studies, since not only parameters strictly related with malaxation conditions could account for the observed discrepancies in VOOs PC and VC content and composition. However, we will try to summarize the most common observations found in the literature, as well as to analyze eventual conflicting outcomes.

Impact of malaxation temperature and duration on phenolic profile

Malaxation time-temperature relationship is crucial for the development of a high-quality VOO, fostering enzymatic activity, with particular attention to the action of β -glu and esterases that promote oleuropein and ligstroside hydrolysis to produce their aglyconic forms, as well as oleacein and oleocanthal. Diamantakos et al. (2020) proposed a mechanism through which such derivatives are produced from oleuropein and ligstroside during malaxation, via the production of intermediate products, i.e., oleokoronal and oleomissional. On the other hand, phenolics oxidation catalyzed by PPO and POD might also be triggered (Miho et al., 2020).

The majority of studies demonstrate that increasing malaxation temperature (MT) tends to enhance VOO phenolic content (de Torres et al., 2016; Diamantakos et al., 2020; Jolayemi et al., 2016; Veneziani et al., 2021) with only one study reporting a reduction in total phenolic content (TPC) (Marx et al., 2021) and another paper stressing the existence of a positive or negative relationship with temperature, depending on the considered temperature range (Zanoni et al., 2018). However, not all phenolics are equally affected by temperature wherein a reduction in phenolic acids and flavonoids was noted alongside an increase in TPC and secoiridoid derivatives by rising the malaxation temperature from 22 to 30 °C (Lukić et al., 2017). This might have been probably due to the different thermal stability of phenols and the release of low-molecular-weight compounds because of β -glu hydrolytic activity. De Torres et al. (2016) found MT to be more important than malaxation time (Mt), irrigation and ripening stage in determining VOO phenolic content. Notably, they highlighted a significant increase in both derivatives of oleuropein (3,4 DHPEA-EA and oleacein) and ligstroside (p-HPEA-EA and oleocanthal) at 60 °C, probably due to thermal inactivation of PPO, even though such aspect

was found to be a particularly genetics-related feature (Lukić et al., 2017). Nevertheless, the authors did not study the impact of MT on lipid oxidation and VC development and losses. More feasible temperatures were tested by Veneziani et al. (2021) who exploited the rise in MT from 18 °C to 25 °C for the whole operation, and for the last step of malaxation as well. Increasing MT to 25 °C for the last 15 min of malaxation was enough to observe a significant increase in secoiridoid derivatives for the three tested cultivars (Canino, Peranzana and Moraiolo). Authors concluded that the impact of higher temperatures on PC solubilization and partitioning was more relevant compared to the strengthening of PPO activity, as observed in a cited study (Taticchi et al., 2013). Diamantakos et al. (2020) underlined that an increase in MT from 22 to 28 °C and from 22 to 32 °C determines a rise in TPC that is evident only in VOOs from late harvesting olives. Indeed, ripening degree is a paramount factor, strongly affecting the activity of PPO, POD and β -glu enzymes (Hachicha Hbaieb et al., 2015). In contrast, Marx et al. (2021) observed a reduction of TPC at increasing MT from 22 to 28 °C and from 22 to 34 °C. In this case, the positive impact of mass transfer improvement at increasing MT was lower in respect to the oxidative action of PPO and POD. This result was supported by the finding of a previous study (Peres et al., 2016), which highlighted that the studied cultivar is characterized by a higher PPO activity. Zanoni et al. (2018) performed malaxation at different temperature-time combinations and noticed that oleacein content followed an unusual trend, with an increase in the range of 22-27 °C, a decrement at 27-36 °C and another rise at higher temperatures. The result observed corroborated with the involvement of multiple mechanisms: a descending trend probably due to enzymatic oxidations and an ascending trend probably due to improved physical and enzymatic release of PC from cellular tissues (Trapani et al., 2017). A compromise between oleacein content and oil yield was achieved both at low temperature (22 °C) for a longer time (40-60 min), as well as at high temperature (35-37 °C) for shorter times (30-40 min) (Zanoni et al., 2018). However, the information about the impact of malaxation on TPC would have been more suitable to be evaluated together with oleacein content.

Regarding the malaxation time (Mt), it is difficult to individually analyze such aspect as it is strictly related with MT (Tamborrino, 2014), but a negative effect of long treatments on TPC is extensively reported in the literature even though it is less significant than MT (de Torres et al., 2016; Diamantakos et al., 2020; Lukić et al., 2017; Miho et al., 2020; Yorulmaz et al., 2017). De Torres et al. (2016) found that oleuropein secoiridoid derivatives tend to decrease at increasing Mt, while ligstroside derivatives slightly increase probably due to the different sensitivity to PPO and POD compared to oleuropein derivatives. Moreover, ligstroside derivatives show lower polarity compared to oleuropein: thus, increasing Mt might have

improved their extractability in the oil phase. However, a negative impact of increasing Mt was highlighted in most models obtained at different maturity stages. Another study by Lukic et al. (2017) demonstrated the presence of a strong interaction between Mt and olive ripeness: only in the case of the lowest ripening degree a reduction of TPC was observed, while a TPC increase was registered with olives at higher maturity stage. Interestingly, the activity of PPO and POD tends to weaken along with olive ripening (Hachicha Hbaieb et al., 2015), thus increasing Mt tends to significantly reduce phenolic content in the case of VOO from early harvested olives, as confirmed by Diamantakos et al. (2020). Moreover, the authors investigated the behavior of individual PC, revealing some discrepancies since oleocanthal increased up to 45-60 min of malaxation, depending on both MT and ripening stage, whereas oleacein generally began decreasing after 30 min of process probably because of higher susceptibility to enzymatic oxidation due to its orthodiphenolic chemical structure (Fregapane & Salvador, 2017). Remarkably, authors also found an interaction of Mt with MT where it was possible to perform malaxation for longer times when lower MTs were selected. However, in general, the maximum TPC was registered within 15-30 min of malaxation, which could probably be suggested as adequate time for β -glu to catalyze the transformation of secoiridoids into their aglyconic forms, oleocanthal and oleacein, in view of negligible degradation. Nonetheless, also the activity of β -glu was proven to be strictly cultivar dependent and regulated by genes affected by agroclimatic conditions (Romero-Segura et al., 2012; Velázquez-Palmero et al., 2017). According to Yorulmaz et al. (2017), the positive impact given by phenols solubilization is stronger compared to the negative activity of PPO and POD that decrease during the treatment, and raises until 40 min of treatment, respectively. Miho et al. (2020) also found that the sum of oleocanthal and oleacein increases until 30 min of treatment, followed by a decrease for longer malaxation time. However, some dissimilarities were noticed between the studied varieties strengthening the hypothesis of an interaction between Mt and genetics of the olives evaluated. Additionally, climatic aspects like temperature and rainfall were found to affect olive fruit ripening process and to modulate genetic factors (Mafrica et al., 2021); in fact, Lia et al. (2019) found that olives belonging to the same cultivar but grown in different countries showed different phenolic profiles.

Impact of headspace composition on phenolic compounds

Unlike enzymes related with phenols degradation, the activity of β -glu is not dependent on headspace oxygen concentration during malaxation (Servili et al., 2008). For this reason, a higher TPC should be expected in case of low-oxygen atmosphere, which hinders the action of PPO and POD, without altering the activity of β -glu. This is of main importance, since relevant

secoiridoid derivatives such as oleocanthal and oleacein were found to be the most affected by oxidative processes (Servili et al., 2008). Literature confirms that the adoption of a low-oxygen atmosphere during malaxation helps to increase TPC and secoiridoid derivatives concentration (Iqdiam et al., 2019; Macaluso et al., 2021; Miho et al., 2020). The application of vacuum to OP kneading significantly enhanced oleuropein and ligstroside aglyconic forms content, as well as oleacein and oleocanthal by 20-23 % in respect to atmospheric conditions (Miho et al., 2020). In another work (Macaluso et al., 2021), the use of an inert gas (argon or nitrogen) during malaxation was tested. Two main phenomena were reported to occur when an inert gas is blown in the malaxer: oxygen removal and deep gas diffusion. Atmosphere modification significantly enhanced the stability of all oleuropein derivatives. Slightly better results were achieved using argon, probably due to a higher diffusivity into OP. Iqdiam et al. (2019) malaxed OP for 35 min at different oxygen concentrations (2, 5, 10, and 21 %) and the results distinctly showed a TPC increase of about 7-12 % as oxygen was reduced. However, a decrease in oil extractability index was found for oxygen concentrations lower than 5 %, which was then proposed as optimal compromise between oil yield and quality.

Impact of malaxation temperature and time on volatile compounds

The formation de novo of VC responsible for both positive and negative sensory notes of VOO is primarily connected to biochemical and chemical reactions taking place in the OP during malaxation. Since there are several enzymes involved in the development and transformation of VC, different optimal malaxation conditions might be defined depending on the sensory notes that producers want to bring out. Specifically, low MTs boost the development of aldehydes, while higher MTs promote their conversion to the corresponding alcohols (Lukić et al., 2017; Veneziani et al., 2017; Veneziani et al., 2021), which may be further transformed to esters. The application of low MT (18 °C) for the whole malaxation step resulted in an increased content of C₆ aldehydes through improved activity of HPL (Veneziani et al., 2021), which were evidenced to have a maximum action at 15 °C (Clodoveo et al., 2014). Specifically, HPL are only involved in the final step of hydroperoxides cleavage. In fact, the pathway is initiated by the LOX, which use free fatty acids released by lipases as a substrate. LOX and lipases have an optimum of temperature close to 30 °C (Lukić et al., 2017; Ridolfi et al., 2002). In contrast, an increase in temperature in the last part of malaxation led to a reduction in aldehydes and an increase in alcohols, due to the higher activity of ADH which promoted the development of green and leaf notes linked to (E)-2-hexenal, as well as the reduction of fruity and sweet notes (Veneziani et al., 2021). Similar results were presented by Lukic et al. (2017), with a maximum aldehyde (hexanal and (E)-2-hexenal) production at 22 °C, while a

temperature rise to 30 °C resulted in higher alcohols production. In this context, genetics is a crucial factor, and the described behavior was more evident in some of the cultivars tested by Veneziani et al. (2017). Differently, Lukic et al (2018) registered an opposite trend with a rise in total aldehydes at increasing temperature which was attributed to an enzymatic pattern where the activity of LOX is prevalent to that of HPL. A further study on the enzymatic activity of the selected cultivar would have been useful to confirm such theory. Other studies highlighted that both cultivar and ripening stage affect the activity of enzymes belonging to the LOX pathway, such as HPL and ADH (Kalua et al., 2007; Veneziani et al., 2021). Furthermore, the concentration of undesired VC might be increased during OP kneading (Conte et al., 2020). Lower temperatures are generally suitable in this regard as particularly proposed by Cevik et al. (2016): a MT of 28 °C was defined as the best compromise considering the pathways involved in the development of both favorable and detrimental VC.

In contrast, the relevance of Mt to VC production seems to be much lower than MT (Lukić et al., 2017, 2018; Yorulmaz et al., 2017). According to Cevik et al. (2016) Mt had no statistically significant effects on most aldehydes, whereas increasing kneading duration up to 60-80 min was beneficial for the production of alcohols (mainly 1-hexanol), as in fact this conversion is a subsequent step that takes place once C₅ and C₆ aldehydes are generated. In another study (Lukić et al., 2018) VOOs obtained after a malaxation period of 30 min were reported to have a better sensory profile compared to those malaxed for 60 min, despite the fact that just few VC displayed a statistically significant difference. Cevik et al. (2016) found that unpleasant VC are not significantly produced until 30 min of malaxation and suggested a malaxation time of 40 min as optimal compromise to minimize the production of undesired VC, while keeping a good production of desired ones.

Impact of headspace composition on volatile compounds

Since molecular oxygen is required as co-substrate in the LOX pathway (Raffo et al., 2015), a reduction in the concentration of VOO VC should be expected as a consequence of reduced partial oxygen pressure in the malaxer. Reducing oxygen concentration through flushing inert gas inside the chamber determined a significant decrease in the concentration of some aldehydes (hexanal and *cis*-2-hexenal) and alcohols (1-hexanol and *trans*-2-hexen-1-ol) (Raffo et al., 2015). As an alternative, a high-vacuum malaxer was tested by Taticchi et al. (2021) in which the decrease in oxygen concentration hindered the formation of VC derived from the LOX pathway with a reduction of aldehydes (12-21 %), alcohols (15-17 %), and esters (6-22 %) that was dependent on the explored cultivar. This finding was justified by the oxygen-dependency of LOX pathway enzymes and the potential stripping of VC. In addition, also the

higher concentration of PC, more protected against oxidative damages, could have contributed, since these compounds weaken the activity of LOX pathway enzymes (Salas et al., 1999). Interestingly, some VC that may generate negative sensory notes such as ethanol, ethyl acetate and acetic acid were seen to decrease even more rapidly as kneading was carried under vacuum conditions (Taticchi et al., 2021).

Concluding remarks

The activity of the enzymes involved in the transformation and degradation of PC and in the production of VC are strongly affected by malaxation parameters, i.e., temperature, duration, and headspace composition, with genetic and agronomical factors playing key roles as well. As a general recommendation, malaxation should be performed at temperature ranging 20-22 °C for a short time (30-40 min), which is the minimum to achieve the technological objective of malaxation, in order to inhibit the activity of PPO and POD and to promote the development of volatile aldehydes through the activity of HPL and LOX. However, an increase up to 25-27 °C in the last 15 min of kneading is suggested, as it is sufficient to favor PC solubilization and partitioning in the oil phase, secoiridoids derivatives production, and the partial conversion of aroma-active aldehydes to the corresponding alcohols, thus enriching VOO sensory notes. Such temperature increase would result also in an improved nutritional quality of VOO, due to a greater production of oleacein and oleocanthal, which also contribute to bitter and pungent notes so it is mainly recommended for consumers that may appreciate such notes. A decrease in oxygen concentration up to 5-10 % may be helpful: despite a slight reduction in the activity of LOX pathway enzymes reducing the final concentration of VC, it inhibits both PC degradation and lipid oxidation. A lower oxygen concentration (< 5 %) is not encouraged, due to an undesirable impairment of VC generation as well as oil extractability. These findings bring out the opportunity to adjust malaxation parameters in order to produce VOOs with tailored nutritional and sensory characteristics for each olive cultivar at varying pedoclimatic and agronomical factors: further research should be aimed at studying how these aspects affect the initial PC and VC composition and the transformations taking place during malaxation.

III: ASSISTING MALAXATION WITH PHYSICAL ADJUVANTS AND NON-THERMAL TECHNOLOGIES

As previously described, OP kneading at relatively high temperature might elevate VOO nutritional quality since oleacein and oleocanthal are produced in higher amounts, but generally determines a decrease of sensory quality due to reduced activity of LOX pathway enzymes and

the production of undesired sensory notes. For this reason, some researchers explored the opportunity to add specific adjuvants during malaxation like talc, calcium carbonate, or enzymes, and to assist mechanical extraction with non-thermal technologies like pulsed electric field (PEF) and ultrasound (US), which could allow to achieve satisfactory results with regard to VOO yield and quality without an excessive increase of MT and Mt.

Malaxation adjuvants

Malaxation adjuvants are often employed to improve VOO yield and chemical composition. Their exclusively physical action is expected to facilitate emulsion breakdown and phase separation, thus making easier the extraction of oil, and possibly micro-components (Caponio et al., 2016). The tested adjuvants are talc (Peres et al., 2017; Squeo et al., 2020; Vidal et al., 2018), calcium carbonate (Peres et al., 2017; Squeo et al., 2020; Tamborrino et al., 2017), sodium chloride (Peres et al., 2017) and enzymatic preparations (Moustakime et al., 2016; Polari & Wang, 2020). The application of the cited adjuvants is permitted by the law: the current European Union Regulation allows the use of talc powder with a maximum calcium carbonate concentration of 6 % (w/w) (Sánchez et al., 2022).

As described by Vidal et al. (2018), talc addition did not determine significant improvements of phenolic and volatile content and composition; however, some discrepancies in the behavior of individual PC were described, with a reduction of phenolic alcohols and an increase of phenolic acids, probably owing to different partition coefficients between water and oil phases. A talc concentration of 0.6 % increased the amount of VC, also reducing ethanol content. Authors also found that the talc dosage increased the amount of VOO secoiridoid derivatives when equal to 2 %, while a detrimental effect was observed at a lower concentration. Squeo et al. (2020) found that talc addition impairs the activity of oxidases responsible for PC degradation at all the tested temperatures, while LOX activity was lowered by talc, leading to a reduction of VC.

Whereas, calcium carbonate (CC) was reported to affect PC partition coefficients, bringing about an increase of 3,4-DHPEA-EA and a reduction of oleacein and oleocanthal; VC composition was slightly affected by CC addition, reducing some C₆ alcohols, but the differences were not significant (Tamborrino et al., 2017). A negative correlation between CC addition and PC and VC content was highlighted by Squeo et al. (2020), related with increased oxidases activity. Interestingly, an interaction between CC concentration and MT was hypothesized which could justify the described results wherein the solubility of the salt indeed was clearly favored at higher temperature.

Sodium chloride increases the ionic strength of the aqueous phase, which could determine a variation in the solubility of VOO micro-components and in the activity of vital enzymes (Peres et al., 2017).

On the other hand, enzyme preparations such as cellulases, hemicellulases, and pectinases hinder PC complexation with polysaccharides (Peres et al., 2017) and promote cell wall degradation (Polari & Wang, 2020), reducing the amount of trapped oil and micro-components. The addition of hydrolytic enzymes (500 ppm) during malaxation significantly improved TPC at all tested conditions, including cultivar, maturity stage, and crushing circumstances (Polari & Wang, 2020). The effect was particularly pronounced at low ripening levels, probably due to a more decisive impact of the enzymes like during ripening the progressive breakdown of cell wall components was mentioned, namely pectin degradation and other polysaccharides solubilization (Negi & Avtar, 2008). Significant increases of oleuropein, oleocanthal, and oleuropein and ligstroside aglyconic forms were reported (Polari & Wang, 2020). These results were consistent with the study performed by Moustakime et al. (2016), who observed that the use of pectolytic enzymes increases VOO TPC up to 50 %. Also, the employment of an olive leaf aqueous extract (20 %, w/w) was studied by Kiritsakis et al. (2017), who noticed a significant increase in TPC and positive sensory notes, i.e., fruity, bitter, and pungent as a result of the extract addition.

In conclusion, the addition of physical adjuvants during OP malaxation may improve VOO quality, but a clear trend is not always underlined. Talc improves oil extraction efficiency, slightly decreases the activity of endogenous oxidases degrading PC, and reduces the production of undesired VC related with fermentations, but it was found to inhibit the enzymes of the LOX pathway. CC, on the contrary, enhances the activity of LOX enzymes, but also of oxidases through OP pH increase. It was also demonstrated to increase OP viscosity, without affecting process efficiency. Proteolytic and pectolytic enzymes increase extraction efficiency and VOO phenolic content through the degradation of the membrane structures and the cell wall, respectively.

Non-thermal technologies

Non-thermal technologies are widely studied as a possible tool to assist malaxation with the objective to ameliorate cell wall degradation without excessive OP heating, thereby enhancing VOO extractability and quality, and to modulate enzymatic activity.

PEF is a well-known non-thermal technology promoting the permeabilization of the cytoplasmatic membrane, which might be reversible or not (Pérez et al., 2021). During a PEF treatment, pores of different shape and diameter are produced on the cell membrane depending

on processing parameters like pulse amplitude and/or duration (Saulis, 2010), hence triggering the release of endogenous enzymes and micro-components. Based on the average pore diameter, cell electroporation or electropermeabilization (for higher-size pores) take place, as described by Saulis (2010). PC seem to be more affected by PEF treatment than VC (Tamborrino et al., 2020; Veneziani et al., 2019). In detail, Tamborrino et al. (2020) stressed a slight increase of oleacein and oleocanthal because of PEF application during kneading, whereas most VC were not affected, except alcohols that decreased. Veneziani et al. (2019) treated OP with PEF during the last phase of malaxation and pointed out an increase of TPC, oleacein, and 3,4-DHPEA-EA, probably linked to enhanced PC release and solubilization. On the other hand, no significant differences were registered with respect to VC, since LOX activity had already produced most of volatile aldehydes in the first step of malaxation. These authors also emphasized the relevance of the studied cultivar on the treatment. Furthermore, both covalent changes linked to altered primary structure of the enzymes and conformational changes leading to different spatial distributions have been described as a result of PEF treatments, which might modify their affinity for the substrates (Zhao et al., 2012).

As for the US technology, it stimulates cell wall breakdown and improves mass transfer by means of acoustic cavitation, particularly in case of high-power treatments (18-40 kHz) (Pérez et al., 2021). Specifically, during US treatments acoustic cavitation bubbles are produced. Depending on pressure amplitude, stable (non-inertial) or transient (inertial) cavitation arise: the latter determines bubbles growth until a critical size and consequent implosion, with the development of extremely high temperature and pressure conditions and the production of reactive free radicals (Wu & Nyborg, 2008). Along with cell wall damages, also the opportunity to modulate the activity of both beneficial and deleterious enzymes is described through US technology (Pérez et al., 2021). A TPC increase is generally described when malaxation is combined with US, mainly linked to enhanced content of oleuropein derivatives (Almeida et al., 2017; Taticchi et al., 2019) for which a higher activity of β -glu might be implicated, together with improved mass transfer. Likewise, US-induced cell degradation was reported to impair PC complexation with polysaccharides and proteins (Khan et al., 2018). Clodoveo et al. (2017) observed an increased TPC when malaxation was combined with US, which was justified by the inactivation of PPO and POD. The same group developed the first continuous ultrasonic full-scale device for VOO industry (Amirante et al., 2017). However, some conflicting results have been described in the literature, with no significant differences or slight phenols reductions as a result of US treatment during malaxation (Bejaoui et al., 2016, 2018). This is most likely due to the selection of different olive cultivars, agronomical aspects, or the application of

particularly severe processing parameters (e.g., treatment time or US power) (Nardella et al., 2021): it would be suitable to design a new experiment where all of these aspects are included, in order to define the best US conditions at varying genetic, pedoclimatic, and agronomical factors. The content of LOX VC is usually unaffected by US (Bejaoui et al., 2016, 2018). However, a work by Almeida et al. (2017) suggested a positive effect of the treatment on LOX pathway enzymes, leading to VOOs with more intense bitter, pungent, and fruity notes, consistent with the observed PC and VC increase. This finding supports the opportunity to modulate the activity of LOX pathway enzymes through US application. Table 3 summarizes the main effects observed on PPO, POD, β -glu and LOX after US treatment of different food matrices at different processing conditions. Also, the opportunity to combine more novel technologies during malaxation may be evaluated. Tamborrino et al. (2019) combined US and microwave with heat exchange during kneading where it was observed that PC content did not rise, but it was possible to reduce Mt to 20 min.

Therefore, the combination of conventional malaxation with innovative technologies generally improves VOO nutritional and sensory quality. The application of PEF increases cell wall breakdown and membrane permeability, leading to higher extraction efficiency and release of micro-components, but sometimes a decrease of some odorants is underlined. US technology improves oil yield and VOO phenolic content, with the opportunity to inactivate oxidases and modulate the activity of LOX enzymes, but generally favors OP heating, which triggers off-flavors development and PC degradation.

Concluding remarks

The application of physical adjuvants and/or non-thermal technologies during malaxation might significantly improve phenolic and volatile content of the produced VOOs. Talc and pectolytic enzymes were reported to enhance PC composition, via the variation of partition coefficients and cell damages, respectively. PEF and US treatments improve the breakdown of cell constituents, facilitating the release of oil and micro-components and might also, depending on processing parameters, stimulate or inhibit enzymatic activities connected to PC and VC development, transformation, and degradation. Furthermore, mass transfer strengthening allows to reduce kneading time and thus lays the groundwork for making olive oil mechanical extraction process continuous.

CONCLUSIONS

The studied literature corroborates the relevance of malaxation conditions to VOO nutritional and sensory quality, due to the impact on PC and VC generation, transformation, degradation, and partitioning. Although there are several factors affecting the optimal malaxation conditions, including genetic and agronomical aspects, an attempt to deduct common findings was performed, and can be summarized as follows:

- temperature of 18-22 °C, followed by an increase to 25-27 °C for the last 15-20 min of kneading favors PC solubilization, the production of oleacein and oleocanthal, the partial conversion of C₅ and C₆ aldehydes to alcohols, and a limited activity of PPO and POD;
- treatment time of 30-40 min is enough to achieve oil droplets coalescence, PC solubilization and VC production, without significant PC degradation and development of unpleasant VC;
- headspace oxygen concentration of 5-10 % limits PC losses, while keeping a sufficient activity of LOX pathway enzymes;
- addition of talc and pectolytic enzymes positively affects micro-components partitioning and cell wall degradation;
- application of non-thermal technologies in the last stages of malaxation, like PEF and US through electroporation and cavitation phenomena respectively, are generally favorable for OP malaxation.

Further research should be aimed at defining specific malaxation optimization charts for each olive cultivar and ripening stage, since these factors may significantly affect the behavior of crucial enzymes at changing malaxation conditions and thereby the characteristics of the produced VOOs. The optimized malaxation charts should be employed at an industrial scale and used for process standardization. Systems for quality prediction and process monitoring should be utilized as well for in-line adjustment of relevant parameters in the context of both Industry 4.0 and 5.0 paradigms, leading to a strict cooperation between humans and machines.

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TABLES

Table 1. *The main sensory attributes given by virgin olive oil volatile compounds generated during the lipoxygenase pathway*

Chemical class	Volatile compound	Sensory attributes	References
Aldehydes	Hexanal	Cut grass, sweet, banana	(Campestre et al., 2017)
	<i>Trans</i> -2-hexenal	Cut grass, green apple, almond, fruity	(Lukić et al., 2019; Romero et al., 2017)
	<i>Cis</i> -3-hexenal	Leaf, green, apple	(Lukić et al., 2019)
	<i>Trans</i> -2-pentenal	Green, apple, almond, bitter	(Lukić et al., 2019)
Alcohols	Hexan-1-ol	Fruity, sweet, tomato	(Campestre et al., 2017; Romero et al., 2017)
	<i>Cis</i> -2-penten-1-ol	Green fruit, fresh olive	(Lukić et al., 2019)
	<i>Cis</i> -3-hexen-1-ol	Green, leaf, apple, banana	(Lukić et al., 2019)
	<i>Trans</i> -3-hexen-1-ol	Green	(Romero et al., 2017)
	<i>Trans</i> -2-hexen-1-ol	Green, grass, leaf, sweet	(Lukić et al., 2019)
Ketones	1-penten-3-one	Green, leaf, pungent	(Campestre et al., 2017; Lukić et al., 2019; Romero et al., 2017)
Esters	<i>Cis</i> -3-hexen-1-yl acetate	Green banana, olive fruit	(Lukić et al., 2019)

Table 2. *Effect of MT and Mt on the concentration of phenolic and volatile compounds*

Factor	TPC	Oleacein + Oleocanthal	Total aldehydes	Total alcohols	Reference
Increasing MT in the range 18-30 °C	↑	↑	↓	↑	(Lukić et al., 2017)
	↔	↑	↑	↓	(Lukić et al., 2018)
	↑	↑	↓	↑	(Veneziani et al., 2021)
	↑	↑	↔	↑ ↓	(Veneziani et al., 2017)
Increasing Mt in the range 30-60 min	↑ ↓	↑ ↓	↔	↔	(Lukić et al., 2017)
	↑ ↓	↑ ↓	↔	↔	(Lukić et al., 2018)

↑: Increased concentration; ↓: decreased concentration; ↔: no significant differences; ↑ ↓: conflicting results depending on studied cultivar and/or ripening stage at harvesting

Table 3. *Effect of ultrasound treatment on the activity of the main enzymes involved in phenolic and volatile compounds development, transformation, and degradation*

Enzyme	Food matrix	US Power	Frequency (kHz)	Effects on enzyme activity	Reference
Polyphenol oxidases (PPO)	Apple juice	N.A.	24	Almost complete inhibition after 45 min	(Bot et al., 2018)
	Mushroom	100-500 W	22-40	50-60 % inhibition after 50 min at 40 °C	(Xu et al., 2022)
Peroxidases (POD)	Goldenberry	1000 W	20	Lower residual activity at higher US amplitude	(Etzbach et al., 2019)
	Horseradish	0-500 W	20-100	Higher inactivation rate at increasing US power	(De Gennaro et al., 1999)
β -glucosidases (β -glu)	Soybean	20 W/cm ²	20	Increased activity compared to control	(Falcão et al., 2018)
	Apricot	0-900 W	25-59	Highest activity at 28 kHz and 225 W	(Fan et al., 2019)
Lipoxygenases (LOX)	Hazel	4 mW/cm ²	29	Increased activity until 20 min; then decreased	(Ghanati et al., 2015)
	Soy flour	N.A.	20	Significant reduction only at pH < 6 and for treatment times > 30 min	(Thakur & Nelson, 1997)

N.A.: not available (missing information)

FIGURES



Figure 1. A malaxer (on the left) (Clark, 2016); olive oil droplets coalescence during OP malaxation (on the right) (OKelly, 2010)

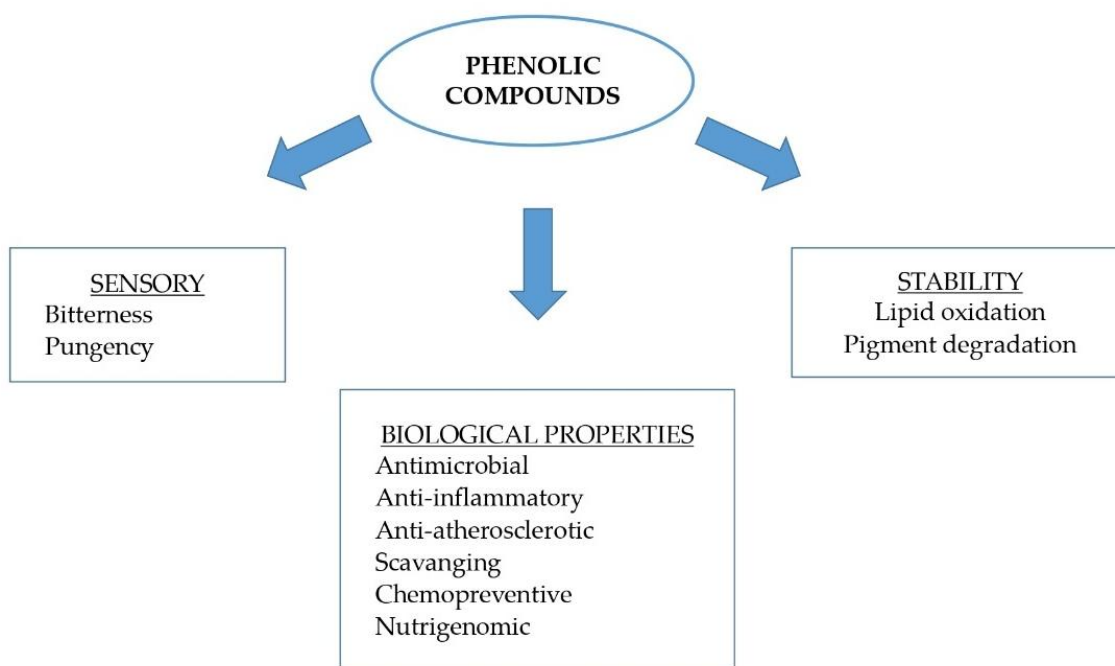


Figure 2. Principal functions of virgin olive oil phenolic compounds

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EVOO SHELF-LIFE 3

COMPUTERS AND ELECTRONICS IN AGRICULTURE - SUBMITTED

PREDICTING EVOO SHELF LIFE USING DEEP LEARNING AND VIS/FT-NIR

SPECTRAL DATA

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Keywords: Artificial Neural Networks, Convolutional Neural Networks, PLS regression, data augmentation, food quality.

Abstract

Extra virgin olive oil (EVOO) possesses qualitative characteristics attributable to its composition. These compounds are subject to deterioration over time, partially influenced by storage conditions. The use of rapid, nondestructive technologies such as spectroscopy to assess EVOO quality is increasingly employed to support traditional methods, although it is not currently an official method. Typically, classical chemometrics is employed for developing predictive models for oil quality assessment. However, recent approaches involve deep chemometrics, potentially eliminating the need for human operators to perform data preprocessing. This study compares PLS (Partial Least Squares) with two types each of CNN (Convolutional Neural Network) and ANN (Artificial Neural Network). The algorithms were

used with FT-NIR (1 000-2 500 nm) and Vis (380-780 nm) spectroscopy to predict acidity, peroxide value, total phenol content, K_{232} , K_{270} , and ΔK during eight months of shelf-life. Storage conditions involved optional thermal stress prior to storage and exposure to light and air.

In general, models for predicting acidity and K_{232} performed poorly using both FT-NIR and Vis spectroscopy. For the other parameters, models based on FT-NIR data exhibited inferior performance with artificial neural networks (ANNs), while convolutional neural networks (CNNs) showed comparable or superior performance to partial least squares developed with data augmentation (PLS-A). This observation was confirmed using Vis spectroscopy, where CNN model performed better than the ANN and PLS-A models for most tested parameters. Among successful models, prediction of peroxides and total phenols achieved the best performance using Vis-CNN-II (x layers) with RMSEP of 2.75 mEqO₂/Kg and 9.37 mg/Kg, and R²P 0.992 and 0.875, respectively.

The study highlights the efficacy of deep chemometrics in obtaining precise and robust models, even when applied to nontraditional analyses such as spectroscopic methods. In conclusion, CNNs, capable of automated hierarchical feature extraction from raw augmented spectral data, demonstrate superior performance compared to ANNs (which lacks this capability) and classical PLS (which required manually selected spectral preprocessing).

Highlights

- CNNs predict EVOO quality parameters from raw Vis/NIR spectra
- Deep Chemometrics (CNN) outperforms classical PLS-A models
- CNNs reduce spectral preprocessing needs for EVOO analysis
- Accurate peroxide prediction achieved using CNNs on Vis/FT-NIR data
- Vis-CNN models generally show better performance than FT-NIR-CNN
- CNN-II architecture yields superior results among neural networks

List of acronyms:

A- Air treatment	ML - Machine Learning
ANN - Artificial Neural Network	MSC - Multiplicative Scatter Correction
AS - Autoscaling	MSE - Mean Squared Error
BIAS - Systematic Error	NN - Neural Network
C - Calibration	P - Prediction
CNN - Convolutional Neural Network	PC - Principal Component
CONV 1D - One-Dimensional	PCA - Principal Component Analysis
Convolutional Layer	PLS - Partial Least Squares
CPI - Cumulative Performance Index	R ² - Coefficient of Determination
CV - Cross-Validation	ReLU - Rectified Linear Unit
DA - Data Augmentation	RMSE - Root Mean Squared Error
DL - Deep Learning	SG - Savitzky-Golay Filter
DNN - Deep Neural Network	SIMPLS - Simplified Partial Least Squares
EVOO - Extra Virgin Olive Oil	Algorithm
FT-NIR - Fourier Transform Near-Infrared	SNV - Standard Normal Variate
Spectroscopy	Vis - Visible Spectroscopy
L - Light treatment	TS - Thermal Stress
LV - Latent Variable	UV - Ultraviolet Spectroscopy
MC - Mean Centering	

INTRODUCTION

Extra virgin olive oil (EVOO) is a premium product in the edible fats market, prized for its superior quality characteristics, sensory attributes, and health benefits (Uncu et al., 2019). In fact, consumers often prefer EVOO to other oils due to its superior organoleptic and nutritional characteristics, which result from its extraction process that preserves the freshness of the olives (EU, 2013). Olive oil is not only appreciated for its flavor and aroma, but it is also rich in unsaturated fatty acids and antioxidants (phenols and tocopherols), which have important health benefits (Lolis et al., 2019). Exposure to light and oxygen, as well as storage temperatures, are important factors in determining the shelf life of the product. Despite its long shelf-life, EVOO is highly perishable and susceptible to chemical (i.e., autoxidation and photooxidation) and enzymatic oxidation (Lanza et al., 2015; Lolis et al., 2020) if not properly stored. These reactions result in the degradation of fatty acids and the formation of compounds that affect the sensory and health properties of the oil. Therefore, the production and quality characteristics of EVOO are well defined by regulations: to be sold as "extra" virgin olive oil, it must meet

specific sensory and chemical parameters (e.g., free acidity, peroxide value, ΔK , phenolic content) (EU, 2022; Gagour et al., 2024). Despite the accuracy of traditional analyses, they have certain limitations, including high costs, long processing times, and the use of chemical reagents that have a negative impact on the environment (Meng et al., 2023). When applied to raw samples, spectroscopic techniques, such as Visible (Vis) and Fourier-Transformation Near Infrared (FT-NIR) spectroscopy, offer an alternative because they are multi-analytical, fast, non-destructive, and environmentally friendly, as they do not generate toxic laboratory waste (Karami et al., 2020). For the development of predictive models, the many spectra obtained in a short time can be correlated with chemical parameters. Specifically, model development involves chemometrics, the science that uses mathematical and statistical tools to evaluate and interpret chemical data (Héberger, 2008; Santos et al., 2019).

In spectroscopy, the use of classical chemometrics is well established. However, raw spectral data are susceptible to issues related to light scattering, band overlap, noise, and baseline shifts (Nicolai et al., 2007), and the selection of appropriate preprocessing techniques can be fundamental for mitigating these problems, while spectral band selection can further improve model performance. In classical chemometrics, feature engineering is crucial for model development and requires human expertise (Acquarelli et al., 2017) to select the best combination of spectral preprocessing, prediction algorithms, feature selection methods, etc. However, due to technical and operational limitations, it is time consuming for an operator to test all possible combinations. Moreover, the operator's decisions may affect the accuracy, precision, and robustness of the final model.

Machine learning (ML) techniques have been increasingly integrated into chemometrics to enhance predictive modeling and handle complex spectral data (Vega-Márquez et al., 2019). Among these, deep learning (DL) techniques are used for spectral data analysis and predictive model development. DL is a subset of ML (Sitorus & Lapcharoensuk, 2024) that has been developed in recent years with the increasing computational power to process high-dimensional data. DL uses neural networks and multiple layers of interconnected neurons to process large data sets. DL includes artificial neural network (ANN) and deep neural network (DNN). The ANNs are widely used and have enabled the development of various predictive models. However, despite their ability to process spectral data, ANNs often require careful hyperparameter tuning to achieve stable model convergence. If not properly optimized, they can suffer from slow training, overfitting, and reduced interpretability (Wang et al., 2025).

DNNs are increasingly being applied in chemometrics, especially for data processing, classification, and regression (Chakravartula et al., 2022). Convolutional neural networks

(CNNs), a type of DNN, were originally developed for image analysis but have recently been applied to one-dimensional datasets, including spectroscopic data. CNNs are particularly advantageous due to their ability to learn hierarchical patterns, which can help distinguish relevant spectral features from background noise and overlapping signals, thereby improving model precision and accuracy (Krohling & Krohling., 2023; Nallan Chakravartula et al., 2022). Consequently, CNNs mitigate some of the limitations of fully connected ANNs by leveraging convolutional layers to extract local features, which improves feature learning and reduces parameter redundancy (Hahn & Choi, 2020; Xu et al., 2021).

To the best of our knowledge, the application of deep learning in chemometrics (i.e. deep chemometrics) to spectral data for the rapid screening of EVOO shelf life has not yet been evaluated. Therefore, the aim of this study was to assess the feasibility of spectroscopic methods (i.e., Vis and FT-NIR spectroscopy) combined with classical and deep chemometrics to develop a predictive model for chemical analytes evaluated during the shelf-life of EVOO stored for 8 months and exposed to air, daylight, and thermal stress, alone or in combination.

MATERIALS AND METHODS

Samples preparation and storage conditions

Filtered EVOO was purchased from local retailers in Viterbo (Latium, Italy), and further filtered through filter paper to remove any remaining impurities, thereby minimizing light scattering during spectral measurements. The oil was stored for 8 months (from December 2023 to July 2024) under various shelf-life conditions to test the effects of the exposure to: i) air (A); ii) light (L); and iii) thermal stress (TS), either alone or in combination (**Tab. 1**). Each factor had two levels (presence and absence), resulting in a total of 8 treatments (**Fig.1**). For each treatment, two biological replicates were conducted. Each sample consisted of approximately 0.95 L of oil, sealed in a 1-L glass jar. The sampling was performed monthly by extracting 40 mL from each jar. The sample was immediately subjected to spectral scans and frozen for subsequent standard chemical analyses. The environmental conditions (i.e., temperature and light intensity) during the 8-months storage were monitored and recorded at 5-min intervals using a custom-built system based on the Arduino UNO R3 board (Arduino srl, Monza, Italy), which was appropriately calibrated. Air exposure was ensured by installing a 40 mm diameter fan on the top of a 3D-printed screw cap designed for the experimentation with a speed range of 1.40-1.75 m/s 10.47 m/s For the samples not subjected to air exposure, the jar was hermetically sealed using a metal screw cap. The thermal stress (TS) was applied prior to storage by exposing the jar to 70 °C for 7 days. The light exposure was ensured by leaving the

glass jar exposed to the laboratory's day-night light cycle, while samples not exposed to light were completely covered using aluminum foil.

Chemical and spectral profiles acquisition

Chemical profile

Determination of acidity, peroxide value, and spectrophotometric indices was performed following the analytical methods outlined in Commission Implementing Regulation 2022/2105 (EU, 2022), with minor modifications. Specifically, acidity was measured by titrating olive oil samples (10 g) with 0.1 M KOH, using phenolphthalein as an indicator. Prior to titration, each sample was dissolved in 100 mL of a neutralized ethanol/ethyl ether solution (1:1 v/v). Results were expressed as the percentage of oleic acid.

Peroxide value (expressed as milliequivalents of oxygen per kg of oil) was measured by titrating 2.5 g of olive oil with 0.01 M Na₂S₂O₃, using a starch solution as an indicator. Prior to titration, the olive oil samples were dissolved in 15 mL of chloroform/acetic acid (2:3 v/v), shaken for 1 min, and left to react in the dark for 5 min with 1 mL of saturated aqueous potassium iodide.

Ultra-violet (UV) spectrophotometric indices were determined by measuring absorbance at 232, 266, 270, and 274 nm using quartz cuvettes with a 1-cm path length and a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Olive oil samples (0.12 g) were first dissolved in isooctane (1 % v/v). The ΔK value was calculated using Equation 1:

$$\Delta K = K_{270} - \left(\frac{K_{268} + K_{274}}{2} \right) \quad (\text{eq. 1})$$

Total phenolic content was determined following the Singleton et al. method using the Folin-Ciocalteu reagent (Singleton et al., 1999). Methanolic extracts were obtained from olive oil samples (1 g) using SPE Strata C18-U columns (Phenomenex, Torrance, CA, USA). The absorbance of the methanolic extract was measured at 765 nm using a UV-1800 spectrophotometer. TPC was calculated using a linear regression equation derived from the standard plot of caffeic acid, which was used as a reference standard. Results were expressed as mg/kg of caffeic acid equivalents. All measurements were performed in duplicate.

Organoleptic profile

The sensory analysis was carried out by eight tasters from the Professional Tasting Panel of Viterbo (Italy). The analysis was performed on samples subjected to the 7 treatment combinations, in addition to the control sample. The evaluation was performed at the beginning, in the middle, and at the end of the storage period. The oil samples were analyzed for the intensity of defects and positive attributes (“fruity”, “bitter”, and “pungent”) according to the

official method of the International Olive Oil Council (IOC, 2018). The resulting data were statistically evaluated, and the median value was calculated for each attribute. Based on the median values obtained for both defects and the “fruity” attribute, the samples were classified into the legally defined quality categories (EU Regulation 2022/2104) of EVOO, virgin olive oil (VOO), and Lampante olive oil (LOO). All statistical tests to discriminate the effect of different treatments for each sampling time were carried out with the XLSTAT Premium Version 2024 (Addinsoft, Paris, France) software using one-way ANOVA.

Spectral profile

The Vis spectrum was acquired within the range of 380-780 nm (~1-nm resolution) using a UV-Vis spectrophotometer mod. Lambda 850+ (PerkinElmer, USA). An FT-NIR spectrophotometer, mod. Antaris II (Thermo Fisher Scientific, USA), was used to collect spectra in the FT-NIR range 1000–2500 nm (~0.48 nm resolution), with each spectrum obtained as the average of 64 interferometer sub-scans. All scans were performed at controlled temperature. Spectra were acquired monthly from month 1 to month 8 for all treatment combinations (each with two biological replicates). At the time zero, representative spectra were obtained from the initial non- and thermally stressed oils. Consequently, the dataset comprised 130 samples (128 from monthly scans and 2 from time zero). For each sample measurement, two technical replicates were performed.

Data handling and data mining

Data overview

A summary representation of the variability of chemical parameters during shelf life was analyzed. Descriptive statistics were calculated for minimum, maximum, mean, and standard deviation values. The trend of chemical values during shelf life was represented with a heatmap, in which the most intense colors were associated with a higher numeric value of the corresponding chemical parameter. The assessments were conducted in RStudio (version 4.2.1).

Principal Component Analysis (PCA) was applied to visualize and summarize the spectral data in a simplified plot. This approach was chosen to effectively represent the variance within the dataset while handling the large volume of spectral data. Specifically, the PCA was applied on the difference between the initial (0 months) and final (8 months) spectra for each treatment, rather than on the raw spectra. This approach was intended to highlight the spectral evolution over time, allowing the PCA to better capture the variance associated with the different storage

treatments instead of the variance merely due to the storage period. The analysis was separately performed on Vis and FT-NIR spectral data.

Data split in calibration and prediction sets

To develop a predictive model while balancing the trade-off between underfitting and overfitting, the dataset was split into calibration (C) and prediction (P) sets, comprising 67 % and 33 % of the total data, respectively. The split was performed using the Kennard-Stone algorithm, which ensures an even distribution of spectral data across both calibration and prediction sets (Zeaiter & Rutledge, 2009).

Spectral pre-treatments

Classical chemometric models require spectral preprocessing to reduce noise, baseline shifts and scattering effects, and to enhance relevant spectral features (Nicolai et al., 2007). Several preprocessing methods were tested both individually and in combination. Specifically: i) signal-to-noise ratio improvement and baseline correction were achieved using the Savitzky-Golay (SG) filter, applied with 0th, 1st, and 2nd derivatives and polynomial orders of 1, 2 and 3, respectively, over a smoothing filter window of 25 to 35 points; ii) scatter removal was performed using either Standard Normal Variate (SNV) or Multiplicative Scatter Correction (MSC); and iii) data normalisation was performed using either mean centering (MC) or autoscaling (AS).

Data Augmentation

The application of deep chemometrics requires larger datasets than classical chemometrics in order to capture complex patterns and relationships in the data, avoid overfitting and ensure accurate, precise and robust predictions. Data augmentation (DA) is a common technique used to artificially increase the size of datasets by generating new calibration samples through mathematical transformations, such as additive and multiplicative effects, applied to the raw spectra.

In this study, data augmentation was performed following the method proposed by Bjerrum et al. (2017), where only the calibration set was subjected to: i) an offset variation of ± 0.6 times the standard deviation; ii) a baseline shift with a slope variation in the range of $\sigma \pm 0.1$; and iii) an additive/multiplicative scattering effect within an intensity variation of 1 ± 0.6 times the standard deviation. This procedure generated nine augmented spectra per raw spectrum, increasing the size of the calibration set tenfold.

The primary objective of the data augmentation was to allow a fair and unbiased comparison between the PLS and CNN models by maintaining the same number of calibration

samples. Since the size of the data set can significantly impact performance metrics, ensuring comparable calibration set sizes was crucial. A secondary objective was to assess the effect of data augmentation on PLS regression.

Chemometrics

Classical chemometrics

The models were developed using Partial Least Squares (PLS) regression with the SIMPLS algorithm (Moscetti et al., 2021). Leave-one-level-out cross-validation based on shelf-life levels was performed to carefully determine the optimal number of latent variables (LVs), thereby preventing both overfitting and underfitting, as well as ensuring better model generalization (Bedini et al., 2023). The most effective models were then evaluated using the prediction dataset.

The classical chemometric analyses were conducted using Matlab R2024b, coupled with PLS_Toolbox v9.5 (Eigenvector Research Inc., WA, USA).

Deep chemometrics

Figure 2 shows the basic structure of the Artificial Neural Network (ANN) used in this study. The input layer processes the raw spectral data (i.e., predictors), while the output layer generates the analytical measurement (i.e., predictand). To mitigate the risk of overfitting, a dropout layer was incorporated to randomly drop a fraction of the input units during training; dropout rates of 0 and 0.5 were tested. The simplest ANN architecture implemented only a hidden dense layer composed of fully connected neurons, with each neuron applying a Rectified Linear Unit (ReLU) activation function to its input. In this configuration, the hidden layer was tested with 64, 128, and 256 neurons. The ReLU activation function was chosen to allow the model to capture complex non-linear patterns. During training, this hidden dense layer learns the relationships between the predictor and the predictand by adjusting both the weights and the biases of the neurons through backpropagation. Finally, a dense output layer of a single neuron computes the final output based on the features extracted by the hidden layer. In addition to the configuration described above, ANNs with multiple hidden dense layers were tested to assess whether deeper architectures could produce better prediction performance. These deeper networks were categorized as Type I and Type II based on the arrangement of the hidden dense layers and their nodes. In Type I, up to four dense layers were explored, with each successive layer using fewer neurons than the previous one. Starting with the first hidden layer containing n neurons, the second layer used $n/2$ neurons of the first, the third used $n/4$, and the fourth used $n/8$. In Type II, the number of layers was extended up to six layers. The second layer contained

2n neurons, the third had n neurons, the fourth n/2, the fifth n/4, and the sixth n/8. In both types, each hidden layer used a ReLU activation function.

A CNN differs from a standard ANN by incorporating additional layers dedicated to the automatic extraction of relevant features from the input data. In the present study, the CNN architecture includes a GaussianNoise layer, a Reshape layer, two 1D Convolutional layers (Conv1D) utilizing ReLU activation, and a Flatten layer, followed by hidden dense layers. The dense-layer configurations used in the CNNs were identical to those tested in the ANNs (i.e., Type I and Type II), ensuring a consistent comparison between the two architectures. The most significant difference between CNNs and ANNs in this study was the inclusion of two Conv1D layers, which played a crucial role in extracting features from the data, enabling the network to identify local patterns and structures within the spectra. By applying a set of learned filters, a Conv1D layer can capture features such as peak shapes, widths, relative intensities, and thus meaningful chemical information. Therefore, CNNs were tested alongside ANNs to evaluate their relative effectiveness, with the Conv1D layers offering a significant advantage in spectral data processing compared to standard fully connected architectures.

For both ANN and CNN architectures, the following common settings were tested: (i) batch sizes of 8, 64, and 128; (ii) optimizers options (Adam or AdaDelta); (iii) EarlyStopping with a patience of 10 epochs and a delta of 0; (iv) kernel regularization options, either None or L2 with a coefficient of 0.01; and (v) a loss function defined as Mean Squared Error (MSE). All possible combinations of these settings were explored, resulting in the training of over 4000 models for each architecture.

Performance metrics

To assess the performance of each model, the following performance metrics were considered: Root Mean Square Error (RMSE), systematic error (BIAS), and the coefficient of determination (R^2) for calibration (C), cross-validation (CV), and prediction (P). In addition, to facilitate the selection of the most robust model, the following ratios and differences were computed: RMSEC:RMSEP, RMSECV:RMSEP, $R^2C:R^2P$, $R^2CV:R^2P$, $|BIASC - BIASP|$, and $|BIASCV - BIASP|$. Overall, the PLS and NN models produced 15 and 9 error metrics, respectively. For NNs, the number of considered metrics was lower because cross-validation metrics are not computed during model development. As a further step, an ad hoc Cumulative Performance Index (CPI) was developed to facilitate the selection of the most effective models by aggregating the performance metrics into a single index value. It is important to consider that the CPI was computed individually for the performance matrix obtained from each model category (i.e., Delta K, K_{232} , K_{270} , acidity, peroxide, and total phenols).

The CPI calculation involved the following steps: i) BIAS threshold adjustment - an arbitrary BIAS threshold was applied. Specifically, absolute BIAS values below the threshold were replaced with the threshold value. This step penalized the models with BIAS above the established threshold. In other words, the threshold was crucial to ensure that models with very low BIAS, but high RMSE did not outscore models with acceptable BIAS and lower RMSE; ii) metrics scaling and Principal Component Analysis (PCA) - all performance metrics included in the CPI calculation were autoscaled to ensure comparability. PCA was then applied, and the principal components (PCs) accounting for at least 95 % of the total variance were retained. This step reduced the data dimensionality and removed collinearity between metrics; iii) weighting by variance proportion - the absolute value of the PCA loadings for the retained PCs were weighted by multiplying them by the proportion of variance explained by their corresponding PC. This generated a matrix reflecting the variance-weighted of each metric contribution to each significant PC; iv) derivation of final metric weights (i.e. k coefficients) - the resulting matrix was normalized by dividing it by the sum of all its elements. Subsequently, the final weights (k_j for the j -th metric) were obtained by summing the normalized variance-weighted loadings across the retained PCs for each performance metric. This ensures the weights (k_j) reflect the overall contribution of each metric, and that their sum was equal to 1 across all metrics; v) calculation of the CPI - prior to the CPI calculation, the absolute value of the input metrics (M_{ij}) normalized by dividing by the maximum value observed for that metric across all models ($\max(M_j)$), scaling them to the 0-1 range. Finally, the CPI for each model was computed as the sum of max-normalized metrics, each multiplied by their corresponding weight (k_j), as shown in Equation 2:

$$CPI_i = \sum_{j=1}^n \frac{M_{ij} k_j}{\max(M_j)} \quad (\text{eq.2})$$

where, CPI_i is the Cumulative Performance Index for model i ; M_{ij} is the absolute value of the j -th performance metric for model i ; $\max(M_j)$ is the maximum value of the j -th metric across all models (used for normalization); k_j is the normalized weight (derived from PCA) for the j -th metric; and n is the total number of metrics.

The approach described was developed to provide a robust and holistic technique for evaluating complex models by integrating diverse performance metrics into a single, interpretable index. However, it must be considered that, like any composite index, its outcome is influenced by the set of evaluated models. In other words, it represents a useful data-driven tool but it does not allow the comparison of different pools of models. The R code for the CPI is open source and available on GitHub (Moscetti, 2025).

The overall workflow showing sample preparation, data acquisition, processing, modeling, and selection is summarized in Figure 3.

RESULTS AND DISCUSSION

Chemical profile overview

The experimental storage period lasted for 8 months, during which both environmental conditions and EVOO quality parameters were continuously monitored. During this period, the average illumination recorded was 79 lux, with variations between 2 and 181 lux, excluding night hours (0 lux). Moreover, a gradual increase in light exposure was observed: from ~ 9 h day⁻¹ in December (start) to ~ 15 h day⁻¹ in July (end). The environmental temperature also showed an increasing trend, with an average of 27 °C and fluctuations between 18 and 35 °C.

The EVOO quality analysis exhibited a wide distribution of data as a result of implementing eight distinct storage conditions (**Tab. 2**). This result was highly expected, as the objective was to achieve broad variability, and thus a comprehensive data range, which is critical for developing robust prediction models. Data show that during storage some samples exceeded the chemical limits defined for the extra virgin category (EU, 2022). In fact, oxidative rancidity was observed in some cases where oils surpassed the limits for peroxides (20 mEqO₂ kg⁻¹), K₂₃₂ (2.5), and K₂₇₀ (0.22). In contrast, both acidity and ΔK remained below their legal limits (i.e., 0.8 and 0.01, respectively).

Figure 4 is a heatmap that allows a visual evaluation of the impact of the storage conditions on the EVOO quality attributes during shelf life. Acidity and ΔK were excluded from the plot due to the aforementioned absence of a significant variation, which would not provide an interesting impact of exposure factors on them. In general, as expected, thermal stress (TS) initially had a pronounced detrimental effect on oil quality, especially for total phenols and K₂₃₂; however, over the entire storage period, its impact did not lead to the highest overall degradation. In fact, among factors, the exposure to light (L) showed the most important impact on the final oil quality either when applied alone or in combination. Conversely, exposure to air (A) exhibited a negligible effect on the overall EVOO. In detail, samples exposed to L and TS showed the highest decrease in total phenols content. Moreover, the depletion of phenolic compounds led to an increased susceptibility to oxidation, as the oil lacked intrinsic antioxidant mechanisms (Farhoosh & Hoseini-Yazdi, 2013; Migliorini et al., 2013). In fact, the heatmap for peroxides content showed that photooxidation, catalysed by the exposure to light, was a major contributor to the development of peroxides (Li & Wang, 2018; Lolis et al., 2019). Visually evaluating the heatmap, the peroxides content observed in the L samples at the third

month was greater than the final peroxides content observed in treatments absent of light exposure. As evidenced by the higher K_{232} values, the primary oxidation reactions were promoted by TS (Gagour et al., 2022; Malvis et al., 2019; Li & Wang, 2018); this effect is particularly evident during the first three months of storage, after which the K_{232} index decreased and aligned with other treatments, probably because of the degradation of primary oxidation products such as hydroperoxides, conjugated dienes, carboxylic compounds, and conjugated trienes. In the absence of TS, a gradual increase in K_{232} was observed during storage, although these values never reached the initial TS levels. Secondary oxidation, quantified by K_{270} (Gagour et al., 2022; & Wang, 2018), occurred in all oils during storage. However, oxidation was particularly pronounced in oils where TS and L were present. As with total phenols, the slowest increase in K_{270} was observed in the control and oil exposed only to air.

Organoleptic profile overview

Sensory analysis, combined with physicochemical parameters, is considered essential for the assessment of olive oil quality grades. The tasters provided evaluations for the most common positive attributes, such as “bitter”, “pungent”, and “fruity”. In addition, for oils classified as EVOO, the tasters detected notes of artichoke, herbs, and almonds, which remained relatively stable during storage. Generally, the analysis corroborated the results of the chemical analysis. However, in most cases, the intensity of positive attributes decreased during the 8 months of storage, being partially “masked” by the development of defects, as confirmed by the statistical analysis (one-way ANOVA) in **Table 3**. From a sensory point of view, the main defect detected during storage was rancidity. The greatest deterioration in sensory quality was observed in all the samples subjected to thermal stress (TS) treatment. Moreover, exposure to light (L) negatively affected the positive attributes and promoted the formation of compounds responsible for rancidity. The degrading effect of light confirmed the observations reported from the chemical analyses performed on the different samples. As observed in another study (Lobo-Prieto et al., 2020), the only treatment group that remained extra virgin (i.e., free of defects) until the end of storage was the sample exposed only to air (A), with fruitiness at the highest values (2.8 and 3.0 respectively) and rancidity at 0, while in all other samples developed rancidity defect. The deterioration of quality in other treatment groups led to a downgrading of the category (i.e., from “extra virgin” to “virgin” and even “lampante”). In other words, these olive oils no longer met the requirements for the “extra virgin” category.

Spectral data overview

Prior to performing the PCA, the FT-NIR data underwent Asymmetric Least Squares baseline correction and Savitzky-Golay (SG) filtering, over a smoothing window of 25 to remove spectral noise. Vis spectra were processed as raw data. The resulting graphical output (**Fig. 5**) is presented as score plots that show the clustering of treatments based on their spectral features, alongside loading plots that illustrate the contribution of individual wavebands to the observed clustering.

The spectra obtained in FT-NIR were mostly related to the fatty acid composition of the oil (Stella et al., 2015). The PCA (**Fig. 5a**) was able to highlight the effect of thermal stress and light exposure during shelf life, as represented by the first and third PCs, respectively. The PC2 was not considered because it did not characterize any treatment (data not shown). PC1 and PC3 accounted for 80.64 % of the total variance. In particular, PC1 (78.39 %) and PC3 (2.25 %) mainly captured the variance related to thermal stress and light exposure, respectively. In addition, it is interesting to note that, in alignment with the chemical analyses showing no remarkable differences between A+L and L samples, the FT-NIR-based PCA did not clearly distinguish them. Looking at the loading plot (**Fig. 5b**), Loadings 1 and 3 show two peaks in the range of 1 300-1 500 nm, which correspond to the second overtone of the carbon-hydrogen (C-H) bond, likely associated with changes in linoleic acid and straight alkane compounds due to L and TS treatments (Casale & Simonetti, 2014; Meng et al., 2023; Vieira et al., 2021). Specifically, the peak at 1 384 nm shows a positive relationship with both light exposure (Loading 3) and thermal stress (Loading 1), while the peak at 1 470 nm shows a negative correlation with light exposure (Loading 3). The first overtone of C-H stretching vibrations in the 1 700-1 800 nm region is associated with the degradative effect of light on mono- and polyunsaturated fatty acids, which exhibit absorption in this range (Casale & Simonetti, 2014; Hourant et al., 2000). The 1900-nm band, corresponding to the first overtone of O-H bonds with deformation combination and attributed to phenolic compounds in olive oil (Li et al., 2025), is positively related to light and negatively related to thermal stress. The spectral band from 2 000 to 2 500 nm is associated with combination bands of C-H vibrations, vibrational modes of cis double bonds in unsaturated fatty acids, and C-O vibrations (Meng et al., 2023; Vieira et al., 2021). In this region TS has a positive correlation at 2 140 nm and a negative one at 2 387 nm, while L has a negative correlation at 2 080 nm and a positive one at 2 427 nm. Unsaturated fatty acids also exhibit absorption at 2 308 and 2 427 nm, showing a positive relationship with both Loadings 1 and 3, as these compounds are sensitive to the effect of both light exposure and thermal stress.

PCA on the Vis spectra (**Fig. 5c**) showed similarities to that obtained using FT-NIR data, but with a clearer separation between light exposure and thermal stress. The first two PCs accounted for 99.08 % of the total variance. It is interesting to note that the four possible combinations of the L and TS factors are located in separate quadrants. The effect of air exposure is negligible again. As expected in the analysis of spectral data in the visible range, the loading plot (**Fig. 5d**) showed spectral bands characteristic of chromophores (i.e., carotenoids, and pheophytin a and b) typical of EVOO. It is important to remember that the PCA analysis was performed on the spectral differences between spectra at the beginning and the end of shelf-life. Thus, while Loading 2 is positive in the carotenoids region, the score plot shows that samples subjected to thermal stress fall on the negative axis of PC2. This indicates that the spectral differences in the carotenoids region were smaller for thermally stressed samples. In other words, TS heavily reduced carotenoid content at the beginning of storage, resulting in smaller spectral differences between the initial and final time points. Consequently, a positive correlation with Loading 2 was observed in non-thermally stressed samples, which had a higher carotenoid content at the beginning of storage (i.e., more to lose). In contrast, following the same logic, light exposure produced the largest spectral differences during storage by degrading carotenoids; consequently, L samples are positively related to Loading 1. Regarding the chlorophyll and pheophytin contents, the results indicate that thermal stress had a predominant effect (Domenici et al., 2014), primarily affecting the spectral bands at 401 and 665 nm.

Regression models

FT-NIR and Vis-based predictive models for EVOO chemical parameters over 8 months of shelf life were developed using both classical chemometrics (PLS) and deep chemometrics (ANN and CNN). Moreover, the impact of data augmentation, a prerequisite in deep chemometrics when large datasets are unavailable, was investigated with respect to its effect on PLS-based models. The results are reported in **Tables 4** and **5**.

Classical chemometrics

In general, the majority of PLS models were preprocessed using scatter correction techniques such as Standard Normal Variate or Multiplicative Scatter Correction, which are commonly employed to mitigate additive (e.g., baseline shifts) and multiplicative distortions in spectral data (Næs et al., 2004). This highlights that, even with sample filtration performed to remove small particles in the oil matrix and the use of a short optical path length (1 mm), light scattering still affected the spectral profile, and its correction was essential to enhance model

performance. In addition, most models required 1st or 2nd derivatives to remove baseline shifts and resolve overlapping spectral features (Nicolai et al., 2007). The signal-to-noise ratio was enhanced in most models by using the Savitzky-Golay filter, with a smoothing window size ranging from 25 to 43. Specifically for models calibrated using data-augmented spectra, a smaller smoothing window was required. In FT-NIR models, the choice between autoscaling (AS) and mean centering (MC) varied based on chemical parameters. In contrast, MC was the most commonly used centering method in Vis-based models. In general, the number of latent variables (LVs) adopted for the FT-NIR-based models ranged from 5 to 10; among them, the peroxide prediction model required only 5 LVs and was the best-performing one. For Vis spectra, the number of LVs ranged from 3 to 8, except for K₂₃₂, which required 11 LVs despite showing poor performance. In the Vis region, the optimal number of LVs generally increased with the application of data augmentation (DA), whereas for FT-NIR, DA had no effect on the required number of LVs. In terms of model performance metrics, the use of the Cumulative Performance Index (CPI) allowed the identification of the most robust models (data not shown). As a consequence of its usage, models showing the most consistent values across calibration (C), cross-validation (CV), and prediction (P) steps were selected.

Regarding the FT-NIR-based models (**Tab. 3**), high accuracy (i.e., small RMSEP) was observed for the prediction of peroxide (3.64 mEqO₂ kg⁻¹) and total phenolic content (14.26 mg kg⁻¹) using data-augmented models. Their systematic error of prediction (BIASP) was 18 % and 23 % of RMSEP, respectively. The superior performance of the peroxide model was confirmed by an excellent coefficient of determination in prediction (R²P) of ~0.99. On the contrary, the prediction model of the total phenolic content showed a R²P of ~0.73, indicating a smaller capability of the model to explain the variance of the predictand. PLS models for K₂₇₀ and ΔK parameters showed fair R²P (~ 0.80), while the best model for olive oil acidity showed poor performance (R²P of 0.66), likely due to an oleic acid concentration range too narrow (0.2-0.3 %) for an effective model calibration using FT-NIR. The K₂₃₂ model completely failed. Regarding data augmentation (DA), it improved the robustness and predictive performance of both peroxide and total phenols models and slightly modified their regression vector: i.e., the β-coefficients of a model that describe the positive or negative relationship of spectral features to the prediction (Nallan Chakravartula et al., 2022). As an example, **Figure 6** shows the regression vectors of non-augmented and augmented peroxide prediction models, respectively. Notably, both regression vectors identified spectral regions of interest similar to the vibrational bands associated with the functional groups highlighted by PCA loadings (**Fig. 5b**, Section 3.1). In particular, wavelengths above 2 000 nm made a substantial contribution, particularly at 2

067 and 2 098 nm (**Fig. 6a**). In the data-augmented model (**Fig. 6b**), where autoscaling (AS) pretreatment influenced the magnitude of the β -coefficients, the key wavelengths remained consistent with those identified in the model without DA. However, the peaks at 1 164, 1 210, 1 440, 1 500, 1 660, 1 908, and 1 965 nm showed an increased contribution in the regression vector. These differences may be attributed to DA's capability to alter the distribution and variance of spectral features, thereby emphasizing distinct patterns within the data (Gong et al., 2019). However, the role of AS in influencing the apparent contribution of these peaks should also be considered and further investigated.

Table 4 shows the models based on Vis spectroscopy. The application of data augmentation led to a reduction in RMSEP and BIASP only for the total phenols model, while performance remained unchanged or slightly deteriorated for the other parameters. Similar to FT-NIR, the peroxide prediction model was the best-performing within the Vis range, with an R^2P higher than 0.98, followed by ΔK (0.88) and total phenols (0.85). However, for the peroxide content, Vis model ($3.73 \text{ mEqO}_2 \text{ kg}^{-1}$) yielded a higher RMSEP compared to the FT-NIR model, while the $|\text{BIASP}|$ was notably small in both of them, suggesting a low impact of the systematic error on the models' accuracy. In the case of ΔK , both RMSEP and BIASP values were similar between FT-NIR and Vis. For total phenols, the Vis model performed better than FT-NIR, with lower RMSEP (10.50 mg kg^{-1}) and $|\text{BIASP}|$ ($6.7 \times 10^{-3} \text{ mg kg}^{-1}$). This is the case in which the effect of DA improved Vis model performance, reducing both RMSEP (from 12.34 to 10.50 mg kg^{-1}) and $|\text{BIASP}|$ (from 2.7 to $6.7 \times 10^{-3} \text{ mg kg}^{-1}$). In Vis spectroscopy, the β -coefficient plot (**Fig. 6c**) for the peroxide model showed that the magnitude was higher in the regression vector of the DA-based model. This effect can also be a consequence of a higher number of LVs used (2 more than the non-augmented model). Nevertheless, a general overlap was observed between the regression vectors derived from the datasets with and without DA. In agreement with what speculated observing the PCA loadings (**Fig. 5d**), the primary spectral contributions to the regression were attributed to carotenoids in the 380-500 nm range, while an additional smaller contribution was observed in the 660-680 nm region, associated with chlorophyll a and pheophytin a, although this region coincided with a high-noise area of the spectrum.

In summary, FT-NIR spectroscopy combined with PLS regression yielded outcomes comparable to those obtained with Vis spectroscopy. In both cases, the most accurate regression models were achieved for the prediction of peroxide content in EVOO. The implementation of data augmentation demonstrated potential improvements in accuracy and robustness within the NIR domain; however, no clear advantages were observed when applied to the Vis dataset. This discrepancy may be attributed to the different physical principles underlying the two spectral

regions: Vis spectroscopy is primarily influenced by electronic transitions, whereas NIR spectroscopy is governed by molecular overtones. As a result, the DA strategy adopted in this study may not be equally suitable for both spectral domains. Therefore, further research is required to optimize data augmentation approaches tailored to the specific characteristics of Vis and NIR spectroscopy.

Deep chemometrics

The present section is dedicated to the analysis of the performance of FT-NIR and Vis-based models trained using ANN and CNN. In this specific case, the dataset was composed of raw data subjected only to data augmentation. Thus, spectral preprocessing was not applied.

Specifically for FT-NIR (**Tab. 3**), only CNN models showed performances comparable or better than those observed in PLS models trained on NIR spectra. In this context, ANN showed unsatisfying performances. This result was possible because CNN incorporates convolutional layers, capable of performing hierarchical and position-invariant feature extraction, which are inaccessible from raw spectra to fully connected ANNs. Consequently, an ANN-based model is likely to require pre-processed spectra to compete with PLS. However, this does not make sense if the aim is to eliminate the need for spectral pre-processing, as in the present work. Among the tested CNNs, the type II architecture, characterized by a greater number of layers and nodes. Despite the greater structural complexity of the network, CNNs with a high number of hidden dense layers performed better and did not show any overfitting problems. Similarly to PLS models, only prediction models for peroxides and total phenols showed notable prediction accuracy. In detail, CNN-II reduced the RMSEP for both peroxides (from 3.64 to 2.96 mEqO₂ kg⁻¹) and total phenols (from 14.26 to 11.32 mg kg⁻¹), and also improved both BIASP and R²P. Models for K₂₇₀, ΔK and acidity remained weak, and K₂₃₂ was not predictable.

In the Vis range (**Tab. 4**), CNNs still showed superior performance, but ANNs were capable of competing with PLS. This was possible because visible spectra are less affected by the baseline shifts and scattering effects, which are characteristic of NIR spectroscopy. Consequently, the features of raw Vis spectra can be more directly accessible to ANNs even without any pre-treatment. Differently from what was observed in FT-NIR, the type of CNN used did not heavily affect the model performance, and in certain cases CNN-I was slightly better than CNN-II (e.g., total phenols and acidity). It means that the simpler architecture of type I provided adequate feature extraction capabilities for the visible data, without the need of the greater complexity of the type II that was required for the FT-NIR. Optimal performance has been observed for some models with a single hidden dense layer. As for PLS, CNN models trained on visible data were slightly better than those calibrated using FT-NIR. Specifically

looking at the performance metrics, the use of CNNs improved the coefficient of determination of all models and the prediction accuracy of peroxides (from 4.02 to 2.75 mEqO₂ kg⁻¹), K₂₇₀ (from 0.016 to 0.011), total phenols (from 10.50 to 8.78 mg kg⁻¹), and acidity (from 0.012 % to 0.009 %). No important improvements for the systematic error of prediction were noted.

As an example, **Figure 7** shows the visual comparison of the best predicted parameter (i.e. peroxides) comparing PLS-A and CNN-II models applied to FT-NIR and Vis spectra. For the FT-NIR spectral range, the CNN-II model (**Fig. 7b**) demonstrated superior predictive accuracy compared to the PLS-A model (**Fig. 7a**). This is evident from the lower RMSEP, a considerably reduced BIASP, and a slightly improved coefficient of determination. Visually, the data points in **Fig. 7b** align closely with the ideal regression line ($x=y$). In the Vis spectral range, the advantage of the CNN-II architecture is even more pronounced. The CNN-II model (**Fig. 7d**) significantly outperformed the PLS-A model (**Fig. 7c**), achieving the lowest RMSEP (2.75 mEqO₂ kg⁻¹) and the highest R²P (0.992) among the four models presented. The Vis PLS-A model yielded the highest RMSEP (4.03 mEqO₂ kg⁻¹). Overall, for peroxide content prediction, the CNN-II architecture applied to raw augmented spectra consistently provided better results than the PLS-A models for both FT-NIR and Vis data, with the Vis CNN-II model ensuring the most accurate and precise predictions.

CONCLUSIONS

The present study successfully evaluated the feasibility of using Vis (380-780 nm) and FT-NIR (1 000-2 500 nm) spectroscopy in combination with classical (PLS) and deep learning (ANN, CNN) chemometrics for predicting key quality parameters (peroxides, K₂₃₂, K₂₇₀, ΔK, total phenols and acidity) during the shelf life of EVOO subjected to different storage conditions. The results demonstrate the different capabilities of the chemometric approaches used. While PLS regression required careful spectral pre-processing (e.g. scatter correction, noise removal, derivatives) to achieve optimal performance, CNNs were effective when applied to raw augmented spectral data, especially for the FT-NIR region. This finding highlights the potential of CNNs to automate feature extraction and bypass the time-consuming and potentially subjective, pre-processing selection that is typically required in classical chemometrics. ANNs without convolutional layers performed poorly on FT-NIR raw data, but showed competitiveness with PLS on the less complex raw Vis spectra.

Across spectral ranges and chemometric methods, models predicting peroxide content consistently achieved the highest accuracy and robustness. CNN models, particularly the Type II architecture, generally showed superior predictive performance compared to PLS models

developed on augmented data (PLS-A). For FT-NIR, CNN-II significantly reduced the RMSEP for peroxides and total phenols. For Vis, CNNs improved prediction metrics for peroxides, K_{270} , total phenols and acidity compared to PLS-A, with Vis CNN-II providing the overall best peroxide model ($\text{RMSEP} = 2.75 \text{ mEqO}_2 \text{ kg}^{-1}$). However, reliable prediction models for K_{232} and acidity remained a challenge for all tested approaches, probably due to the nature of the spectral information or the limited range of the calibration data.

Data augmentation improved the performance of certain PLS models (e.g. FT-NIR peroxides and phenols) and was a necessary step for training the NNs with the available dataset size. However, the universal DA strategy used may not be optimal for all spectral ranges or models, showing the need for further investigation into tailored DA techniques.

In conclusion, deep chemometrics represents a promising and powerful alternative to classical methods for rapid EVOO quality assessment using spectroscopy. Its ability to effectively process raw spectral data reduces the reliance on manual pre-processing, potentially simplifying model development. Future research should focus on i) exploring more advanced NN architectures (e.g. transformers); ii) optimising data augmentation strategies; iii) calibrating and validating models across broader datasets (e.g. different seasons, varieties); iv) investigating spectral fusion; v) and exploring the UV range without using harmful organic solvents.

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TABLES

Table 1 Summary of the factors (Air, Light, Thermal stress) of each treatment used in the study.

Factors	Combination of treatments							
	CNT	TS	L	L+TS	A	A+TS	A+L	A+L+TS
Air (A)	-	-	-	-	✓	✓	✓	✓
Light (L)	-	-	✓	✓	-	-	✓	✓
Thermal stress (TS)	-	✓	-	✓	-	✓	-	✓
Number of factors	0	1	1	2	1	2	2	3

Table 2. Descriptive statistics of chemical parameters measured over 8 months of shelf life

	Acidity	Peroxides	Total Phenols	K ₂₃₂	K ₂₇₀	ΔK
	(% oleic ac.)	(mEqO ₂ /kg)	(mg/kg)			
Min	0.200	7.50	136.00	1.79	0.100	-0.003
Mean	0.212	43.22	181.27	2.12	0.171	0.000
Max	0.300	163.90	257.00	2.54	0.250	0.007
St.Dev	0.032	37.17	28.67	0.15	0.032	0.002

Table 3. Sensory data of olive oils (median values for each positive and negative attribute) and their respective quality grade classifications. ANOVA was used to assess differences between treatments applied to samples at each specific time of analysis.

Sensory attributes	Time	Treatments							
		CNT	A	L	A+L	TS	A+TS	L+TS	A+L+TS
Fruity	start	3.0 a							0.8 b
	middle	3.0 b	3.6 a	1.5 d	1.0 e	2.0 c	1.0 e	0.8 f	0.5 g
	end	2.8 b	3.0 a	1.3 d	1.0 e	1.5 c	1.0 e	0.5 f	0.5 f
Bitter	start	3.0 a							1.0 b
	middle	3.0 b	3.5 a	1.5 d	1.2 e	2.0 c	1.0 f	1.0 f	1.0 f
	end	2.5 a	2.2 b	1.0 d	1.0 d	2.0 c	1.0 d	0.5 e	0.5 e
Pungent	start	3.5 a							2.0 b
	middle	3.5 b	3.8 a	2.9 c	3.5 b	2.7 d	2.5 e	2.0 f	2.0 f
	end	2.5 cd	3.0 a	2.4 d	2.6 bc	2.7 b	2.0 e	0.5 f	0.5 f
Artichoke	start	3.0 a							1.0 b
	middle	3.0 a	2.2 b	1.5 c	1.0 d	0.5 e	0.0 f	0.0 f	0.0 f
	end	3.0 a	2.2 b	1.5 c	1.0 d	0.5 e	0.0 f	0.0 f	0.0 g
Almond	start	4.0 a							2.0 b
	middle	4.0 a	3.5 b	3.0 c	2.0 d	1.5 e	0.5 f	0.0 g	0.0 f
	end	4.0 a	3.5 b	3.0 c	2.0 d	1.5 e	0.5 f	0.0 g	0.0 g
Herbs	start	2.5 a							1.0 b
	middle	2.5 a	1.5 b	1.0 c	0.0 d	0.0 d	0.0 d	0.0 d	0.0 d
	end	2.5 a	1.5 b	1.0 c	0.0 d	0.0 d	0.0 d	0.0 d	0.0 d
Rancid	start	0.0 b							4.0 a
	middle	0.0 f	0.0 f	1.5 e	1.5 e	2.0 d	2.5 c	4.2 b	4.5 a
	end	0.0 g	0.0 g	1.8 f	2.0 e	2.2 d	2.8 c	5.0 b	6.0 a
Olive oil quality (panel 's response)	start	EVOO							LOO
	middle	EVOO	EVOO	VOO	VOO	VOO	VOO	LOO	LOO
	end	EVOO	EVOO	VOO	VOO	VOO	VOO	LOO	LOO

Table 4. Preprocessing details and performance metrics (RMSE, BIAS, R²) in calibration (C), cross-validation (CV), and prediction (P) for PLS, ANN, and CNN models predicting olive oil quality parameters using FT-NIR spectroscopy

Adulterant	Acronym	n. layer	SG				Preprocessing				Model				Results			
			Deriv.	Poly.	SC	Norm.		C	CV	P	C	CV	P	C	CV	P		
Peroxide	PLS	33	1st	2	SNV	MC	AS	5	3.848	4.297	4.212	2.1E-13	-8.1E-02	-4.3E-01	0.991	0.988	0.982	
	PLS-A	25	1st	2	MSC	AS		5	3.192	3.571	3.642	1.4E-14	2.1E-02	6.7E-01	0.993	0.992	0.987	
	ANN-I	2							5.133		5.182	3.9E-01		5.6E-02	0.983		0.973	
	ANN-II	3							5.233		5.264	3.3E-01		5.1E-01	0.982		0.972	
	CNN-I	3							3.258		3.230	5.9E-01		1.4E-02	0.993		0.989	
	CNN-II	6							2.666		2.963	1.8E-01		2.0E-01	0.995		0.991	
K ₂₃₂	PLS	25	1st	2	MSC	MC		6	0.112	0.138	0.117	3.1E-15	6.0E-03	8.5E-03	0.543	0.334	0.205	
	PLS-A	25	1st	2		MC		9	0.112	0.135	0.115	5.3E-15	5.5E-03	4.2E-03	0.542	0.358	0.203	
	ANN-I	4							0.138		0.113	2.0E-02		6.7E-03	0.299		0.133	
	ANN-II	6							0.143		0.111	4.0E-03		2.0E-02	0.257		0.176	
	CNN-I	3							0.133		0.114	1.9E-03		3.4E-03	0.349		0.132	
	CNN-II	3							0.138		0.109	2.9E-03		5.0E-03	0.301		0.197	
K ₂₇₀	PLS	29	1st	2		AS		10	0.009	0.011	0.014	-6.1E-16	5.8E-04	-3.5E-03	0.923	0.871	0.793	
	PLS-A	25	1st	2		MC		10	0.009	0.011	0.014	1.4E-15	6.0E-04	-3.6E-03	0.915	0.874	0.793	
	ANN-I	1							0.018		0.022	3.6E-03		3.1E-03	0.672		0.471	
	ANN-II	6							0.015		0.023	5.8E-04		3.3E-03	0.782		0.455	
	CNN-I	3							0.016		0.019	3.9E-04		9.1E-04	0.740		0.623	
	CNN-II	6							0.014		0.019	4.4E-04		3.2E-03	0.796		0.634	
ΔK	PLS	41	2nd	2	MSC	MC		8	0.001	0.001	0.001	3.3E-18	4.9E-05	-1.6E-04	0.895	0.813	0.807	
	PLS-A	33	1st	2		AS		8	0.001	0.001	0.001	4.1E-18	3.9E-07	-9.1E-05	0.904	0.835	0.802	
	ANN-I	3							0.002		0.001	2.4E-04		7.2E-05	0.513		0.593	
	ANN-II	3							0.001		0.002	1.1E-04		1.3E-04	0.703		0.432	
	CNN-I	4							0.001		0.001	2.9E-04		1.0E-04	0.791		0.674	
	CNN-II	6							0.001		0.001	1.3E-04		7.7E-05	0.839		0.718	
Total Phenols	PLS	39	2nd	2	SNV	MC		9	11.866	15.163	15.074	1.7E-13	-1.3E+00	4.9E+00	0.835	0.736	0.714	
	PLS-A	27	1st	2	MSC	AS		7	12.232	15.897	14.260	8.5E-14	-5.4E-01	3.3E+00	0.825	0.709	0.727	
	ANN-I	4							14.942		16.098	2.9E+00		3.8E+00	0.739		0.632	
	ANN-II	6							10.942		13.851	9.7E-01		2.5E+00	0.860		0.728	
	CNN-I	4							11.240		11.608	1.7E+00		9.2E-01	0.852		0.809	
	CNN-II	6							11.291		11.322	1.1E+00		1.1E+00	0.851		0.818	
Acidity	PLS	39	1st	2	SNV	AS		8	0.010	0.016	0.013	-5.6E-17	1.9E-04	-7.2E-07	0.796	0.502	0.666	
	PLS-A	25	1st	2	SNV	AS		10	0.008	0.016	0.014	1.9E-16	-3.9E-05	3.0E-05	0.868	0.489	0.647	
	ANN-I	3							0.019		0.019	5.8E-04		3.2E-04	0.263		0.308	
	ANN-II	3							0.019		0.019	2.8E-05		3.4E-04	0.256		0.318	
	CNN-I	3							0.014		0.014	5.2E-04		2.6E-03	0.573		0.614	
	CNN-II	6							0.011		0.013	1.1E-03		1.1E-03	0.737		0.689	

Table 5. Preprocessing details and performance metrics (RMSE, BIAS, R²) in calibration (C), cross-validation (CV), and prediction (P) for PLS, ANN, and CNN models predicting olive oil quality parameters using Vis spectroscopy.

Adulterant	Acronym	n. layer	Preprocessing				LYs			RMSE			BIAS			R ²		
			SG	Deriv.	Poly.	SC	Norm.			C	CV	P	C	CV	P	C	CV	P
Peroxide	PLS		27	2nd	2	MSC	MC	4		3.256	3.819	3.782	-7.1E-15	-1.0E-02	-3.3E-03	0.993	0.991	0.986
	PLS-A		37	2nd	2	MSC	MC	6		4.625	5.260	4.025	-1.4E-14	7.1E-03	1.2E-01	0.986	0.982	0.984
	ANN-I	4								3.071		3.977	3.2E-01		4.4E-01	0.994		0.984
	ANN-II	5								3.558		3.780	2.9E-01		4.2E-01	0.992		0.986
	CNN-I	2								2.814		2.807	5.3E-01		3.9E-02	0.995		0.992
	CNN-II	6								2.545		2.754	8.3E-01		1.8E-01	0.996		0.992
K ₂₃₂	PLS		25	2nd	2	SNV	MC	10		0.059	0.086	0.078	0.0E+00	2.4E-03	3.2E-03	0.871	0.740	0.615
	PLS-A		43		2	MSC	MC	11		0.069	0.082	0.090	-4.4E-15	5.0E-03	4.9E-03	0.824	0.758	0.534
	ANN-I	1								0.061		0.078	3.5E-03		3.2E-03	0.863		0.593
	ANN-II	5								0.056		0.081	9.6E-03		7.9E-03	0.885		0.556
	CNN-I	3								0.060		0.068	5.6E-03		7.5E-05	0.870		0.693
	CNN-II	3								0.040		0.068	1.8E-03		3.7E-03	0.941		0.692
K ₂₇₀	PLS		25		2	SNV	MC	4		0.012	0.014	0.017	2.8E-17	-7.1E-05	-1.1E-03	0.855	0.808	0.699
	PLS-A					SNV	MC	6		0.011	0.013	0.016	0.0E+00	3.7E-04	1.1E-04	0.870	0.831	0.724
	ANN-I	1								0.008		0.014	3.1E-04		2.1E-03	0.931		0.802
	ANN-II	3								0.006		0.015	3.6E-04		2.6E-04	0.966		0.770
	CNN-I	2								0.004		0.011	1.0E-04		3.3E-04	0.981		0.870
	CNN-II	6								0.004		0.011	2.7E-04		5.3E-04	0.984		0.870
ΔK	PLS					SNV	MC	4		0.0006	0.0007	0.0008	-3.0E-19	-3.8E-06	-2.3E-05	0.941	0.913	0.871
	PLS-A		25		2	MSC	AS	8		0.0005	0.0007	0.0007	-6.4E-18	1.1E-05	1.5E-05	0.948	0.915	0.885
	ANN-I	1								0.0008		0.0009	3.1E-06		6.1E-05	0.892		0.826
	ANN-II	6								0.0004		0.0010	8.4E-05		1.8E-04	0.965		0.772
	CNN-I	1								0.0005		0.0007	1.9E-05		3.1E-05	0.950		0.893
	CNN-II	6								0.0004		0.0007	3.3E-06		8.0E-06	0.969		0.902
Total Phenols	PLS		25		2		MC	3		13.033	14.221	12.335	2.8E-14	-1.9E-02	2.7E+00	0.801	0.765	0.798
	PLS-A					SNV	MC	5		12.035	13.906	10.501	8.5E-14	-3.0E-02	-6.7E-03	0.831	0.775	0.846
	ANN-I	3								9.671		14.672	1.2E+00		1.1E+00	0.891		0.695
	ANN-II	6								7.452		11.717	1.7E+00		1.5E+00	0.935		0.805
	CNN-I	1								8.739		8.778	1.2E+00		1.2E+00	0.911		0.891
	CNN-II	6								9.410		9.371	8.8E-01		4.7E-01	0.896		0.875
Acidity	PLS		25	2nd	2		MC	8		0.009	0.011	0.012	0.0E+00	1.2E-03	-1.6E-04	0.851	0.742	0.724
	PLS-A		25	2nd	2		MC	8		0.009	0.011	0.012	-2.8E-17	1.1E-03	-1.6E-04	0.851	0.738	0.722
	ANN-I	3								0.012		0.014	1.3E-05		1.4E-03	0.727		0.612
	ANN-II	6								0.004		0.015	3.5E-04		4.3E-04	0.965		0.549
	CNN-I	2								0.005		0.009	1.4E-03		3.3E-04	0.951		0.837
	CNN-II	5								0.003		0.012	4.0E-04		2.0E-04	0.982		0.736

FIGURES

Figure 1. *Experimental setup illustrating different treatment combinations applied to olive oil samples. Conditions include air exposure (fans on jars), light exposure (clear versus foil-wrapped jars), and thermal stress (brown colored oil).*

Neural Network architecture**Common hyperparameters**

- > Batch size {128, 64, 8}
- > Optimizer {adam, AdaDelta}
- > EarlyStopping (patience = 10, delta = 0)
- > Kernel regularizer {None, L2 = 0.01}
- > Loss function (MSE)

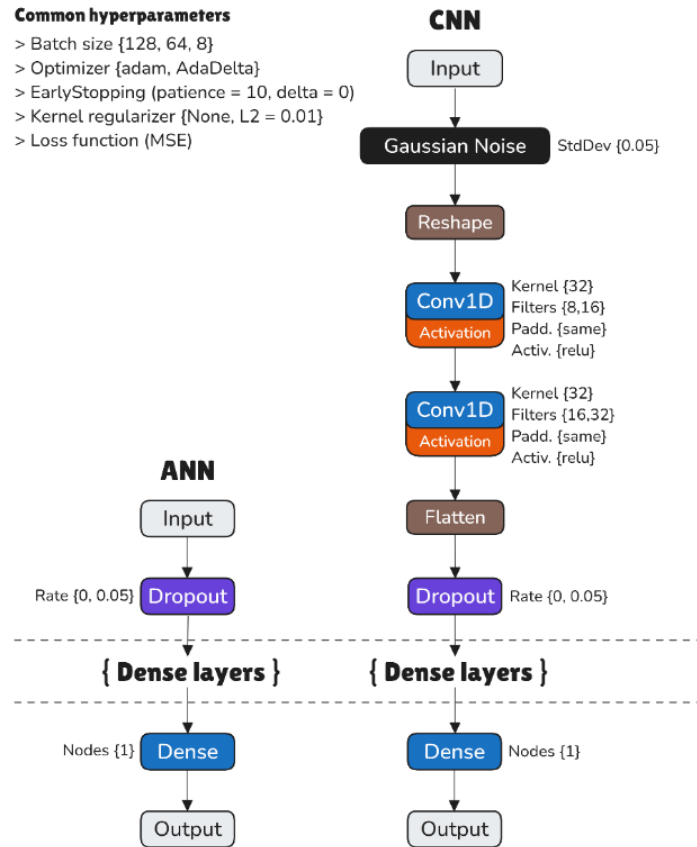
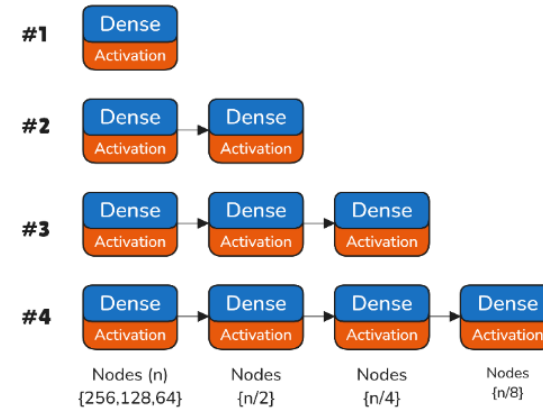
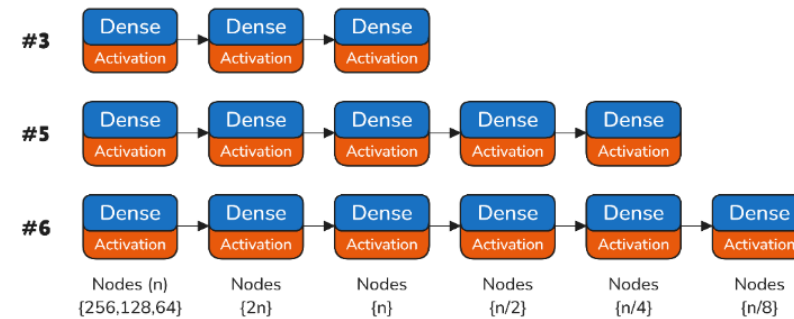
**Dense layers configuration (activation ReLU)****> Type I – decremental number of nodes****> Type II – incremental and decremental number of nodes**

Figure 2. Schematic overview of the Deep Neural Network architectures evaluated. On the left i) the common hyperparameter settings applied during training and ii) the core structures for the ANN and CNN. On the right, the detailed configurations (Type I and Type II) tested for the hidden block {Dense layers} are shown, where '#' in the configuration label (e.g., #1, #2) denotes the number of dense layers used. Type I configurations involve up to four layers with progressively fewer nodes (n , $n/2$, $n/4$ and $n/8$). Type II configurations involve up to six layers with varying nodes (specifically n , $2n$, n , $n/2$, $n/4$ and $n/8$). The initial number of nodes (n) was tested with values of 256, 128, or 64. All dense layers utilized the ReLU activation function.

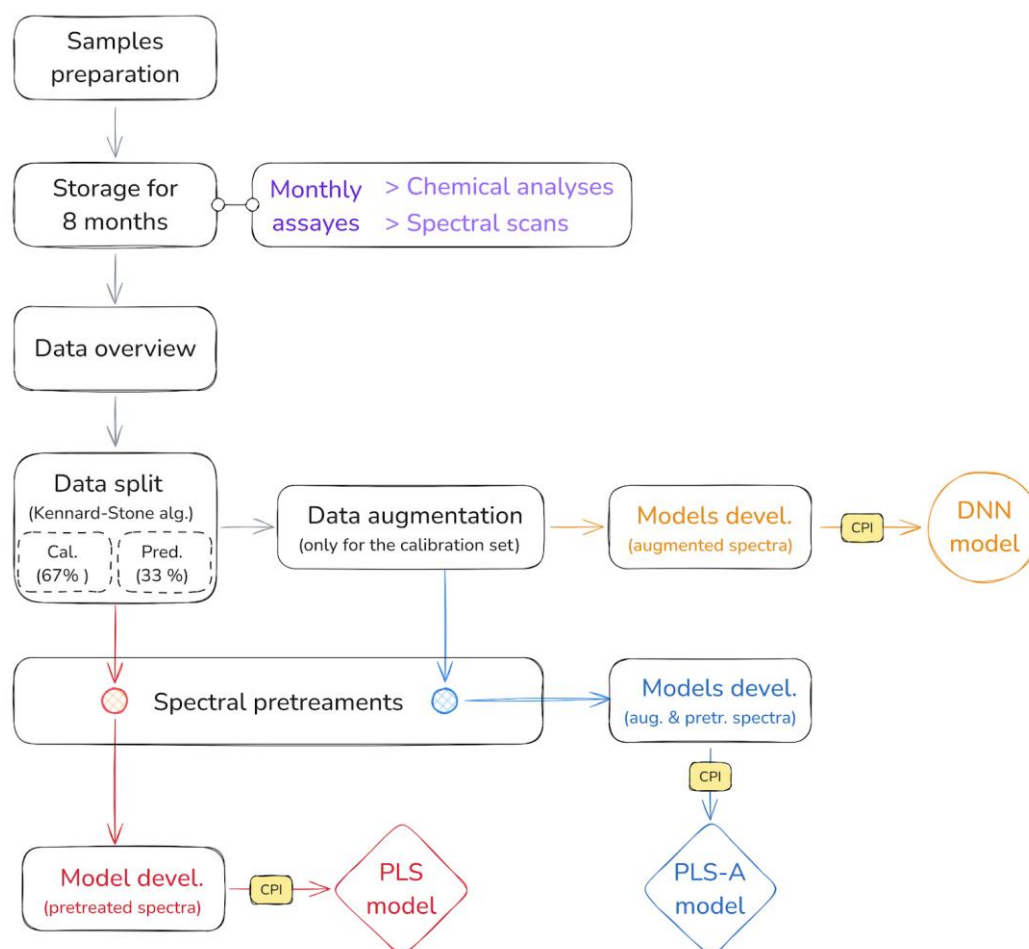


Figure 3. Workflow diagram of the experimental pipeline, showing all steps and including the three modeling paths: i) PLS models development using preprocessed data (red path); ii) PLS-A models development using augmented and preprocessed data (blue path); iii) Deep Neural Network (i.e. ANN and CNN) models development using augmented raw data (orange path). Best model selection for each approach was performed using the Cumulative Performance Index (CPI).

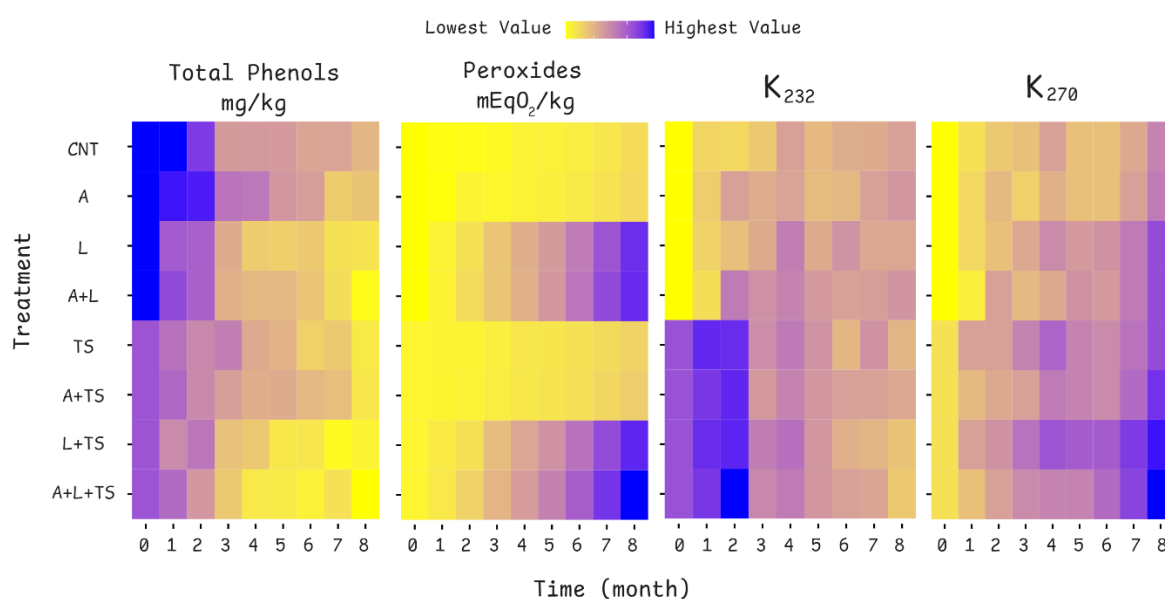


Figure 4. Heatmaps of chemical parameters (i.e., total phenols, peroxides, K_{232} , and K_{270}) analyzed during 8 months under different treatments (Control, and combinations involving A: air exposure, L: light exposure, TS: thermal stress). The color scale indicates parameter values, ranging from yellow (lowest) to blue (highest).

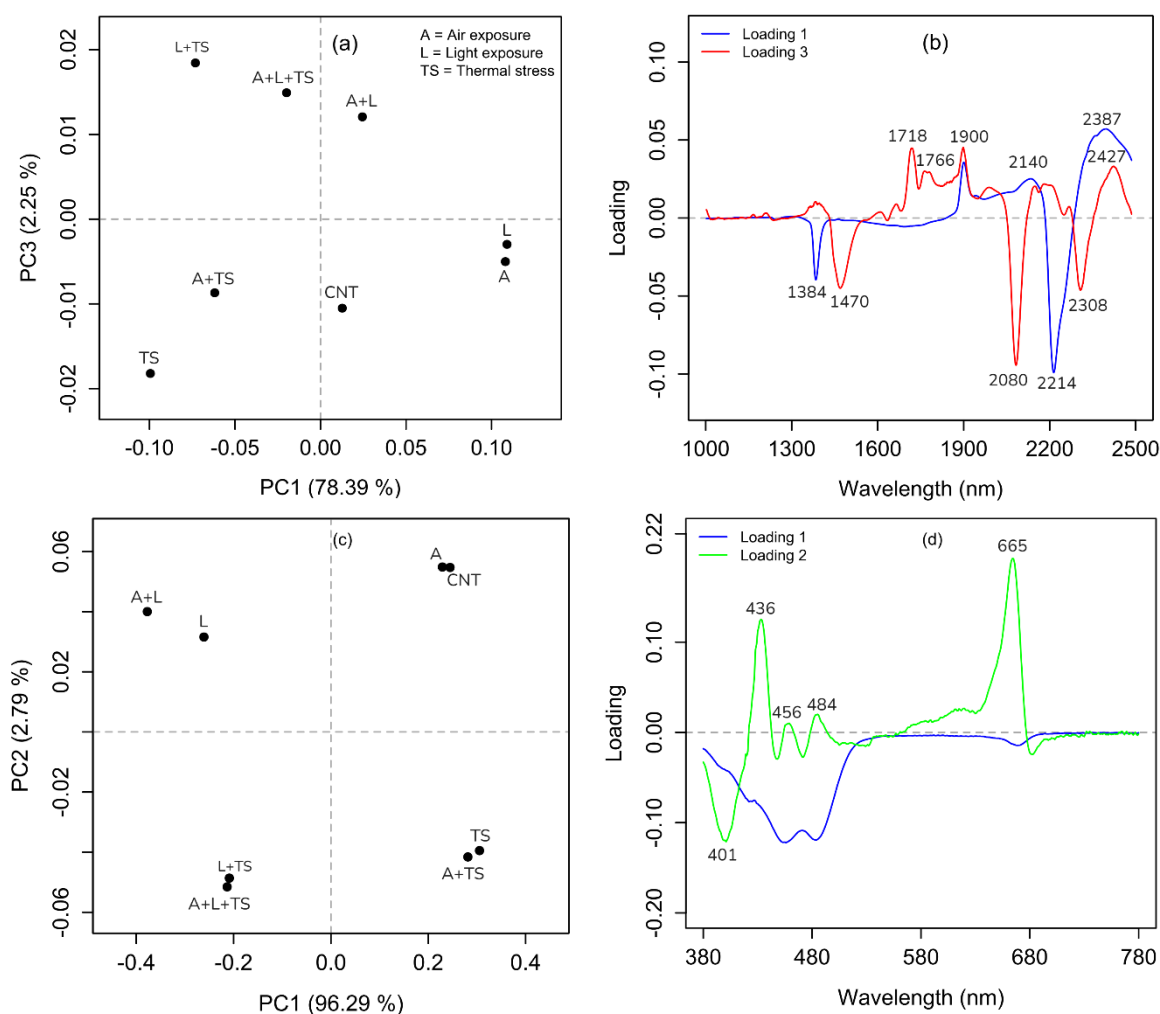


Figure 5. Principal Component Analysis (PCA) performed on FT-NIR and Vis spectra for all treatment combinations: a) PCA score plot (PC1 vs PC3) derived from FT-NIR spectra; b) corresponding loadings for PC1 (blue line) and PC3 (red line) from the FT-NIR data; c) PCA score plot (PC1 vs PC2) derived from Vis spectra; and d) corresponding loadings for PC1 (blue line) and PC2 (green line) from the Vis data.

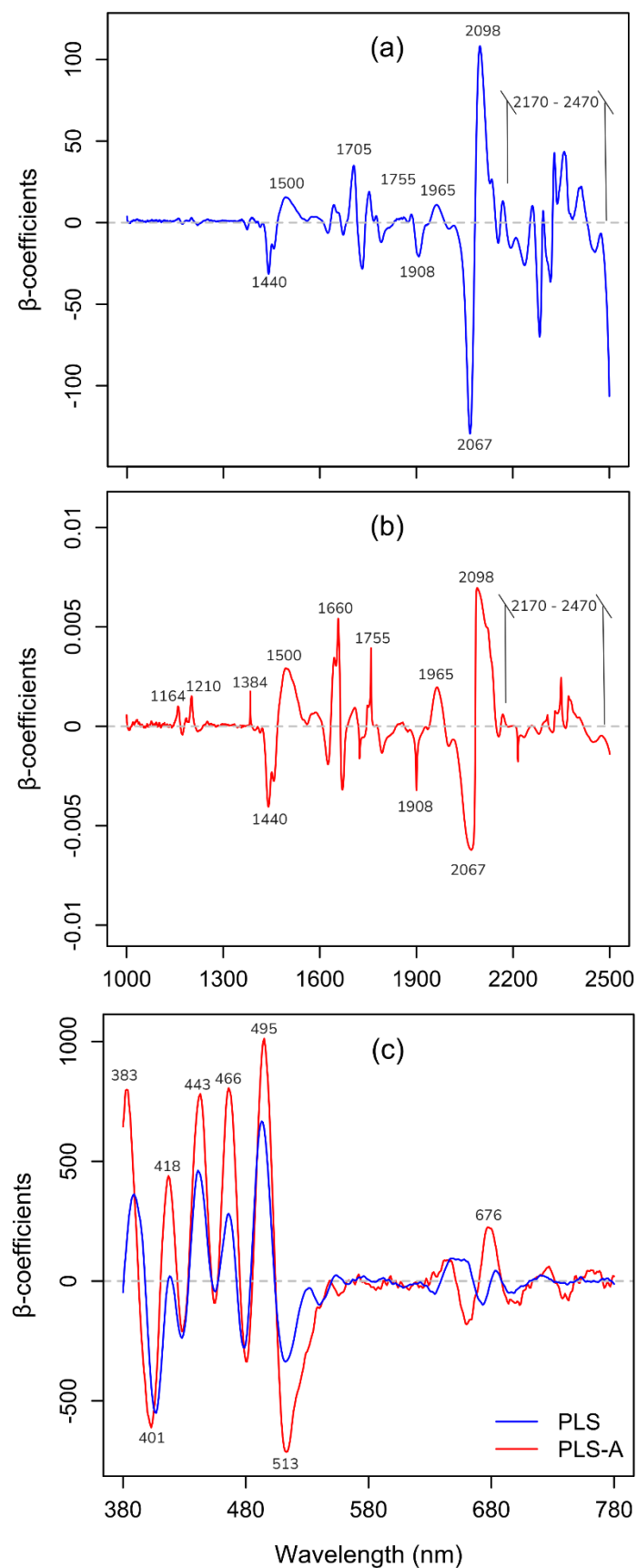


Figure 6. Regression vectors (β -coefficients) for peroxide prediction models: a) FT-NIR model with non-augmented spectra (PLS); b) FT-NIR model with augmented spectra (PLS-A); and c) Vis models comparing non-augmented (PLS, blue line) and data-augmented (PLS-A, red line) spectra.

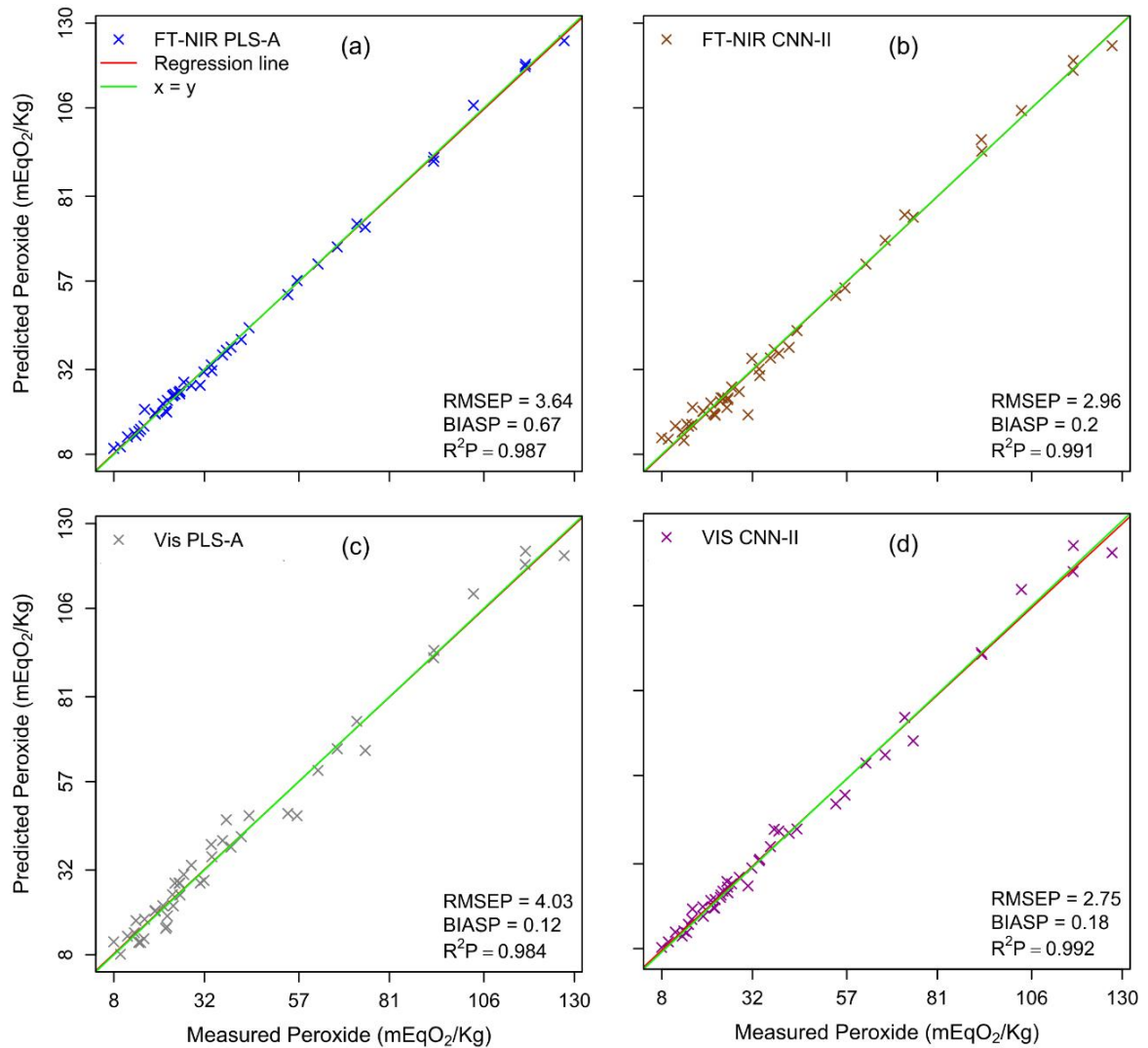


Figure 7. Regression plots comparing peroxides model performance. (a) FT-NIR PLS with augmented data (PLS-A); (b) FT-NIR CNN-II. (c) Vis PLS with augmented data (PLS-A); and (d) Vis CNN-II. Prediction metrics (RMSEP, BIASP, R²P) are shown for each model.

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CHEMICAL PROFILE OF OILS 4

AIIA 2025 INTERNATIONAL CONFERENCE - EXTENDED VERSION

DEEP CHEMOMETRICS FOR OLIVE OIL PROFILING: FROM CLASSICAL TO DEEP CHEMOMETRICS

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Keywords: Extra Virgin Olive Oil, Chemical analysis, Convolutional Neural Network, Partial Least Squares, FT-NIR spectroscopy, Vis-spectroscopy.

Abstract

The demand for rapid and non-destructive product analysis has been increasing. This trend is also observed in the oil market, especially for olive oil, where the high economic value of the product requires faster analysis times. Fourier transform near-infrared (FT-NIR) and Visible (Vis) spectroscopy are techniques that address these needs and allow the development of predictive models to assess quality parameters. However, the efficacy of these solutions can be affected by the human choice of spectral pre-treatment selection during the feature engineering phase. This research investigates the potential of deep chemometrics to mitigate these challenges, potentially reducing the reliance on manual spectral pre-treatment selection. Specifically, convolutional neural networks (CNNs) were evaluated and compared with classical partial least squares (PLS) chemometric algorithms. Through collaboration with a refinery that provided the samples and wet chemistry data obtained using gas chromatography,

it was possible to develop models that correlated FT-NIR and Vis spectra with oil parameters. In general, CNNs outperformed PLS in almost all models developed. Furthermore, a considerable number of chemical parameters were found unsuitable for robust modeling, as initial screening using PLS models yielded coefficients of determination (R^2) lower than 0.75. In general, FT-NIR spectroscopy was able to predict a larger number of parameters, many of them related to fatty acids or sterols. However, for the subset of parameters predictable by both techniques using CNN models, Vis spectroscopy often yielded lower Root Mean Square Error (RMSE) compared to FT-NIR models, suggesting higher predictive accuracy despite the narrower spectral range.

INTRODUCTION

The olive oil market has gained increased economic value due to its attributes and nutritional properties (Esposito et al., 2021), which result from the presence of bioactive compounds (phenols, tocopherols, polyphenols) and monounsaturated fatty acids (Li & Wang, 2018). Different categories of oil derived from olives can be discriminated based on production characteristics, but unquestionably, Extra Virgin Olive Oil (EVOO) is one of the most valuable. A comparison of EVOO with other oils (e.g., refined oil, lampante oil, and pomace oil) reveals its unique chemical and sensory characteristics, defined by law (EU, 2022). According to the European Union regulation (EU, 2013), EVOO must be produced "from the fruit of the olive tree exclusively by mechanical means, which have not undergone any treatment other than washing, decantation, centrifugation, or filtration". Correct oil categorization is essential for determining its commercial value and, consequently, the producer revenues. Quality parameters are generally determined through traditional analytical methods that assess chemical, physical, and sensory properties (Grigoletto et al., 2025). Despite their advantages, classical analyses are costly, time-consuming, and not eco-friendly due to the use of reagents and chemical waste production (Meng et al., 2023). The need for rapid screening in the industry demands the implementation of techniques that are multi-analytical, rapid, economical, and non-destructive. Spectroscopy is included among these techniques for product quality detection, particularly near-infrared (NIR) combined with Fourier transform (FT) and visible (Vis) spectroscopy (Hashempour-baltork et al., 2024). Correlations between spectral data and analyte concentrations enable the development of predictive models. In this context, multivariate regression techniques are frequently employed. Classical chemometrics, which utilize mathematical and statistical techniques to evaluate and interpret chemical data, are commonly used to develop predictive models (Acquarelli et al., 2017). However, human selection during

the feature engineering phase can influence the results of classical chemometrics. This phase is necessary to overcome problems inherent in spectroscopy, such as light scattering, overlapping bands, noise, and baseline shifts (Nicolai et al., 2007).

To address the challenges related to operator choices in feature engineering, deep learning (DL) as a branch of machine learning that utilizes deep neural networks (DNNs) to develop predictive models, offers an alternative approach. Technically, DNNs are neural networks characterized by the use of multiple (deep) layers of interconnected neurons to process large datasets. Among DNNs, Convolutional Neural Networks (CNNs) are widely used due to their auto-learning capability, which may simplify data preprocessing in spectroscopy. In fact, CNNs are compatible with one-dimensional datasets, such as spectroscopic data, and allow to achieve high precision and accuracy even with noisy and overlapping signals (Krohling & Krohling, 2023; Nallan Chakravartula et al., 2022; Sitorus & Lapcharoensuk, 2024). In addition, CNNs utilize techniques like convolutional filters and regularization, which allow them to automatically learn relevant features from raw or minimally processed spectra and potentially reduce data redundancy, thus minimizing the need for manual feature engineering and selection steps (Hahn & Choi, 2020; Xu et al., 2021). Due to these properties, CNNs are applicable to different contexts (Wu et al., 2024) in the food industry (quality evaluation, food adulteration, etc.).

The literature indicates that the application of CNNs for detecting different analytes in olive oil (and derivatives) has not been sufficiently explored. This study aims to evaluate the performance of both classical (PLS) and deep chemometric (CNN) techniques in developing FT-NIR and Vis-based predictive models for quantifying key chemical parameters (e.g., free fatty acids, peroxide value, specific absorption coefficients like K_{232} and K_{270}) across various categories of olive-derived oil, including extra virgin, virgin, lampante, and refined oils.

MATERIALS AND METHODS

Samples preparation

A total of 126 samples were provided by the refinery UmbriaOlii s.p.a (Perugia, Umbria, Italy), together with their analytical reports provided by the industry. The dataset comprised 126 samples of different types of oils and derivatives, categorized as follows: i) 52 samples of Extra Virgin Olive Oil (EVOO); ii) 5 samples of virgin olive oil (VOO); iii) 43 samples of lampante olive oil (LO); iv) 15 samples of olive oil (OO); v) 6 samples of pomace olive oil (PO); vi) 2 samples of fatty acids (FA); vii) 2 samples of grape seed oil (GPO) and viii) one sample of oleins (OL). The non-olive oil samples (FA, GPO, and OL) were included to test the

specificity of the models and, thus, challenge them with different matrices. The chemical parameters, determined by the refinery according to official methods (e.g., IOC/EU regulations where applicable) or validated internal procedures, included acidity, peroxide value, fatty acids ethyl esters, waxes content, ΔK value, K_{232} value, K_{270} value, sterol composition (i.e. cholesterol, brassicasterol, campesterol, campestanol, stigmasterol, β -sitosterol, $\Delta 7$ -stigmasterol, $\Delta 7$ -avenasterol, sum erythrodiol-uvaol, total sterols), and the fatty acid profile (i.e., myristic acid, palmitic acid, palmitoleic acid, heptadecanoic acid, stearic acid, oleic acid, linoleic acid, arachidic acid, linolenic acid, eicosenoic acid, behenic acid, erucic acid and lignoceric acid). The specific chemical parameters analyzed depended on the type of oil the refinery's requirements; therefore, the number of samples available for evaluating each parameter varied between oil categories. Each sample was filtered with fast filter paper and stored in the dark at room temperature until analysis. All samples were analyzed using both Vis and FT-NIR spectrophotometers.

Spectral data acquisition

The oil was vortexed for 10 seconds prior to scanning and then placed in a 1 mm quartz optical cell. A UV-Vis spectrophotometer mod. Lambda 850+ (PerkinElmer, USA) was used to acquire the Vis spectrum in the range of 380-780 nm (~ 1 -nm resolution); an FT-NIR spectrophotometer mod. Antaris II (Thermo Fisher Scientific, USA) was used for the NIR measurement in the range of 1 000-2 500 nm (~ 0.48 nm resolution). Scans were performed at a controlled temperature. Three technical replicates (spectra) were acquired for each sample, and subsequently averaged to obtain a representative spectrum for modeling.

Data handling and data mining

Data split in calibration and prediction sets

To develop a model capable of performing good predictions, it is necessary to find the optimal balance between under- and over-fitting. Accordingly, the dataset used in this study was divided into calibration (C) and prediction (P) sets, assigning 67 % of the samples to the calibration set and the remaining 33 % to the prediction set. The division of the dataset was carried out using the Kennard-Stone algorithm, applied to spectra data. It is designed to ensure representative distribution of spectral variability across both calibration and prediction sets (Zeaiter & Rutledge, 2009).

Spectral pre-treatments

For the classical chemometrics models (i.e., PLS) developed in this study, spectral pre-treatments were applied before model development. These pre-treatments are essential for

reducing noise, baseline shifts, and scattering effects, but also facilitate the enhancement of relevant spectral features (Nicolai et al., 2007). In the present study, a variety of pre-treatment combinations were evaluated, including: i) signal-to-noise ratio enhancement and baseline correction through the application of the Savitzky-Golay (SG) filter, using 0th, 1st, or 2nd derivatives with a polynomial order of 2. A range of 25 to 35 smoothing points (window size) was tested; ii) scatter removal using either Standard Normal Variate (SNV) or Multiplicative Scatter Correction (MSC); iii) data normalization by either mean centering (MC) or autoscaling (AS). All the listed pre-treatments were applied individually or in combination, always including a normalization step.

Data Augmentation

In the context of deep chemometrics, large datasets are often considered essential to identify complex patterns and relationships within the data. The objective is to reduce the risk of overfitting and facilitate accurate, precise, and robust predictions. A data augmentation (DA) technique was employed to increase the effective dataset size. This method involved producing new calibration samples through the implementation of mathematical transformations, such as additive and multiplicative effects, on the original raw spectra. The impact of the increased dataset size on performance metrics and its influence on the outcomes was considered. The increase in data provided an equivalent sample size in the calibration process for both PLS and CNN approaches, allowing for two comparisons: i) the performance of PLS with and without data augmentation; and ii) the performance comparison between PLS and CNN using the same augmented calibration sets. DA in this research followed the methodology described by Bjerrum et al. (2017) and was applied only to the calibration set. The specific transformations were applied sequentially or randomly to each raw calibration spectrum to generate augmented spectra. These included: i) additive offset variation of ± 0.6 times the standard deviation (calculated across the spectrum); ii) linear baseline shift with slope variation within the range of $\sigma \pm 0.1$ (where σ is the spectral standard deviation); and iii) multiplicative scattering within the intensity variation of 1 ± 0.6 times the standard deviation. Nine augmented spectra for each raw spectrum were computed, thus increasing the size of the calibration set by a factor of 10.

Prediction models development

The development of prediction models involved implementing both Partial Least Squares (PLS) and Convolutional Neural Network (CNN) chemometric approaches. PLS models were developed using two types of datasets: non-augmented and augmented. In classical chemometrics, spectral pre-treatments were applied as described above. CNN models, in

contrast, were trained directly on raw augmented spectra without prior pre-treatment. In summary, the training approaches compared were as follows: i) PLS trained on pre-treated data (PLS-P); ii) PLS trained on augmented and pre-treated data (PLS-AP); and 3) CNN trained on raw augmented data.

Classical chemometrics

PLS regression models were developed using the SIMPLS algorithm (Moscetti et al., 2021). A Venetian blinds 10-fold cross-validation was performed to select the optimal number of latent variables (LVs). Cross-validation helps to avoid over- and underfitting, leading to enhanced model generalization (Bedini et al., 2023). The optimal regression models were evaluated using the independent prediction dataset. Classical chemometric analysis was performed using Matlab R2024b, coupled with PLS_Toolbox v9.5 (Eigenvector Research Inc., WA, USA).

Deep chemometrics

In the present study, the CNN architectures used were the same tested and described in the research activity conducted on the EVOO shelf-life. They consisted in an Input layer matching the spectral dimension, followed by a GaussianNoise layer (standard deviation 0.05), a Reshape layer, two 1D Convolutional layers (Conv1Ds) each followed by an activation function, a Flatten layer, a Dropout layer, Hidden Dense layers, and an Output layer. and The Conv1D layers serve for automatic feature extraction from the input data. Both used a kernel size of 32, “same” padding, and were followed by a Rectified Linear Unit (ReLU) activation function. The number of filters was varied during hyperparameter tuning: the first Conv1D layer used either 8 or 16 filters, and the second Conv1D layer used either 16 or 32 filters. They identified local patterns and structures within the spectra, capturing features such as peak shapes, widths, and relative intensities, thereby providing meaningful chemical information. The Dropout layer in the network was included to reduce the probability of overfitting by randomly setting a fraction of the input units to zero during the training phase. The Dropout layer was tested at rates of 0 and 0.5. The Dropout layer was followed by one or more Hidden Dense layers, composed of fully connected neurons. Each neuron in these Dense layers applied a Rectified Linear Unit (ReLU) activation function to its input. The number of neurons in the first Hidden Dense layer was tested with values of 64, 128, or 256. The ReLU function was selected for its ability to capture complex non-linear patterns. The training process involved adjusting the weights and biases of the neurons through backpropagation, allowing the model to learn the relationships between the input spectra (predictors) and the chemical parameters (predictands). To evaluate

the efficacy of deeper architectures in producing superior prediction performance, CNNs with multiple hidden dense layers were assessed. CNNs were categorized as Type I and Type II based on the arrangement of the hidden dense layers and their nodes. In Type I, a maximum of four hidden dense layers were investigated, with each subsequent layer having half the number of neurons compared to the previous one. If the first hidden layer contained n neurons, the second layer employed $n/2$ neurons, the third layer used $n/4$ neurons, and the fourth layer used $n/8$ neurons. In Type II, the number of hidden dense layers was increased to a maximum of six. Compared to the first hidden layer with n neurons, the second layer contained $2n$ neurons, the third had n neurons, the fourth had $n/2$ neurons, the fifth had $n/4$ neurons, and the sixth had $n/8$ neurons. In both types, each hidden layer used a ReLU activation function. The output layer of the network structure computed the final prediction based on the features extracted by the preceding hidden layers. The CNN hyperparameters and training configuration included the following settings, which were systematically explored: i) batch size of 8, 64, or 128; ii) optimizer either Adam or AdaDelta; iii) early stopping criterion monitoring the MSE, with a patience of 10 epochs and minimum change (delta) of 0; iv) kernel regularizer either none or L2 regularization with a factor of 0.01; v) mean squared error (MSE) as loss function. These hyperparameters were systematically explored through a comprehensive combination search, which ultimately led to the training of over 2 000 distinct model configurations for each chemical parameter.

Performance metrics

The Cumulative Performance Index (CPI) algorithm, tested in the previous EVOO adulteration and EVOO shelf-life activities, was used to select the best performing models. This index integrates the multiple performance metrics into a single score. The CPI calculation involves scaling the metrics, using Principal Component Analysis (PCA) to derive weights based on each metric's contribution to performance variation, normalizing the metrics, and calculating a weighted sum, as shown in Equation 1:

$$CPI_i = \sum_{j=1}^n \frac{M_{ij} k_j}{\max(M_j)} \quad (\text{eq. 1})$$

where, CPI_i is the Cumulative Performance Index for model i ; M_{ij} is the absolute value of the j -th performance metric for model i ; $\max(M_j)$ is the maximum value of the j -th metric across all models (used for normalization); k_j is the normalized weight (derived from PCA) for the j -th metric; and n is the total number of metrics. The CPI provides a consolidated ranking but is relative to the specific models evaluated. In addition, the CPI prioritized robustness (e.g., similarity between calibration and prediction errors) over achieving the absolute lowest

prediction error (RMSE). The R code and details about the CPI are available on GitHub (Moscetti, 2025).

RESULTS AND DISCUSSION

Chemical and spectral data overview

A descriptive statistical analysis was conducted to summarize the distribution of the chemical data (**Tab. 1**). In addition, the average FT-NIR and Vis spectra from the different categories of oils were graphically compared (**Fig. 1**).

In Vis spectroscopy, specific bands were observed for grape seed oil, EVOO, lampante oil, virgin olive oil, and especially pomace olive oil (**Fig. 1a**). The absorptions in the Vis range are correlated with the presence of pigments, and grapeseed and pomace oils were particularly rich in these compounds. The carotenoid region, ranging from 400 to 500 nm, showed several absorption bands. The most pronounced absorption band was centred around 414 nm, which is usually correlated with pheophytin a, pheophorbide a, and pyropheophytin a (Cayuela-Sánchez et al., 2019). A further significant spectral absorption was observed at 670 nm, corresponding to chlorophyll (Ayuso et al., 2004), with the highest intensity detected in pomace olive oil.

The average FT-NIR spectra for each oil category were baseline-corrected using Asymmetric Least Squares algorithm to allow an optimal visual comparison. The spectral bands of the samples reflected the characteristic absorptions of fatty acids, as confirmed by the pure fatty acid spectrum (brown line). The spectral profile was generally similar for all the oils studied, with higher intensities detected for EVOO and virgin oil, except for grapeseed oil at 1164 and 2145 nm. The peak absorptions in the region between 1000 and 1500 nm (**Fig. 1b**) were characteristic of the second overtones of the carbon-hydrogen bond (C-H) stretching or combination vibrations of methyl, methylene, and ethylenic groups, which are characteristic of oleic and linoleic fatty acids (Meng et al., 2023; Vieira et al., 2021). The bands at 1725 and 1760 nm (**Fig. 1c**) were indicative of the presence of stretching or combination bands of the first overtone of C-H (Casale & Simonetti, 2014), which are related to trans-unsaturated triglycerides. These bands have also been tentatively linked to trans-unsaturation in some contexts (Sato et al., 1991), although cis-unsaturation dominates in olive oil. The peaks observed at 2125 nm and 2145 nm were due to combinations of vibrational modes including the stretching mode of the COOR group, and combination bands involving double bond carbon (C=C) and C-H stretching mode vibrations of cis-unsaturated fatty acids (Mendes et al., 2015; Vieira et al., 2021). In this area, the spectral absorption is associated with fatty acids that have cis double bonds, typical of those found in vegetable oils. The terminal region of the FT-NIR

spectra (**Fig. 1d**) exhibited increased absorption, particularly at 2 308 and 2 348 nm, which is indicative of the combination of C-H stretching vibrations typically associated with long-chain compounds and the saturated groups of fatty acids (Hourant et al., 2000; Vieira et al., 2021). In summary, visual inspection of **Figure 1** suggests differences in the NIR region, potentially reflecting variations in the degree of unsaturation between oil categories.

Model development

Regression models using PLS and CNN were developed to predict each individual chemical parameter from the spectral data. An initial screening using PLS selected models with an R^2 in cross-validation (R^2_{CV}) greater than 0.75 was performed. Models that exceeded the threshold were then further developed and evaluated using both classical (PLS) and deep (CNN) chemometrics. Subsequently, the best PLS/CNN model for each chemical analyte (predictand) was selected and presented in **Table 2**. For both fatty acids and the sterol fraction, except for “erythrodiol + uvaol”, only FT-NIR-based models yielded good regression performance. Models based on the Vis spectral range outperformed FT-NIR models for acidity, peroxides, waxes, K_{232} , and K_{270} .

Classical chemometrics (PLS) provided the best performance only for the prediction of linoleic and oleic acid content using FT-NIR spectroscopy. These PLS models performed optimally when data augmentation was applied. The optimal performance was obtained using AS normalization, suggesting that enhancing subtle spectral features was crucial for predicting these parameters, as opposed to relying solely on the largest spectral variations using MC normalization. Applying the 2nd derivative was necessary to correct for baseline shifts and overlapping peaks (Nicolai et al., 2007). Considering the high dimensionality of the spectra (3 112 variables), noise reduction was performed with relatively small smoothing windows (maximum size of 41 points). The most effective scatter correction method was found to be the SNV filter. Six LVs were generally sufficient to capture most of the relevant information from the spectral data while avoiding the risk of overfitting. The performance metrics of the best PLS models were comparable across the calibration (C), and prediction (P) sets, with R^2 values consistently higher than 0.99.

Deep chemometrics (CNN) was performed on the augmented raw dataset (i.e., without spectral pre-processing). Due to the fact that CNN outperformed PLS in most of the cases, it can be speculated that Conv1D layers effectively perform feature extraction from raw data. In general, the CNN models outperformed the corresponding PLS models, ensuring lower RMSE values (detailed comparison not shown). CNN models based on FT-NIR spectra generally achieved good R^2_P values (>0.90); however, as aforementioned, for some analytes, Vis-based

CNN models often performed better than the corresponding FT-NIR models. This improvement was reflected in better accuracy and precision, with predicted values closer to the reference values. For example, for the prediction of peroxide value using CNN, the RMSEP was reduced from 1.12 mEqO₂/kg for the FT-NIR model (**Fig. 2a**) to 0.34 mEqO₂/kg for the Vis model (**Fig. 2b**), and the R²P exceeded 0.99 for the Vis model, while the BIAS remained similar (data not shown in **tab.2**).

Finally, although the CNN results generally outperformed the PLS models in this study, a larger dataset will likely be required to develop robust predictive models for all chemical parameters studied using this deep learning approach.

CONCLUSIONS

This study focused on the development of predictive models for oil quality parameters using a deep learning approach, specifically Convolutional Neural Networks (CNNs). The CNN models were compared with those obtained using classical chemometrics (PLS).

Predictive models were developed only for parameters where an initial PLS screening yielded an R² value in cross-validation greater than 0.75; thus, models were not developed for all analytes. Overall, the models developed using CNN often achieve competitive or superior predictive performance compared to PLS models for both FT-NIR and Vis spectroscopy, except for a few analytes (oleic acid and linoleic acid predicted from FT-NIR). FT-NIR spectroscopy enabled the successful prediction of a larger number of parameters compared to Vis spectroscopy. However, for several parameters that could be predicted using both spectral ranges, CNN models often achieved better performance metrics when using Vis spectroscopy data, compared to the corresponding FT-NIR data, despite the narrower spectral range of Vis.

In conclusion, deep learning approaches like CNN show significant potential as an alternative or complement to classical chemometrics for predicting olive oil quality parameters from spectral data. However, a larger dataset comprising more unique samples is needed to thoroughly evaluate the robustness and generalizability of the CNN models particularly for parameters where predictive performance was moderate (e.g., R²P < 0.90) in the current study. In addition, further investigation into different data augmentation strategies may be beneficial to potentially further improve model accuracy. Future work could include the exploration of methods for selecting informative spectral regions, the application of data fusion techniques combining FT-NIR and Vis data, and the evaluation of alternative neural network architectures.

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TABLES

Table 1. Descriptive statistics of the reference chemical parameters provided by the UMBRIAOLII refinery

Chemical parameter	unit	n	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.	st.dev
Acidity	%(m/m)	126	0.1	0.4	0.7	4.4	5.5	80.5	10.3
Peroxides	(mEqO ₂ /kg)	44	0.1	7.0	9.0	8.7	11.0	16.5	3.5
Fatty acids ethyl ester	mg/kg	41	4	11	14	16	19	44	8
Waxes	mg/kg	72	47	126	198	214	291	542	105
K ₂₃₂		112	1.47	1.83	2.12	2.23	2.49	4.44	0.55
K ₂₇₀		96	0.13	0.18	0.23	0.38	0.38	1.73	0.34
ΔK		111	0.000	0.000	0.002	0.022	0.024	0.232	0.042
Sterol composition									
Cholesterol	%	67	0.04	0.10	0.13	0.16	0.20	0.80	0.11
Brassicasterol	%	67	0.06	0.13	0.15	0.16	0.19	0.29	0.05
Campesterol	%	67	2.49	3.06	3.30	3.33	3.60	4.40	0.40
Campestanol	%	36	0.02	0.05	0.09	0.10	0.12	0.23	0.06
Stigmasterol	%	67	0.56	1.09	1.48	1.62	1.9	3.68	0.71
Beta-sitosterol	%	66	90	93	94	94	94	96	1
Δ7-stigmastenol	%	67	0.1	0.25	0.4	0.40	0.47	1.8	0.22
Δ7--avenasterol	%	67	0.17	0.39	0.51	1.79	0.68	83	10
Total Sterols	mg/Kg	67	123	159	1202	1284	1634	11195	1664
Erythrodiol + Uvaol	%	67	1.76	2.69	3.19	4.47	3.95	25.24	4.44
Fatty acid composition									
Myristic acid	%	73	0.01	0.01	0.02	0.02	0.02	0.06	0.01
Palmitic acid	%	74	7.3	12.1	13.4	13.5	15.2	19.8	2.1
Palmitoleic acid	%	74	0.15	0.93	1.11	1.20	1.47	2.45	0.44
Heptadecanoic aci	%	74	0.07	0.14	0.20	0.23	0.31	0.49	0.11
Stearic acid	%	74	1.64	2.54	2.69	2.73	2.82	4.48	0.50
Oleic acid	%	74	18.05	69.37	71.50	69.90	73.66	78.29	9.52
Linoleic acid	%	74	4.7	7.3	9.0	10.9	11.5	69.7	10.0
Arachidic acid	%	74	0.12	0.36	0.41	0.39	0.43	0.50	0.07
Linolenic acid	%	74	0.30	0.61	0.64	0.65	0.70	0.80	0.08
Eicosenoic acid	%	74	0.13	0.23	0.26	0.68	0.28	32	3.67
Behenic acid	%	74	0.03	0.10	0.12	0.12	0.14	0.22	0.03
Erucic acid	%	69	0.00	0.02	0.02	0.05	0.02	2.00	0.24
Lignoceric acid	%	73	0.03	0.04	0.05	0.05	0.06	0.08	0.01

n, number of samples

Table 2. Performance metrics (RMSE, BIAS, and R^2) on Calibration (C), and Prediction (P) sets for the best regression models of chemical parameters using FT-NIR and Vis spectroscopy.

Adulterant	Spectrosc.		Preprocessing					LVs	RMSE			BIAS			R ²		
			SG	Deriv.	Polin.	SC	Norm.		C	CV	P	C	CV	P	C	CV	P
Acidity	FT-NIR	CNN-II	-	-	-	-	-	-	0.214	-	0.217	1E-02	-	5E-03	0.99	-	0.99
	Vis	CNN-II	-	-	-	-	-	-	0.195	-	0.197	9E-03	-	5E-03	0.99	-	0.99
Peroxides	FT-NIR	CNN-II	-	-	-	-	-	-	0.911	-	1.118	2E-01	-	4E-02	0.93	-	0.89
	Vis	CNN-II	-	-	-	-	-	-	0.250	-	0.335	2E-02	-	7E-02	0.99	-	0.99
Ethyl ester	FT-NIR	CNN-II	-	-	-	-	-	-	1.493	-	2.450	6E-01	-	5E-01	0.97	-	0.76
Waxes	FT-NIR	CNN-II	-	-	-	-	-	-	26.08	-	36.96	1E-02	-	3E+00	0.94	-	0.84
	Vis	CNN-II	-	-	-	-	-	-	6.584	-	21.72	4E-01	-	5E-01	0.99	-	0.94
	Vis	CNN-II	-	-	-	-	-	-	0.070	-	0.078	1E-02	-	6E-03	0.99	-	0.97
K ₂₆₈	FT-NIR	CNN-II	-	-	-	-	-	-	0.074	-	0.076	2E-02	-	1E-02	0.96	-	0.92
	Vis	CNN-II	-	-	-	-	-	-	0.020	-	0.037	9E-04	-	2E-03	0.99	-	0.98
K ₂₇₀	FT-NIR	CNN-II	-	-	-	-	-	-	0.057	-	0.057	4E-03	-	5E-04	0.98	-	0.95
	Vis	CNN-II	-	-	-	-	-	-	0.029	-	0.029	4E-03	-	2E-03	0.99	-	0.99
ΔK	FT-NIR	CNN-II	-	-	-	-	-	-	0.008	-	0.011	1E-03	-	1E-03	0.96	-	0.94
	Vis	CNN-II	-	-	-	-	-	-	0.010	-	0.012	6E-04	-	2E-04	0.94	-	0.92
Linoleic acid	FT-NIR	PLS-A	41	2nd	2	SNV	AS	6	0.692	0.694	0.621	5E-15	-1E-05	-2E-01	0.99	0.99	0.99
Oleic acid	FT-NIR	PLS-A	27	2nd	2	MSC	AS	6	0.877	0.879	0.987	-2E-14	3E-04	4E-01	0.99	0.99	0.99
Palmitic acid	FT-NIR	CNN-II	-	-	-	-	-	-	0.552	-	0.547	5E-02	-	4E-02	0.93	-	0.92
Palmitoleic-acid	FT-NIR	CNN-II	-	-	-	-	-	-	0.138	-	0.139	3E-03	-	9E-04	0.90	-	0.91
Arachidic acid	FT-NIR	CNN-II	-	-	-	-	-	-	0.019	-	0.029	1E-03	-	4E-03	0.93	-	0.76
Campestanol	FT-NIR	CNN-II	-	-	-	-	-	-	0.015	-	0.030	3E-03	-	4E-03	0.94	-	0.75
Stigmasterol	FT-NIR	CNN-II	-	-	-	-	-	-	0.274	-	0.275	1E-01	-	1E-01	0.84	-	0.86
Total Sterols	FT-NIR	CNN-II	-	-	-	-	-	-	227.9	-	372.5	8E+00	-	8E+00	0.98	-	0.94
Erythrodiol + Uvaol	FT-NIR	CNN-II	-	-	-	-	-	-	0.714		0.794	2E-02	-	6E-02	0.98	-	0.95
	Vis	CNN-II	-	-	-	-	-	-	0.284		0.295	2E-03	-	1E-02	0.99	-	0.99

FIGURES

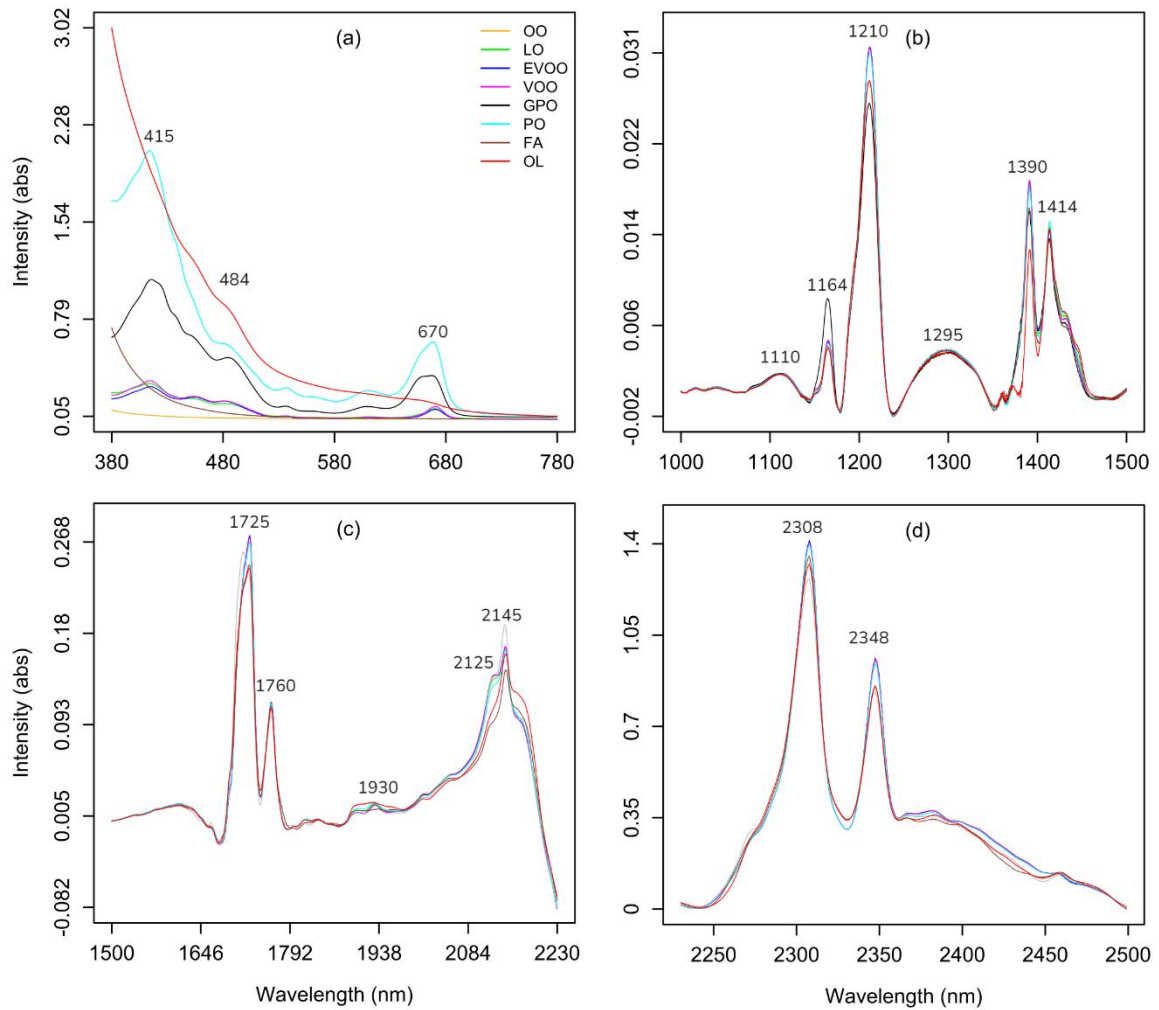


Figure 1. Average spectral profiles of the different oil/derivative categories analyzed. (a) Visible (Vis) absorption spectra (380-780 nm); (b), (c), and (d): baseline-corrected Fourier Transform Near-Infrared (FT-NIR) absorption spectra in the ranges 1000-1500 nm, 1500-2230 nm, and 2230-2500 nm, respectively.

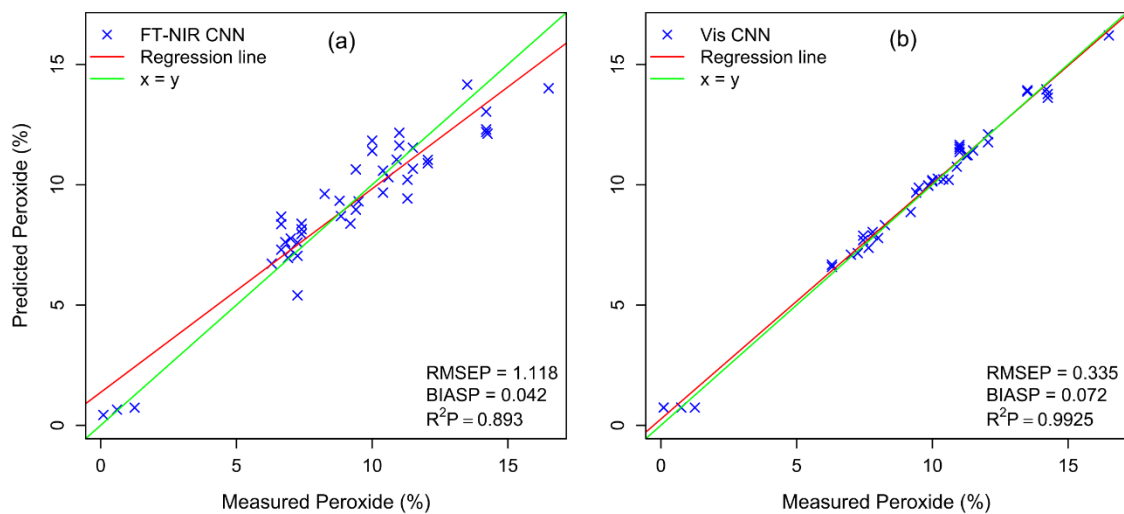


Figure 2. Regression plots for the best FT-NIR (a) and Vis (b) CNN models

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ADULTERATION OF EVOO 5

FOOD CONTROL

COMPARING DEEP AND CLASSICAL CHEMOMETRICS: CAN CNN
ENHANCE THE ACCURACY OF EVOO ADULTERATION FROM
SPECTRAL DATA?

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Keywords: Extra virgin olive oil, Seeds oils, Convolutional Neural Network, Partial Least Squares, FT-NIR spectroscopy, Vis-NIR spectroscopy

Abstract

Extra virgin olive oil (EVOO) has a high economic value and is therefore susceptible to adulteration with oils of lower quality and price. Spectroscopy, although not recognized as an official analysis methodology in EVOO, can be used in rapid screening to detect adulterants. Predictive models are usually developed with classical chemometrics, which require human knowledge in feature engineering. Deep chemometrics overcome human intervention by relying on neural networks. This study compares the use of PLS (Partial Least Squares) and

CNN (Convolutional Neural Network) algorithm in combination with FT-NIR (1000–2500 nm) and Vis-NIR (380–900 nm) spectroscopy to predict EVOO adulteration using four different seed oils (peanut, maize, sunflower and soya). Adulterant concentrations at 0.5 % and 1.5 % were difficult to distinguish, as the subtle spectral changes were often masked by a low signal-to-noise ratio mainly due to high spectral similarity with the pure EVOO, instrumental noise, and intrinsic oil variability. The FT-NIR-based regressions generally showed minimal performance differences between PLS and CNN, regardless of the application of spectral pretreatment or data augmentation (RMSEP = 0.99–2.08 %), indicating that for this spectral range, the added complexity of CNN offered no significant advantage. Only the model obtained using CNN and FT-NIR for peanut adulteration was not able to converge. In contrast, the Vis-NIR models based on CNN significantly outperformed the PLS models, regardless of the use of pretreatment or data augmentation. Therefore, in the present study, deep chemometrics proved not to be a universal replacement for classical chemometrics, but rather a complementary tool that demonstrates its true value where the classical approach is less effective.

Highlights

- The deep chemometrics is feasible in detecting seed oil adulteration in EVOO
- Convolutional Neural Network models outperformed PLS models in the Visible range
- Data augmentation showed potential in improving PLS models' robustness
- Spectral pretreatment was generally useful in enhancing CNN models' performance

List of acronyms:

ANN - Artificial Neural Network	PLS - Partial Least Squares
AS - Autoscaling	PUFA - Polyunsaturated Fatty Acid
ASHA - Adaptive Asynchronous Successive Halving Algorithm	R^2 - Coefficient of Determination
BIAS - Systematic Error	ReLU - Rectified Linear Unit
C - Calibration	RMSE - Root Mean Squared Error
CPI - Cumulative Performance Index	RNN - Recurrent Neural Network
CNN - Convolutional Neural Network	SG - Savitzky-Golay Filter
CONV 1D - One-Dimensional Convolutional Layer	SIMPLS - Simplified Partial Least Squares Algorithm
CV - Cross-Validation	SNV - Standard Normal Variate
DA - Data Augmentation	t-SNE - t-Distributed Stochastic Neighbor Embedding
DL - Deep Learning	UV-Vis - Ultraviolet-Visible Spectroscopy
DNN - Deep Neural Network	Vis-NIR - Visible and Near-Infrared Spectroscopy
EVOO - Extra Virgin Olive Oil	
ELU - Exponential Linear Unit	
FC - Fully Connected Layer	
FT-NIR - Fourier Transform Near-Infrared Spectroscopy	
GAN - Generative Adversarial Network	
HPC - High-Performance Computing	
LV - Latent Variable	
MC - Mean Centering	
ML - Machine Learning	
MLDE - Machine Learning Development Environment	
MSC - Multiplicative Scatter Correction	
MSE - Mean Squared Error	
MUFA - Monounsaturated Fatty Acid	
NN - Neural Network	
P - Prediction	
PC - Principal Component	
PCA - Principal Component Analysis	

INTRODUCTION

Extra virgin olive oil (EVOO) is one of the most important vegetable-derived edible oils (Aslam et al., 2024). In recent years, global olive oil production has exhibited a fluctuating trend, with an average annual production of around 3 million tons (IOC, 2024). Approximately 61 % of this production comes from the European Union. In Europe, EVOO is subject to a rigorous legislation framework, which regulates the entire product process from the agronomic level up to the final production. In fact, the EVOO must be obtained “from the fruit of the olive tree solely by mechanical, which have not undergone any treatment other than washing, decantation, centrifugation or filtration” (EU, 2013). Furthermore, in order to be classified as “Extra”, the product must also satisfy relevant chemical and sensory requirements (EU, 2022).

The EVOO market is expanding due to its healthy properties and sensorial attributes, which contribute to its high commercial value; it is rich in monounsaturated fatty acids and antioxidant compounds, including phenols and tocopherols (X. X. Li & Wang, 2018). Consequently, as with other high-value foods, EVOO is vulnerable to fraudulent practices, especially adulteration with cheaper oils. Unfortunately, the adulteration of EVOO is particularly difficult to detect due to its chemical profile’s similarity with other vegetable oils (Tian et al., 2023). For example, the phenolic content of EVOO is comparable to that of pumpkin seed and sunflower oils and a high percentage of oleic acid has also been found in peanut, hazelnut, papaya and rapeseed oils. The challenge of efficiently detecting EVOO adulteration has led to the investigation of various analytical techniques and technologies (e.g., sensory analysis, wet chemistry, chromatography, electronic nose, electronic tongue, DNA method, nuclear magnetic resonance) (Meng et al., 2023; Ye & Meng, 2022). Apart from their performance, these approaches can be characterized by significant drawbacks. For instance, gas chromatography and high-performance liquid chromatography can be expensive due to high instrumentation costs and the requirement for solvents and standards. They are also time-consuming due to complex sample preparation procedures and extended run times. Furthermore, most of these methods are destructive, require skilled personnel to operate the instrumentation and interpret the data, and involve the use of harmful and non-environmentally friendly chemicals, such as hexane, chloroform, or acetonitrile, for extraction and as mobile phases. These limitations make traditional methods less suitable for rapid and on-site screening. This highlights the need for analytical strategies that, while not always real alternatives to traditional methods, can significantly speed up the screening process, reducing the reliance on extensive and resource-intensive techniques.

It is well known that spectroscopy has the potential to overcome the aforementioned problems. For oil analysis, techniques such as UV–Vis and FT-NIR spectroscopy are

particularly advantageous due to their fast, non destructive, environmentally friendly, and relatively inexpensive nature, potentially providing accurate and sensitive results with minimal or no sample preparation. Specifically, UV–Vis spectroscopy can investigate characteristics related to chromophoric compounds (i.e., pigments), as well as oxidation status indexes (i.e., K-values), while FTNIR spectroscopy can explore chemical composition and molecular structure (e.g. the chemical fingerprint of C-H, N-H and O-H functional groups) (Valli et al., 2021). For these reasons, the use of electronic and/or vibrational spectroscopy for the identification of adulterants in oil has been the subject of many studies (Uncu & Ozen, 2019). This technique facilitates the acquisition of a high number of spectra in a relatively short time period, which can be correlated with the chemical and physical properties of the product multivariate analysis (Ozdemir & Oztürk, 2020). In particular, correlation can be achieved through chemometrics, a branch of chemistry that uses statistical and mathematical tools (Hashempour-baltork et al., 2024) to develop models that correlate spectral data with analyte concentrations. Classical chemometrics used in spectrophotometry requires a data pre-processing step to improve the accuracy and robustness of the developed models by removing irrelevant information and distortions from spectra. Popular pre-processing methods include baseline correction, scatter correction, data smoothing and scaling (X. Wang et al., 2020). In this context, the selection of the most appropriate pre-processing type, training algorithm, and hyperparameters forms part of the ‘feature engineering’ process, which is fundamental to the extraction and organization of the information obtained from the original data prior to model training (Bengio et al., 2013). However, it should be noted that the choices are inherently subjective and influenced by the operator’s skills and experience (Acquarelli et al., 2017); the great unspoken is that the researcher is forced to compare hundreds, if not thousands, of pre-treatment/algorithm combinations in an attempt to achieve the best model. As a result, the operator may be overwhelmed by the extensive range of potential tests, which can result in an inability to consider all relevant features. This, in turn, can have a detrimental effect on the accuracy, precision, and robustness of the final model. To partially overcome the issue, pioneering studies report tests in spectroscopy using Artificial Neural Networks (ANNs). The basic structure of these machine learning (ML) techniques is built on layers of interconnected nodes, which mimic human neurons and are capable of learning through an iterative process (Vega-M´arquez et al., 2019). The technique can enable the use of raw spectra, potentially reducing reliance on manual pre-treatments; however, suitable pre-processing may still be beneficial, as inadequate feature representation can slow or even prevent model convergence (Krohling & Krohling, 2023; Liu et al., 2017; Mishra et al., 2022; Shen & Viscarra Rossel,

2021). Anyway, ANNs still remain a valid alternative to other ML algorithms applied to spectrophotometry. The fairly recent innovation in the ML field is represented by the growing interest in deep learning (DL) (Sitorus & Lapcharoensuk, 2024), which has risen to prominence in the last two decades or so in parallel with the increasing availability of computational power and the capability to process large amounts of training data (e.g. big data). DL algorithms are generally referred to as Deep Neural Networks (DNNs), and include different categories developed for specific applications (e.g., Convolutional Neural Network (CNN), Recurrent Neural Network (RNN), Generative Adversarial Network (GAN)). The CNNs are widely used in 2D datasets (X. Wang et al., 2020). However, they are also compatible with 1D datasets, including one-dimensional spectroscopic data. Recent studies have demonstrated the effectiveness of 1D CNNs in spectral data analysis by i) achieving high precision and accuracy even with noisy and overlapping signals (Krohling & Krohling, 2023; Nallan Chakravartula et al., 2022; Sitorus & Lapcharoensuk, 2024); ii) solving challenges associated with ANNs, such as overfitting and classifier interpretability (J. Wang et al., 2025); iii) incorporating regularization techniques that reduce linear dependencies in the dataset (i.e., dropout, as a method to prevent overfitting and improve generalization) (Nallan Chakravartula et al., 2022); iv) reducing data complexity by smoothing effect on the spectral data, enabling the selection of spectral regions and reducing dependencies between neurons, which is relevant in spectral data analysis for accurate results (Hahn & Choi, 2020; Xu et al., 2021); and v) being applicable to different contexts in the food industry, including the identification of adulterants in olive oil and their quantification with different technologies (Wu et al., 2022, 2024).

Despite the DNNs started being applied in various manners to the olive oil sector, to the best of our knowledge there are no studies benchmarking classical and deep chemometrics, in combination with Vis-NIR and FT-NIR spectroscopy, to quantify EVOO adulteration with vegetable oils. This study was designed to address this gap. Our goal is not to introduce a novel deep learning methodology, but to determine when the significant computational complexity required by deep learning is actually justified. Therefore, in this study, a head-to-head comparison was made: four different seed oils were individually mixed to EVOO at graded concentrations, and identical datasets were used to test whether a compact CNN can match or surpass the precision and accuracy of its classical counterpart.

MATERIALS AND METHODS

Samples preparation

EVOO, already filtered through diatomaceous earth at the oil-mill plant, and seed oils (i.e., maize, peanut, soy, and sunflower) were purchased from local retailers (Viterbo, Latium, Italy), filtered again in the laboratory with 200 μm filter paper, and stored in the dark at room temperature until the use. The laboratory filtration was included as a precautionary step to remove any accidental solid/suspended particles, whose absence is fundamental for minimizing scattering and baseline variations due to suspension particles during measurements, thereby limiting negative effects on model performance. Adulterated samples were prepared by blending the EVOO with the adulterant at the following percentages: 0 (pure EVOO), 0.5, 1.5, 3, 5, 10, 15, 20, and 100 % w/w (pure adulterant). The 0.5–20 % window was selected to improve on the 2.7–20 % detection limits reported by Vanstone et al. (2018). A 60 % w/w blend was also prepared, but despite vigorous shaking it showed rapid phase separation within minutes; the observed instability, likely due to differences in density or polarity, made these samples non-homogeneous and unreliable for repeatable measurements. Therefore, after some calibration tests, the 60-% spectra were excluded from the model development. In contrast, the 100 % w/w samples were retained. Rather than ensuring linearity across the 0–100 % range, their role was to serve as a regression endmember, defining the boundary condition for the adulterant and helping to orient the regression model. The statistical leverage of the 100 % point and its impact on performance metrics are discussed in Section 3.2.1.

In total, four adulteration batches were prepared, one for each seed oil. For each adulteration mixture sixteen biological replicates (i.e., independent oil samples each prepared from a different batch) were performed, while the pure EVOO (0 %) was subjected to seven biological replicates and the pure seed oil (100 %) to six biological replicates. Finally, each sample was scanned through both Vis-NIR and FT-NIR spectrophotometers.

The study was performed by acquiring samples for two consecutive years (i.e., 2022 and 2023), with the objective of reducing the impact of season variability.

Spectral data acquisition

Prior to scan, the oil mixture was vortexed for 10 s and placed into a 1-mm quartz optical cell. The Vis-NIR spectrum was acquired within the range of 380–900 nm ($\sim 1\text{-nm}$ resolution) using a UV–Vis spectrophotometer mod. Lambda 850+ (PerkinElmer, USA); the NIR measurement was performed at 1000–2500 nm ($\sim 0.48\text{ nm}$ resolution) with a FT-NIR spectrophotometer mod. Antaris II (Thermo Fisher Scientific, USA). These resolutions

reflected the instruments' intrinsic operating modes. The Antaris II operates at a constant resolution of 2 cm^{-1} (0.19–1.21 nm across the range), while the Lambda 850+ uses a standard $\sim 1\text{ nm}$ slitdefined bandpass. Consequently, identical spectral resolutions were not feasible but, most importantly, the chosen settings allowed the two instruments to operate under the most similar conditions.

Each spectrum was obtained as an average of 64 sub-scans. All scans were performed at controlled temperature: i.e., 25 and 32 °C for Vis-NIR and FT-NIR, respectively. The temperatures were selected based on the operational specifications of the respective instruments. The key consideration was to maintain a stable temperature for each instrument throughout the analyses, ensuring reliable and reproducible results. In other words, while temperature can affect spectral features by maintaining a temperature control for each instrument independently, we aimed to minimize any temperature-dependent variability within each dataset. Three technical replicates were performed for each sample, thereby obtaining 375 spectra in total.

Data handling and data mining

Spectral data overview

Prior to model development, a comparison of the spectral data was carried out using graphical summary representations performed with two approaches: i) visualizing the mathematical spectral differences between EVOO and various adulterants over the wavelengths; and ii) using the t-distributed Stochastic Neighbor Embedding (t-SNE) algorithm, based on manifold learning, to reveal differences/similarities due to the type of adulterant and its concentration. Compared to PCA, which is a linear algorithm that preserves the total variance and consequently only its global structure (Luo et al., 2021; Nallan Chakravartula et al., 2022), t-SNE has the advantage to preserve both the global and local structures of the original data. In the present study, based on various preliminary tests and results obtained, the t-SNE was preferred to the PCA. The hyperparameters used for t-SNE included the output dimensionality (i.e., the number of Y variables in which the spectral data are compressed) and the perplexity value (i.e., the balance between local and global aspects of data), which were set to 2 and 9, respectively. The perplexity was set to the maximum value allowed by the sample size. This setting was chosen to best capture the global separation between sample groups, as lower values in preliminary tests produced less informative graphical abstracts.

Data split in calibration and prediction sets

In the development of predictive models, it is essential to find the optimal trade-off between under- and over-fitting. Consequently, the dataset from each batch was split into calibration (C) and prediction (P) which corresponded to 60 and 40 % respectively. The split was performed through the use of the Onion algorithm (Gallagher & O'sullivan, 2012), a sample selection method that split data into subsets by forming successive “layers” of samples based on their distance from the dataset mean. The chosen algorithm ensured an even, stratified distribution of the adulteration percentages in both the calibration and prediction datasets.

Spectral pre-treatments

Models developed through classical chemometrics require spectral pretreatments to reduce noise, baseline shifts and scattering effects, as well as to enhance relevant spectral features (Nicolai et al., 2007). Following preliminary tests to screen various possibilities, which were also based on a study of the literature, a refined set of the most promising pretreatments was selected. Thus, the following combinations were tested: i) to improve the signal-to-noise ratio, the Savitzky-Golay (SG) smoothing filter (0th derivative) was applied. In addition, first and second-order SG derivatives were investigated for their effectiveness in correcting baseline shifts and enhancing the resolution of overlapping spectral features. Polynomial orders of 1, 2, and 3 were tested over a smoothing filter window ranging from 25 to 35 features, with the objective of finding the optimal tradeoff between noise reduction and the loss of spectral information; ii) to mitigate possible light scattering effects, which can be responsible for non-linearities and obscure relevant features, both Standard Normal Variate (SNV) and Multiplicative Scatter Correction (MSC) were tested. Although SNV and MSC belong to the same class of pretreatments, they are mathematically different and may produce different results. Thus, testing both allowed the selection of the most effective one for the sample set; iii) finally, to ensure that each feature properly contributed to the model, the data were normalized by either mean centering (MC) or autoscaling (AS). All pretreatments were applied individually or in combination, with normalization always included.

Data Augmentation

The primary objective of this study was to conduct an unbiased comparison between classical and deep chemometrics. A critical difference between the two approaches lies in their data structure requirements. In fact, deep chemometrics requires larger datasets than classical chemometrics to capture complex patterns and relationships within the data, avoid overfitting, and allow accurate, precise, and robust predictions. One common technique used to artificially

increase the dataset size is data augmentation (DA), which involves generating new calibration samples by applying mathematical transformations, such as additive and multiplicative effects, to the raw spectra. Therefore, to ensure that any performance differences between classical and deep chemometrics were attributable to the algorithms themselves, DA was applied to create identical (augmented) training sets for both approaches. Comparing the performance of classical chemometrics with and without DA was a secondary objective for the present study.

In detail, data augmentation was conducted by using the method proposed by Bjerrum et al. (2017). Thus, the standard deviation was computed over all values in the raw calibration dataset, resulting in a single global value used for augmentation. Subsequently, the following transformations were applied to the calibration set only: i) offset variation of ± 0.6 times the standard deviation; ii) baseline shift with slope variation within the range of $\sigma \pm 0.1$; and iii) additive/multiplicative scattering within the intensity variation of 1 ± 0.6 times the standard deviation. The technique was applied to generate 9 augmented spectra for each raw spectrum, thereby increasing the calibration set size of 10 times (Supplementary **Figs. 1**). Augmented spectra were generated only for the calibration set to avoid overoptimistic prediction results that arise when highly correlated (augmented) duplicates are introduced into the prediction set. This approach aligns with the fundamental principle, as reviewed by Mumuni and Mumuni (2022), that data augmentation is a tool specifically for enriching the training data to improve model generalization. In fact, the presence of synthetic spectra, instead of only unseen samples, would make the prediction test artificially easy, deflating both RMSEP and BIASP and inflating R^2P .

Adulteration prediction models development

Prediction models were developed using the Partial Least Squares (PLS) and the Convolutional Neural Network for the classical and deep chemometrics approaches, respectively. Table 1 shows all the model's configurations tested. In detail, models were developed using two types of datasets: i.e., not augmented (a) and augmented (A). Spectral pretreatments were applied for all models developed through classical chemometrics, while deep learning models were tested using not pretreated (p) and pretreated (P) spectra. In summary, the training approaches used were the following: PLS-aP, trained on pretreated data only; PLS-AP, trained on augmented and pretreated data; CNN-Ap, trained on raw augmented data; and CNN-AP, trained on augmented and pretreated data. All model's configurations were calibrated and validated for each seed oil adulterated batch.

Classical chemometrics

The models were developed using the Partial Least Squares (PLS) regression through the SIMPLS algorithm (Moscetti et al., 2021). A leave-one-level-out cross-validation by adulteration level was performed to carefully select the optimal numbers of latent variables (LVs), thus helping to prevent over- and underfitting by ensuring a better model generalization (Bedini et al., 2023). The β -coefficient vector, also known as regression vector, was computed to assess the relative contribution of each feature or subset of features to the performance of each PLS model. The best performing models were tested using the prediction dataset. Classical chemometrics was performed using Matlab software R2017b coupled with PLS_Toolbox software v8.6.2 (Eigenvector Research Inc., WA, USA).

Deep chemometrics

Deep-Learning tasks require high computational power; thus, CNN models were developed using a High-Performance Computing (HPC) unit (mod. HPE ProLiant DL380) equipped with 2 Intel(R) Xeon(R) Gold 6240R CPUs with 24 cores each, 256 GB of RAM and an Nvidia GPU mod. A100 with 40 GB of VRAM. The underlying software stack present on the machine was composed of Ubuntu v20.04.6 LTS, Nvidia CUDA Toolkit v11.2, Nvidia driver v460.73.01, and Docker v26.1.4. As development software, the Nvidia RAPIDS Notebook (docker image: 23.12-cuda11.2-py3.10) was used as a DL framework based on PyTorch v2.2.0. HPE MLDE (Machine Learning Development Environment) v0.25.1 was used for model calibration, model prediction and the selection of the best hyperparameters' combination (HPE-MLDE, 2025). MLDE is a DL platform that has the aim to help users in all the steps of model development on High Performance Computing (HPC) systems making it easier and more efficient, with particular attention to hyperparameters' tuning.

The starting point for the calibration and prediction of CNN-based models was the rule-of-thumb structure proposed by Cui and Fearn (2018).

- one 1D convolutional layer on top (Conv1D);
- four fully connected layers (FCs), with the last one (FC4) producing the predicted values as output;
- two different types of activation functions to introduce non-linearity and help model to learn complex spectral patterns: an Exponential Linear Unit (ELU) after the Conv1D layer and a Rectified Linear Unit (ReLU) after each FC layer; weight initialization using Kaiming technique, a method to avoid the occurrence of exploding and/or vanishing gradients while calibration;

- ADAM optimizer, a stochastic gradient descent method that adapts the learning rate for each parameter by estimating both first order (mean) and second order (uncentered variance) moments;
- Mean Squared Error (MSE) as loss metric.

Precisely, the aforementioned baseline structure was modified, tested and optimized to improve both accuracy and precision of predicting the percentage of oil adulteration based on spectral data. In our proposed version (**Fig. 1**), the number, type, and order of the layers remained unchanged, while changes were made to other components as follows.

- ELU was applied as an activation function after Conv1D and each FC layer, rather than applying it only after the convolutional layer. This modification made the model more accurate in the approximation of the relation between predictand and predictors at the cost of increasing the computational complexity of the model. Moreover, it increased the efficiency of weights updates and robustness against noise;
- for the optimizer, the Adam algorithm was substituted by the AdamW, which implements weight decay for a better regularization, thus improving the reduction of the risk of overfitting;
- Root Mean Squared Error (RMSE) was used as loss function instead of MSE to make model more sensitive to larger errors, reducing the impact of significant outliers;
- all the weights and parameters were adopted in double precision to obtain models with high sensitivity to improve numerical accuracy, enhancing predictive performance and reliability;
- feature selection implemented through the Pearson Correlation Coefficient calculated for the input features. Using the SelectKbest method from the scikit-learn's feature_selection package (Pedregosa et al., 2011) with f_regression as score function, the features were ranked in ascending order based on their score values. The number of the input variables for the model was chosen by the parameter N_features defining how many of the top ranked features had to be selected during calibration.

The model performances were not only affected by the structure and the optimizer used, but also by the hyperparameters, which can have a huge impact (Yang & Shami, 2020). Testing different values of all hyperparameters to identify the optimal configuration can be time-consuming because it requires calibration and prediction of the model for each combination. This process can be facilitated by using a fit-for-purpose algorithm for the exploration of hyperparameters space. Among them, it was chosen the adaptive Asynchronous Successive Halving Algorithm (ASHA) (L. Li et al., 2018), a modified multi-armed bandit method which

tests multiple hyperparameters' combination at the same time, performing early stopping of the worst performing ones. Adaptive ASHA is one of the search algorithms already implemented in MLDE and it was used to find the optimal combination of the hyperparameters of the CNN model schematized in **Fig. 1**. Each CNN model was calibrated using 200 hyperparameters' combinations with a maximum number of epochs equal to 500.

Performance metrics

Model performance was evaluated in terms of Root Mean Square Error (RMSE), systematic error (BIAS), and coefficient of determination (R^2) of calibration (C), cross-validation (CV) and prediction (P) (Nallan Chakravartula et al., 2022). In addition, the following metrics were computed with the aim of improving the best model selection in terms of robustness: RMSEC:RMSEP, RMSECV:RMSEP, $R^2C:R^2P$, $R^2CV:R^2P$, |BIASC - BIASP| and |BIASCV - BIASP|. A total number of 15 performance metrics were then obtained. As a further step, an ad hoc Cumulative Performance Index (CPI) was developed to facilitate the selection of the best performing models by aggregating the performance metrics into a single, interpretable, index value. For Partial Least Squares (PLS) models, 15 error metrics were considered, while for CNNs the number of metrics was limited to 9 due to the unavailability of cross-validation metrics during the development of the neural network.

The CPI calculation consisted of the following steps: i) BIAS threshold adjustment - an arbitrary BIAS threshold of 0.5 % was applied. Specifically, for BIAS with absolute values below the threshold, the BIAS values were replaced with the threshold value. For BIAS values above the threshold, the actual BIAS values were retained. This step allowed penalizing only those models that exceeded the systematic error limit, which was set based on the acceptable level of accuracy defined for the study. In other words, the threshold was crucial to ensure that models with very low BIAS but high RMSE did not overwhelm models with acceptable BIAS and lower RMSE; ii) metrics scaling and Principal Component Analysis (PCA) - all performance metrics were scaled to ensure comparability. PCA was then applied and the principal components (PCs) accounting for at least 95 % of the total variance were retained. This step was fundamental to reduce the dimensionality of the data and to remove collinearity between metrics; iii) weighting by variance proportion - for each retained PC, the loadings were multiplied by the proportion of the total variance explained by that component. This step ensured that the contribution of each PC to the index reflected its importance in describing the overall variability of the metrics; iv) normalization of weights - the resulting weighted loading matrix was normalized by dividing its elements by the sum of the matrix. This step ensures that the derived weights (k coefficients) were comparable across all metrics and models; v)

calculation of the CPI - the k coefficients were used as weights for the original, normalized performance metrics. Thus, the CPI was computed as the sum of the product of each metric and its corresponding k coefficient. The resulting CPI values ranged from 0 to 1, with higher values indicating better model performance. The CPI formula is shown in eq. (1):

$$CPI_i = \sum_{j=1}^n \frac{M_{ij} \cdot k_j}{\max(M_j)} \quad (\text{eq. 1})$$

where, CPI_i is the Cumulative Performance Index for model i ; M_{ij} is the absolute value of the j -th performance metric for model i ; $\max(M_j)$ is the maximum value of the j -th metric across all models (for normalization); k_j is the normalized weight (derived from PCA) for the j -th metric; and n is the total number of metrics.

Finally, it is important to consider that the CPI calculation was applied individually to the performance matrix obtained from each category of model: i.e., PLS-aP, PLS-AP, CNN-AP, and CNN-AP. The objective was not to compare the performance among the different approaches used, but rather to distill the best models obtained from each one of them.

This methodology provides a robust, standardized approach to evaluate complex models by integrating diverse performance metrics into a single, interpretable index. The R code for the CPI is open source and available on GitHub (Moscetti, 2025). The workflow of the entire development process described in this chapter is summarized in **Fig. 2**.

RESULTS AND DISCUSSION

Spectral differences among oils

The differences between the pure spectra of EVOO and the seed oils were calculated using data from FT-NIR and Vis-NIR spectrophotometry to highlight the macroscopic differences between the EVOO and the adulterants used (**Fig. 3a and b**). Prior to this calculation, the FT-NIR data were subjected to SNV to remove most of the variability due to light scattering. Vis-NIR spectra were used as raw data. Positive values in the profiles obtained correspond to higher values of EVOO.

Fig. 3a shows the observed differences in the NIR spectral region (1000–2500 nm) for EVOO compared to maize and peanut oils. The spectral differences for sunflower and soy were not shown because, on the y-axis scale required to represent all bands, their profiles lie on top of the maize profile. In other words, adding three nearly identical profiles would obscure a by-eye evaluation of macroscopic information. In general, the plot primarily reflects the differences in terms of fatty acid composition, with three distinct spectral regions associated to specific

chemical bonds and molecular structures: i) 1000–1600 nm; ii) 1600–2000; and iii) 2000–2500 nm. However, these differences between EVOO and peanut oil were minimal when compared to the other seed oils. In fact, the plot shows that the spectral differences between EVOO and maize oil were much higher than those observed in peanut oil, covering a wider range of wavelengths, thus facilitating a clearer visual differentiation between the two. In other words, the spectral profile of peanut oil shows a fingerprint very similar to that of EVOO, suggesting that building models capable of quantifying the adulteration with peanut oil may be more challenging. Turning to the plot, the differences in the 1200–1400 nm were attributed to the combination and stretching vibrations of the second overtone resulting from the carbonhydrogen bond (C-H) of the methyl, methylene and ethylene groups of fatty acids, especially oleic and linoleic (Casale & Simonetti, 2014; Vieira et al., 2021). Bands in the 1400–1450 nm range suggested the presence of hydroxyl groups (Zaukuu et al., 2024) of water, free fatty acids, and other trace compounds (e.g., hydroperoxides, mono-/diglycerides, phytosterols, tocopherols, etc.), but their contribution to the oil differentiation was negligible. The 1600–2000 nm region was mainly associated with triacylglycerols and is associated with the first overtone of C-H stretching vibrations in methyl, methylene and ethylene groups. EVOO showed a higher absorbance at 1729–1763 nm, typically associated with monounsaturated fatty acids (MUFAs), which are abundant in olive oil (Hourant et al., 2000). In contrast, maize oil showed a notable and characteristic peak at 1704 nm, indicative of polyunsaturated fatty acids (PUFAs). An opposite trend was observed when EVOO was compared with peanut oil, particularly in terms of MUFA content. Spectral differences observed from 2000 nm to 2500 nm were generally attributed to combined bands of both C-H stretching vibrations of cis double bonds of unsaturated fatty acids and carbon-oxygen (C-O) vibrations of double bonds (Casale & Simonetti, 2014; Hourant et al., 2000; Meng et al., 2023). Maize oil had higher absorptions at 2142, 2179, 2463 and 2481 nm with the most pronounced differences at 2269 and 2329 nm. EVOO oil showed characteristic bands at 2309 and 2348 nm and in the range 2380–2450 nm. As observed at 1700 nm, the spectral differences in the 2000–2500 nm range between peanut oil and EVOO were opposite to those of maize oil and less pronounced.

Fig. 3b shows the spectral differences observed in the Vis-NIR range (380–780 nm) with a small portion of the NIR region (780–900 nm). Since the absorption in the Vis-NIR spectrum is primarily due to electronic transitions within molecules, which are often associated with specific chromophores (e.g., chlorophyll, carotenoids), the observed differences were mainly attributed to specific compounds rather than chemical bonds. Consequently, due to the low amount of pigments in the tested seed oils, their Vis-NIR spectra did not show distinctive peaks

(data not shown), and thus the subtraction from the pure EVOO spectrum resulted in profiles with a shape similar to that of EVOO. The first notable difference arises from the characteristic three-peak structure of carotenoids, often observed around 400–500 nm; in our tests at 416, 455 and 481 nm. These peaks may also overlap with chlorophyll derivatives such as pheophytins (e.g., pheophytin a, pheophorbide a, and pyropheophytin a), which exhibit strong absorption in the blue light region (Cayuela et al., 2014). The 669 nm wavelength is relevant because it is associated with the chlorophyll content. In particular, it corresponds to the Q band of chlorophyll *a*, which is completely absent in the seed oils tested. The same spectral observations obtained for each mixture were confirmed in both years of the study. As a first speculative impression, the content of pigments seems to be a key factor in differentiating EVOO from seed oils in the visible spectral region. However, it is important to consider that the pigment content in EVOO is strongly affected by seasonal factors, olive variety, ripening stage, and production methods, introducing variability that may affect the robustness of models developed using the Vis-NIR spectral region (Musa, 2024). It implies that to preserve the robustness and generalizability of a predictive model, the calibration must be updated periodically by i) adding samples from multiple harvest seasons covering a wide range of cultivars, agronomic conditions, commercial brands, etc.; ii) validating the models on real independent data sets; and iii) applying periodic recalibration transfer if new instruments are introduced. In practice, the calibration set should be further populated with additional samples to capture as much natural variance as possible, remembering that data quality (reliability) is more important than quantity.

Finally, the t-SNE algorithm was employed to obtain a 2D plot summarizing the clustering patterns of EVOO adulterated with the four seed oils at different concentrations, based on the FT-NIR (**Fig. 3c**) and Vis-NIR (**Fig. 3d**) spectral datasets. Each data point was labelled with the concentration of the adulterant (%), and the axes (Y1 and Y2) represent the components identified by the t-SNE algorithm. In the FT-NIR case, t-SNE reveals subtle differences between types of adulteration, but did not clearly show distinct patterns among maize, sunflower and soy oils. On the other hand, peanut oil shows a distinct cluster mainly in the upper-right quadrant (together with pure EVOO, 0 %), but with no clear trend based on concentration. Differently, a fairly clear gradient was observed for maize, sunflower, and soy oils, with lower concentrations (0.5–1.5 %) clustering closer together and higher concentrations progressively shifting towards the bottom-left quadrant. The clustering of the 0.5–1.5- % samples visually demonstrated an analytical challenge: at the lowest adulteration levels, the subtle spectral differences introduced by the adulterant were minimally detected by the spectrophotometers. In addition, these results confirmed both the similarities observed through

the spectral differences between EVOO and peanut oil, as well as among maize, sunflower and soy oils. For the Vis-NIR range, the overlap between samples at low concentrations was confirmed. However, the algorithm allowed a visual differentiation of the samples, with a clear progression across the quadrants. This result highlights the significant impact of pigment concentration on the differentiation of oils adulterated at different levels.

Regression models

This section summarizes the results of regression models developed using FT-NIR and Vis-NIR spectral data, using both classical (PLS) and deep chemometrics (CNN) approaches, and highlights the effect of preprocessing methods and data augmentation in improving the prediction performance. In addition, the strengths and limitations of each approach are discussed. For the intended purposes, the best models selected by the Cumulative Performance Index (CPI) are reported in Table 2 to allow a detailed comparison. Before comparing the two approaches in detail, it is worth noting that in several datasets the prediction error (i.e., RMSEP) is slightly lower than the calibration or cross-validation errors (i.e., RMSEC and RMSECV, respectively), even though the opposite usually occurs. In certain cases, this inversion can imply an underfitting problem. In the present work, the risk of underfitting was carefully managed by using a leave-one-level-out cross-validation procedure to select the optimal number of latent variables for each model (i.e. the number of LVs that minimized the RMSECV value before it increased due to overfitting). Furthermore, the excellent predictive performance ($R^2P \approx 0.99$) suggests that the models successfully captured the underlying data structure. Therefore, the most plausible explanation for the observed inversion is the stochastic nature of the data split. Despite the use of the Onion algorithm to ensure representative subsets, the calibration set may have casually captured slightly more spectral complexity or variability than the prediction set, resulting in what could be considered a fortuitous data split and making the latter marginally easier to predict.

Classical chemometrics

Regarding the FT-NIR based models, the scatter correction pretreatment improved the performance of most of them, with the multiplicative scatter correction (MSC) as the most frequently used method. Since MSC was required, it means that physical phenomena such as light scattering and/or path length differences may have occurred even though the oil was filtered, negatively affecting the chemical variance of the samples in the raw data. In addition, the 1st derivatization was predominant, together with a smoothing window ranging from 25 to 35, for removing baseline offsets, enhancing subtle features and removing high-frequency

noise. Among the tested normalization methods, the mean centering (MC) prevailed over the auto-scaling (AS), leading to the assumption that most of the chemical variations of the spectra were relevant for the prediction of the adulteration, without the need to enhance subtle features by AS at the risk of introducing scaling artifacts into the model. The number of latent variables (LVs) ranged from 4 to 6 for maize, sunflower, and soy models, except for the peanut model that required 10 LVs. The higher number of LVs used in the peanut model can be related to the small spectral differences observed between EVOO and peanut oil; in other words, the model required more LVs to capture the required information from spectral data. Consequently, the model is more complex and subjected to a higher risk of overfitting. Overall, the performance of the PLS models was excellent, with RMSEP ranging from 0.99 to 1.75 %, and a |BIASP| of 0.001–0.297 %. The R^2P exceeded 0.99 for all models (data not shown). The RMSEP range is particularly important as it explains how difficult it is to reliably quantify the lowest levels of adulteration (i.e., 0.5 % and 1.5 %). In fact, when the magnitude of the model's error is similar to or greater than that of the measured predictand, distinguishing adulterated oil from pure EVOO is statistically challenging. This is not a model failure, but a limitation due to fundamental analytical factors: primarily, a low signal-to-noise ratio in spectral regions where the adulterant signal is weak (i.e., at low concentrations) and masked by strong overlapping bands from pure EVOO, instrumental noise, and the intrinsic variability of the matrix.

The best PLS model was obtained for soy, followed by sunflower, peanut and maize, with all metrics showing comparable values across calibration (C), cross-validation (CV) and prediction (P). Thus, the results proved that the models were robust, minimizing the risk of under-/ overfitting problems, even for the peanut model, despite the highest number of LVs. Several studies reported the data augmentation capability to change the distribution and variance of the features, also highlighting specific patterns within the data (Gong et al., 2019). This effect may correspond to an adjustment in the magnitude of β coefficients, which was exactly what was observed in the present study (**Fig. 4a**). However, the observed effect did not improve the predictive performance of models. Finally, a quick leverage analysis on the FT-NIR PLS model was performed to evaluate the impact of the 100 % adulteration level on model performance. The check was conducted for maize adulteration and showed that the 100 % adulteration level primarily affected R^2 . In fact, in the pretreated, non-augmented model (PLS-aP), removing the 100 % w/w sample from the calibration caused R^2P to drop from 0.99 to 0.96, whereas RMSEP changed only from 1.47 to 1.44 % w/w and |BIASP| from 0.001 to 0.020 % w/w. The same pattern was observed for the augmented model (PLS-AP): R^2P declined from 0.99 to 0.95, while RMSEP shifted from 1.52 to 1.56 % w/w and |BIASP| from 0.037 to 0.005

% w/w. These negligible changes in error metrics demonstrate that prediction accuracy is determined by the densely populated 0–20 % region rather than by the single extreme (100 %) sample. However, the exclusion of the 100 % level must be evaluated in further work, assessing the effect produced by the increase of the number of samples, as well as the introduction of intermediate levels (i. e., 20–100 %), trying to solve the mixture problem faced in this study.

Regarding the Vis-NIR range, the scatter correction pretreatment did not improve the models as expected. In fact, light scattering is proportional to the wavelength (Pilorget et al., 2016) and therefore the visible range is less affected by this physical effect compared to NIR. As in NIR, the 1st derivative was predominantly required, with a similar smoothing filter window (25–31), despite the lower spectral resolution. Again, the MC was the preferred normalization method. The number of LVs was usually higher than in NIR, ranging from 6 to 11. However, the higher model complexity did not correspond to best performances. In fact, the RMSEP ranged from 1.92 to 3.67 %, although the |BIASP| was generally lower than in NIR (0.003–0.16 %). The average R²P was equal to 0.98 (data not shown). Similar to NIR, the maize adulteration was the easiest to predict. Soy, peanut and sunflower models followed in descending order of performance. Models were less robust for peanut and sunflower adulterations. These results seem to be in contrast with the visual representation obtained from the t-SNE algorithm. However, as previously discussed, the cluster separation achieved by this algorithm was probably due to pigment-related differences. These variations qualitatively separated the constituents and allowed the presence of adulterants to be detected. However, the pigment analyses were not sufficiently chemically informative for an accurate quantification of adulteration, unlike the compositional information obtained in the NIR region, which could be related to the presence of fatty acids characteristic of one or more oils (Hourant et al., 2000; Musa, 2024). The data augmentation effect was unclear, improving results in some cases and showing no effect in others. On the contrary of NIR, the β -coefficients plot (**Fig. 4b**) showed overlaps between augmented and non-augmented models. It can be speculated that the use of data augmentation on the Vis-NIR dataset probably required a different approach compared to the NIR dataset. In the visible range the measurements are mainly affected by electronic transitions, whereas in the NIR range are due to molecular vibrations. Consequently, the same data augmentation method may not be equally effective for the two spectral regions. Further studies are needed to better understand and improve the method in the Vis-NIR electromagnetic region.

Deep chemometrics

This section not only describes the performance obtained using the CNN algorithm but also compares the results with classical chemometrics. All CNNs were calibrated using augmented data, which is a prerequisite for the development of deep learning models and tested with/without the same pretreatment selected for the best PLS models. Although this approach meets the objective of the present study (i.e., the comparison of PLS and CNN modes using the same dataset), it also represents a limitation. In fact, further studies could focus on improving CNN performance by investigating additional pretreatments, as well as different data augmentation techniques.

In the case of the FT-NIR based models, the impact of spectral pretreatment ranged from beneficial to essential, depending on the type of dataset (i.e., adulterant). For maize, sunflower, and soy, in the absence of spectral pretreatment, the RMSEP and |BIASP| range from 1.67 to 1.98 % and 0.12–1.45 %, respectively. When the pretreatment was applied, the performance of CNNs was slightly improved, with RMSEP values of 1.40–2.06 % and |BIASP| of 0.03–0.65 %. In all cases, R^2P was higher than 0.99, confirming a higher proportion of explained variance, and models showed a very good robustness. The only exception was represented by the peanut adulteration model calibrated on untreated spectral data (i.e., CNN-AP): the CNN was not capable of generalizing. This value is probably due to the high similarity between EVOO and peanut oil, thus the CNN was unable to perform correct predictions. In particular, the CNN tended to assign the same predicted percentage to all samples, resulting in the RMSE being equal to the standard deviation of the calibration dataset (RMSEP = 22.14 %). Only by applying spectral pre-treatment did the peanut model converge and produce valid predictions (Supplementary **Figs. 2**). Consequently, it is possible to speculate that, while pre-treatment could improve performance on easier tasks, it becomes a critical step when CNN training is challenged by highly similar spectral data, as was observed in the differences between the peanut and EVOO spectra. In conclusion, despite the excellent performance, the magnitude of the prediction error of CNN combined with FT-NIR data was slightly higher than PLS, not making them the best choice in terms of performance metrics. In the case of maize adulterant, the CNN-AP model was the best one and showed similar metrics of the PLS-aP model. The graphical representation of the best selected FT-NIR models is shown in **Fig. 5**. The plot did not reveal notable visual differences in the regressions, regardless of the algorithm or pretreatment used. Also observing the plot, it is clear why there was a slight improvement when spectral pretreatment was applied in deep chemometrics; it is quite clear that the worst

performance was due to the impact of a higher BIAS, leaving space for further optimization of the model during the calibration with the objective of identifying possible causes and solutions.

Specifically for the Vis-NIR range, as observed for CNNs calibrated on FT-NIR data, the use of spectral pre-treatment slightly improved model performance. Most importantly, all Vis-NIR based CNNs not only showed superior predictive performance compared to the Vis-NIR based PLS models but, in certain cases (i.e. peanut and maize adulteration), outperformed all other models. This improved performance is probably because CNNs are non-linear models capable of automatic feature extraction. Unlike PLS, which creates linear combinations of the spectrum, CNN are superior at capturing complex relationships within extensive, overlapping and noisy features, such as spectral data. Consequently, it can be speculated that the non-linear changes in the pigment-related peaks observed in the Vis-NIR spectral range may represent a dataset that is better suited to the pattern-recognition capabilities of CNNs, but which is challenging for PLS. The models calibrated on untreated datasets achieved a RMSEP of 1.22–1.55 % and a |BIASP| of 0.12–0.61 %, while those obtained from pretreated spectra showed a RMSEP of 1.17–1.42 % and a |BIASP| of 0.10–0.41 %. The regression plot for Vis-NIR based models (**Fig. 6**) shows the better performances of CNN models. PLS models were not affected by data augmentation, as spectral pretreatments did not improve the performance of the CNN models.

In addition, it is important to consider that the adopted CPI selection method penalized less robust models. In fact, some CNN models with prediction metrics very similar or slightly better than their corresponding PLS models were discarded due to a higher deviation between the calibration and prediction metrics (data not shown). It means there is much room for improvement in terms of accuracy and model robustness for CNNs that, as deep learning algorithms, require substantially larger datasets to fully exploit their potential. Thus, the results obtained with CNNs are very promising and deserve to be further investigated.

Overall, it is important to consider that the calibration design was deliberately imbalanced. In fact, most of the samples were below 20 % w/w, while the remaining range contained only the maximum concentration of 100 % w/w. This choice reflected the practical objective of quantifying economically motivated low-range adulteration, while still capturing the spectral signature of the pure adulterant. Predictions above the 0–20 % (w/w) threshold should be treated qualitatively until additional intermediate-to-high concentrations are incorporated into a future calibration. This effort should be undertaken only if detection of adulteration above 20 % is economically justified and once the observed mixing problem has been resolved.

Finally, this comparative analysis also requires critical trade-offs between classical and deep chemometrics beyond simple performance metrics. In fact, it is also important to consider that PLS models are computationally less expensive and faster to train than CNNs. Furthermore, a PLS model offers a high degree of interpretability through the analysis of regression coefficients, which allows for a direct assessment of which features are mainly involved in the prediction. In contrast, an NN works as a black box, making it difficult to understand how it works. This represents a drawback for scientific validation and requires advanced techniques that were not explored in the present work. Therefore, the potential for superior accuracy with deep learning must be weighed against its higher demands for data, computational cost, and its difficulty in model interpretability.

CONCLUSIONS

The study evaluated and compared the performance of deep chemometrics (CNN) and classical chemometrics (PLS) for the adulteration detection of EVOO with seed oils (peanut, maize, sunflower and soy) by using Vis-NIR (380–900 nm) and FT-NIR (1000–2500 nm) spectroscopy.

Qualitatively, the difference between the FT-NIR spectra of EVOO and seed oils showed characteristic profiles, except for the peanut oil that had a high similarity with EVOO. This observation was confirmed by t-SNE, which showed an overlap between EVOO and peanut oil. Diversely, Vis-NIR spectral differences between seed oils and EVOO were remarkable in all cases, as the latter was characterized by the highest content in carotenoids, chlorophyll and its derivatives. The two-dimensional representation shown by t-SNE in Vis-NIR and FT-NIR indicates a strong similarity between low concentrations of adulterants (i. e., 0.5 and 1.5 %) and pure EVOO.

The difficulty in distinguishing very low adulteration levels was confirmed by the performance metrics of the developed models, which were characterized by RMSEP values closer to 1.5 % for all models, regardless of the adulterant. In detail, both PLS and CNN approaches have been shown to be effective in detecting EVOO adulteration, but their performance varied depending on the spectral region and the specific adulterant.

PLS models were robust and performed well (RMSEP 0.99–1.75 %) in the FT-NIR region showing excellent performance for soy, sunflower, and maize oils. The PLS models based on Vis-NIR data showed poorer predictive performance (RMSEP 1.92–3.67 %). Data augmentation seems not to be capable of improving the accuracy of the PLS models; however, its small contribution was greater in FT-NIR than in Vis-NIR spectroscopy. Because of the

results obtained, in our opinion, data augmentation should be further investigated in classical chemometrics. Indeed, despite its potential to improve model stability, the number of available studies on this topic is very small and deserves more attention from the scientific community.

Conversely, CNNs demonstrated consistent improvements in predictive accuracy in the Vis-NIR region. They achieved the best results for peanut and maize adulterations (RMSEPs of 1.17 and 1.42 %, respectively). The effect of spectral pretreatment on the data used to feed the CNN models showed a slight improvement in performance only for the Vis-NIR range. In the case of FT-NIR, the pretreatment had a negligible effect on all the models, except for the peanut adulteration, where it was fundamental for the correct generalization of the model.

In summary, the answer to the question raised in the title is “yes, but in certain cases”: deep chemometrics can improve the accuracy of EVOO adulteration detection from spectral data. However, in this study, its effectiveness was most evident in spectral data in domains where PLS was unsuccessful, such as the Vis-NIR range. Thus, it is not a universal replacement for PLS, but rather a powerful complementary tool. Moreover, our comparison of raw and pre-treated input confirmed that CNNs still benefit from sensible spectral pre-processing and that future work should pair them with explainable-AI tools (e.g., saliency maps or Grad-CAM) to provide valuable insight by identifying which spectral regions most influence the model prediction. In addition, based on our findings, future research could explore several additional possibilities. For instance, it could be interesting to evaluate if i) other types of neural networks (e.g., transformers), ii) more sophisticated hyperparameter tuning approaches, and iii) advanced data augmentation strategies (e.g., DA based on generative models) are able to improve the performance of the models, regardless of the spectral treatment used. Significant advancements could also be obtained from NNs that are agnostic on the type of seed oil used to adulterate the EVOO, as well as perform feature selection and/or data fusion of various spectra ranges. In particular, the t-SNE analysis of FT-NIR data revealed that maize, sunflower, and soy oils were clearly separated from both pure EVOO and the more challenging peanut oil samples. This suggests the potential for developing a single model of these specific seed oils. In addition, long-term robustness will require calibrations updated across multiple seasons and cultivars, as well as validated on independent data sets.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Andrea Bandiera: Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Armando Camerlingo:** Writing – original draft, Methodology, Formal analysis. **Nico Sanna:** Writing – review & editing. **Costantino Zazza:** Writing – review & editing. **Alessandro Benelli:** Writing – review & editing. **Riccardo Massantini:** Writing – review & editing, Funding acquisition. **Roberto Moschetti:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

DECLARATION OF COMPETING INTEREST

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodcont.2025.111608>.

TABLES

Table 1. Summary of the type of models developed in the study.			
Type of model	Data augmentation	Data pretreatment	Model acronym
PLS	×	✓	PLS-aP
	✓	✓	PLS-AP
CNN	✓	×	CNN-Ap
	✓	✓	CNN-AP

Table 2. Summary of the best adulteration prediction models and their performance metrics.

Adulterant	Spectrosc.	Acronym.	Preprocessing					LVs		RMSE (%)			BIAS (%)		
			SG	Deriv.	Polin.	SC	Norm.		C	CV	P	C	CV	P	
Peanut	FT-NIR	PLS-aP	25	1st	2	MSC	MC	10	1.21	1.31	1.25	-5E-15	0.028	-0.175	
		PLS-AP	25	1st	2	MSC	AS	10	1.03	1.22	1.13	2E-13	0.026	-0.190	
		CNN-AP	-	-	-	-	-	-	20.28		22.14	0.045	-	0.051	
		CNN-AP	25	1st	2	MSC	AS	-	1.52		1.62	0.899	-	0.652	
Vis		PLS-aP	25	1st	2	-	MC	8	2.16	2.48	2.42	-2E-14	-0.009	0.046	
		PLS-AP	31	1st	2	-	MC	9	2.33	2.63	2.53	-2E-14	-0.041	0.117	
		CNN-AP	-	-	-	-	-	-	1.23	-	1.22	0.272	-	0.125	
		CNN-AP	31	1st	2	-	MC	-	1.16	-	1.17	0.264	-	0.203	
Sunflower	FT-NIR	PLS-aP	35	1st	2	-	MC	5	1.02	1.05	1.21	-4E-14	0.002	0.297	
		PLS-AP	31	1st	2	MSC	MC	6	1.05	1.10	1.19	-2E-13	-0.003	0.291	
		CNN-AP	-	-	-	-	-	-	1.66	-	1.67	-0.682	-	-0.439	
		CNN-AP	31	1st	2	MSC	MC	-	1.43	-	1.40	-0.498	-	-0.427	
Vis		PLS-aP	25	1st	2	-	MC	6	2.92	3.31	3.67	-9E-15	0.040	0.160	
		PLS-AP	31	1st	2	-	AS	9	2.63	3.08	3.09	6E-14	-0.022	-0.013	
		CNN-AP	-	-	-	-	-	-	1.40	-	1.40	0.389	-	0.442	
		CNN-AP	31	1st	2	-	AS	-	1.34	-	1.46	-0.401	-	-0.414	
Maize	FT-NIR	PLS-aP	25	1st	2	MSC	MC	5	1.64	1.69	1.47	-2E-14	-0.004	0.001	
		PLS-AP	35	2st	3	MSC	MC	4	1.75	1.79	1.52	8E-14	-0.011	-0.037	
		CNN-AP	-	-	-	-	-	-	2.11	-	1.93	-1.180	-	-1.077	
		CNN-AP	35	2st	3	MSC	MC	-	1.63	-	1.42	-0.014	-	-0.027	
Vis		PLS-aP	25	1st	2	-	MC	11	1.75	2.20	1.92	-2E-14	-0.050	-0.132	
		PLS-AP	31	1st	2	-	MC	11	1.83	2.27	1.95	-1E-13	-0.069	-0.094	
		CNN-AP	-	-	-	-	-	-	1.38	-	1.39	0.341	-	0.189	
		CNN-AP	31	1st	2	-	MC	-	1.36	-	1.34	0.354	-	0.358	
Soy	FT-NIR	PLS-aP	35	1st	2	SNV	AS	4	1.16	1.21	0.99	-5E-14	-0.007	-0.019	
		PLS-AP	35	-	-	MSC	AS	6	1.22	1.27	1.03	6E-13	-0.006	-0.017	
		CNN-AP	-	-	-	-	-	-	1.98	-	1.98	1.475	-	1.453	
		CNN-AP	35	-	-	MSC	AS	-	1.92	-	2.08	-0.012	-	0.101	
Vis		PLS-aP	25	-	-	-	MC	8	2.06	2.27	2.05	-3E-14	-0.022	0.108	
		PLS-AP	25	1st	2	-	MC	7	2.22	2.38	2.32	-6E-14	0.003	0.158	
		CNN-AP	-	-	-	-	-	-	1.58	-	1.55	0.490	-	0.612	
		CNN-AP	25	1st	2	-	MC	-	1.44	-	1.42	-0.089	-	0.103	

PLS, Partial Least Squares; CNN, Convolutional Neural Network; a, not augmented dataset; A, augmented dataset p, untreated dataset ; LV's, latent variables; SG, Savitzky-Golay filter window; SC scatter correction algorithm; SNV, standard normal variate MSC, multiplicative scatter correction; MC, mean center; AS, autoscaling; RMSE, root mean square error ; C, calibration; CV, cross -validation; P, prediction.

FIGURES

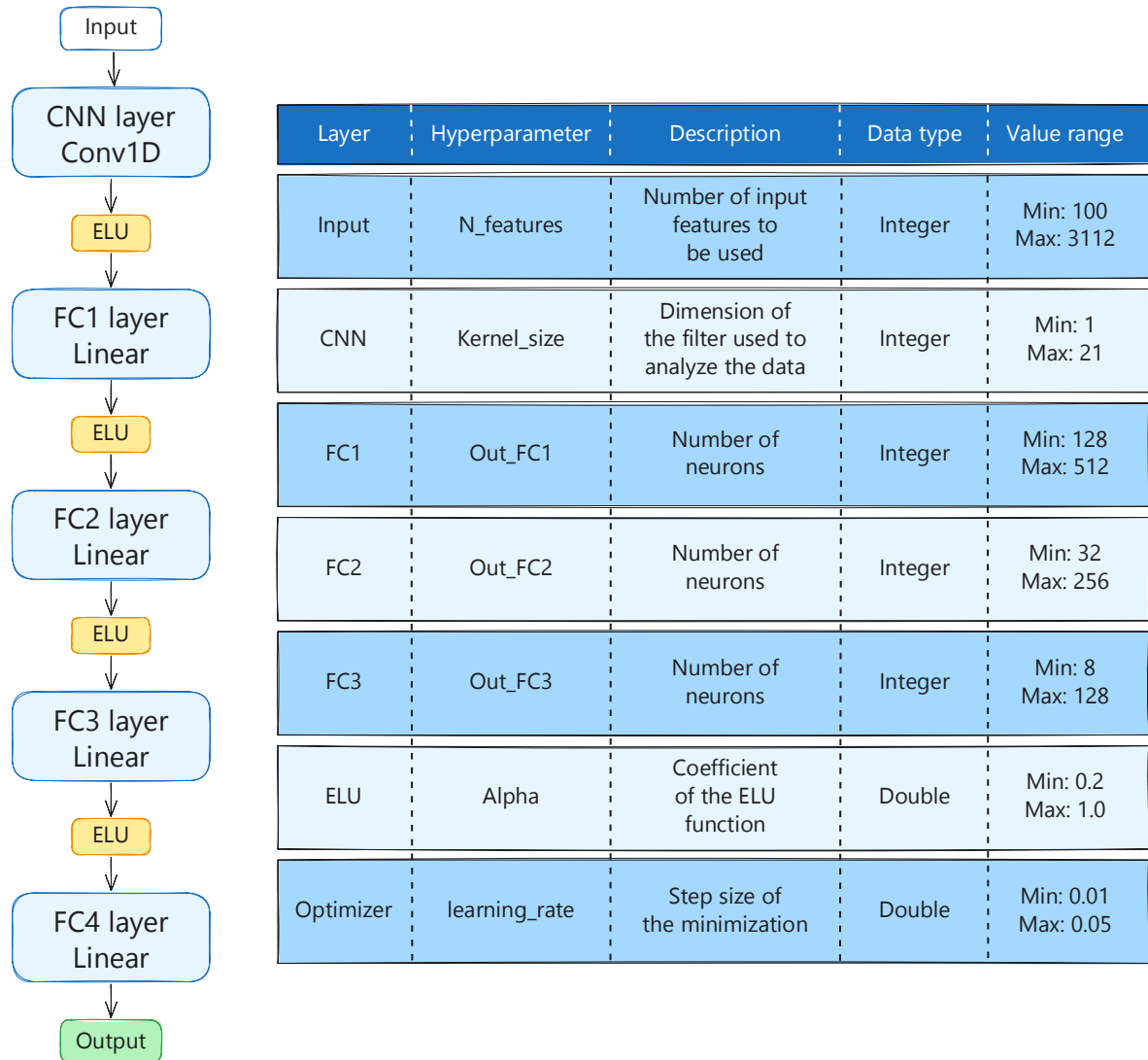


Figure 1. DL model structure and hyperparameters with their description and tested range of values.

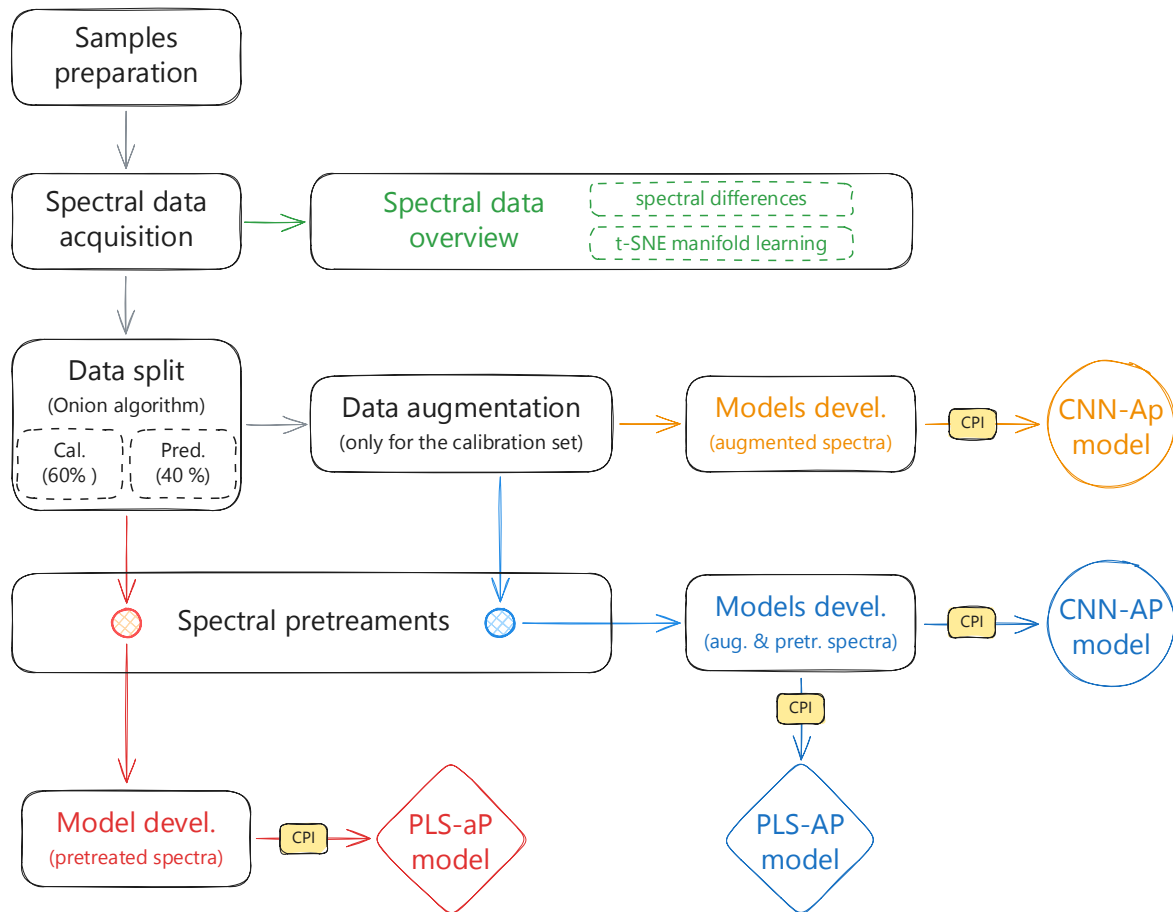


Figure 2. Schematic overview of the data processing and modeling steps used to develop Partial Least Squares (PLS) and Convolutional Neural Network (CNN) models for the prediction of the adulteration level in EVOO using spectral data

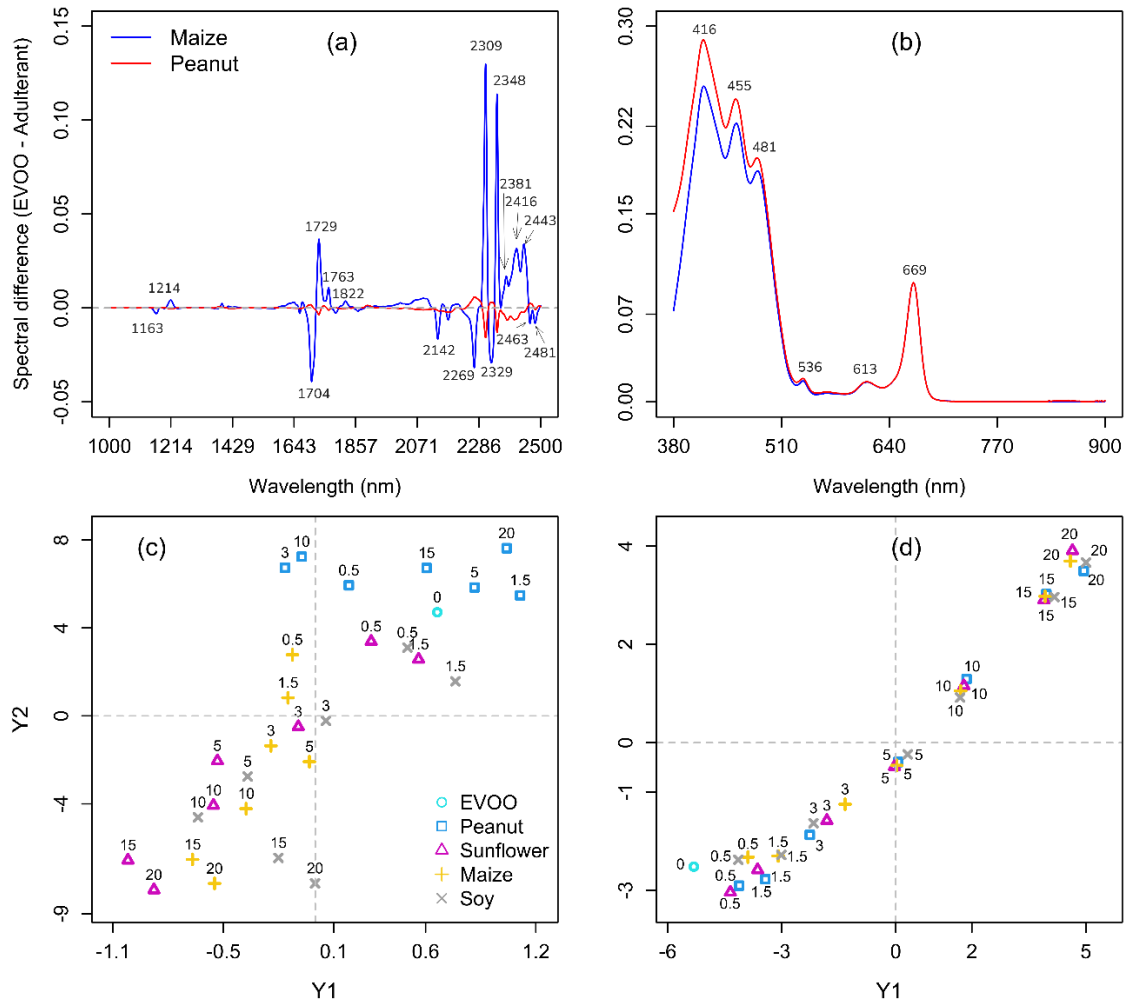


Figure 3. FT-NIR (a) and Vis-NIR (b) spectra for maize (blue line) and peanut (red line) resulting from the difference between the spectra of pure EVOO oil (100 %) and seed oil (0 %). The distributions of the t-SNE analysis for each seed oil-EVOO blend were reported for FT-NIR (c) and Vis (d) spectroscopy

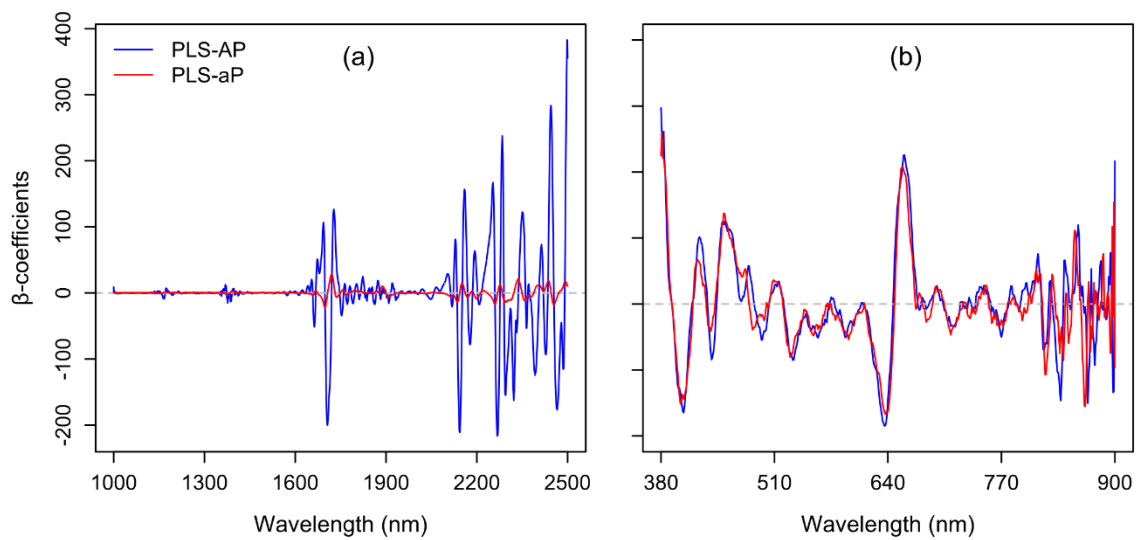


Figure 4. Regression vector for the best FT-NIR (a) and Vis-NIR (b) PLS models in maize obtained with and without data augmentation

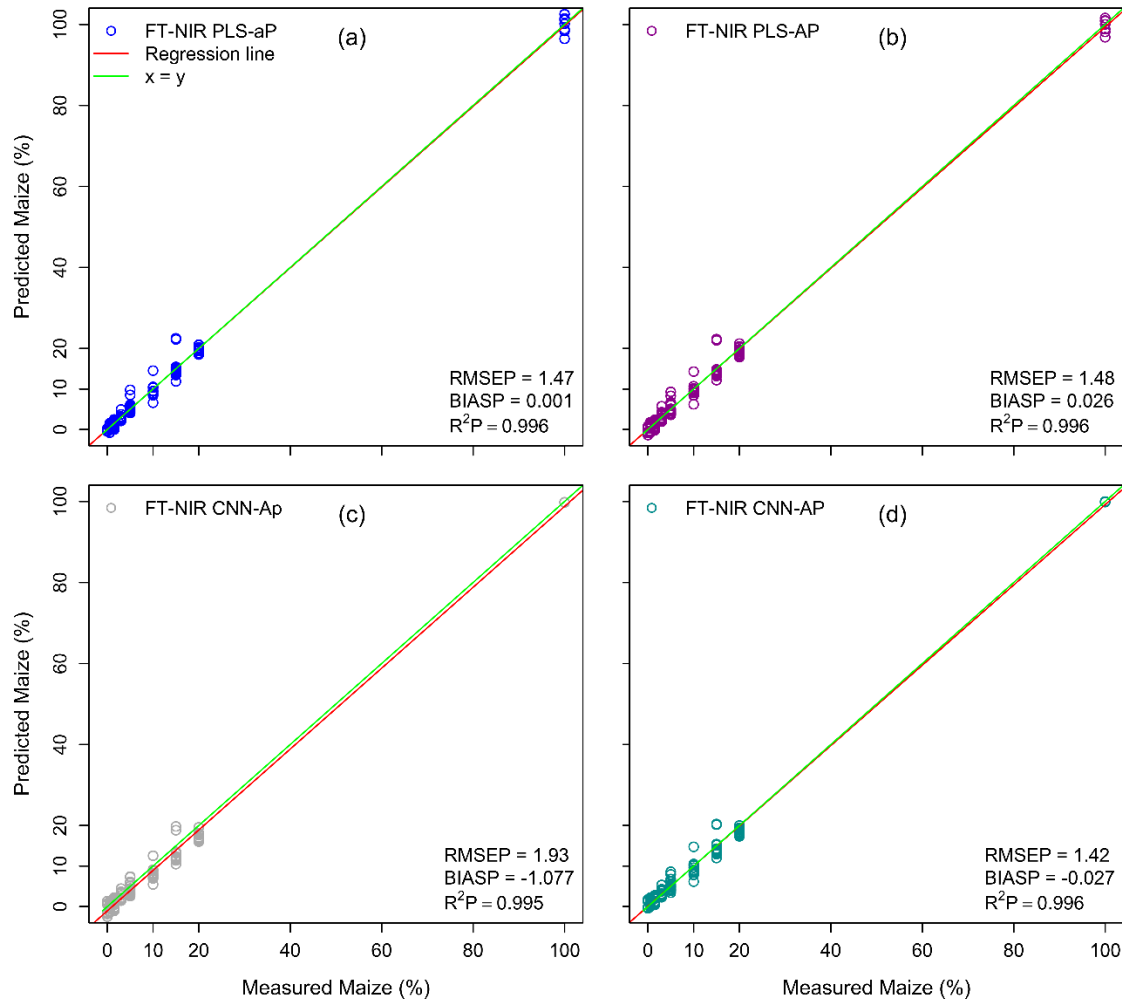


Figure 5. Regression plots for the best FT-NIR-based models obtained using the PLS regression in combination with (a) unaugmented but pretreated spectra (PLS-aP) and (b) augmented and pretreated spectra (PLS-AP), using the CNN algorithm with (c) augmented but untreated spectra (CNN-Ap), and (d) augmented and pretreated spectra (CNN-AP).

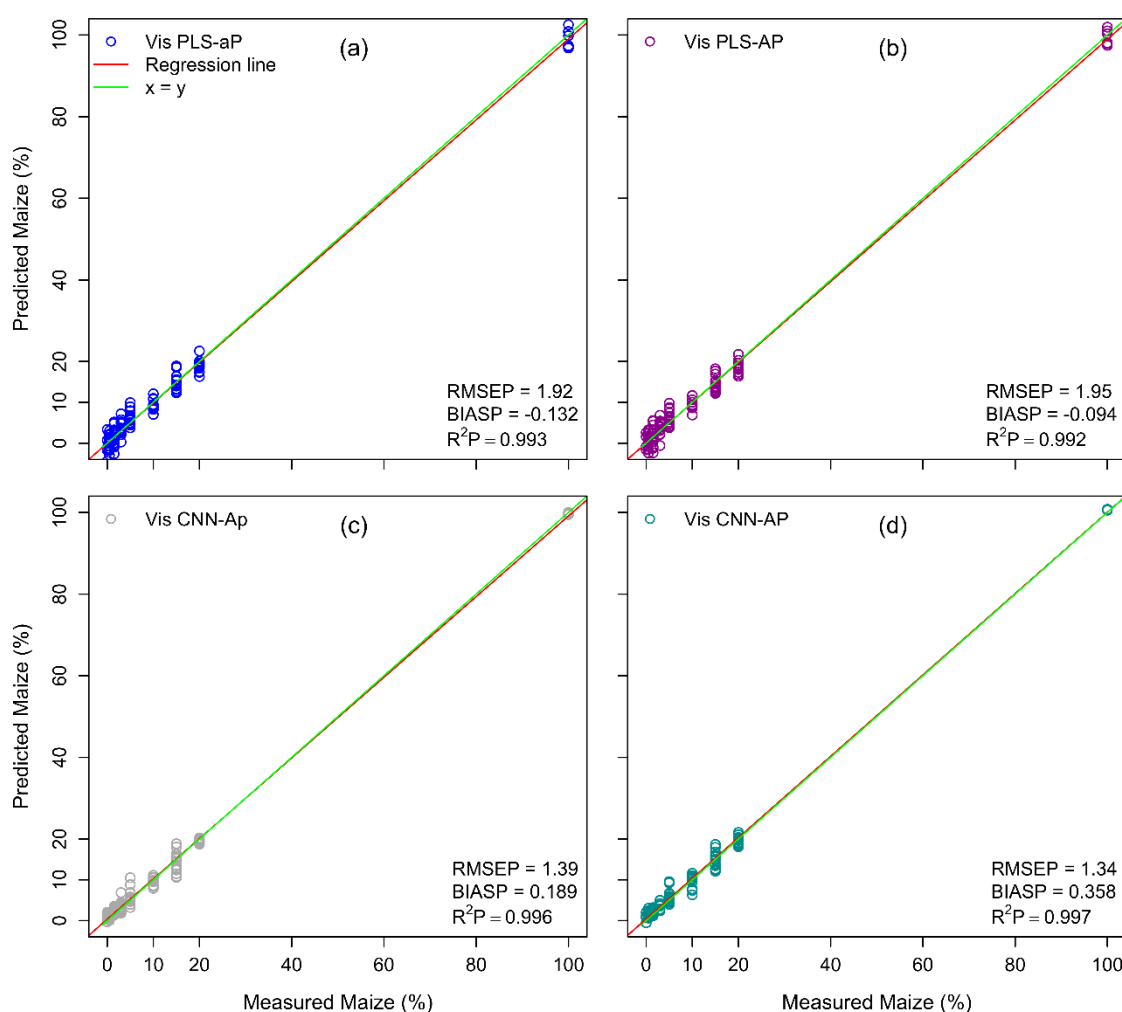


Figure 6. Regression plots for the best Vis-NIR based models obtained using the PLS regression in combination with (a) unaugmented but pretreated spectra (PLS-aP) and (b) augmented and pretreated spectra (PLS-AP), using the CNN algorithm with (c) augmented but untreated spectra (CNN-AP), and (d) augmented and pretreated spectra (CNN-AP).

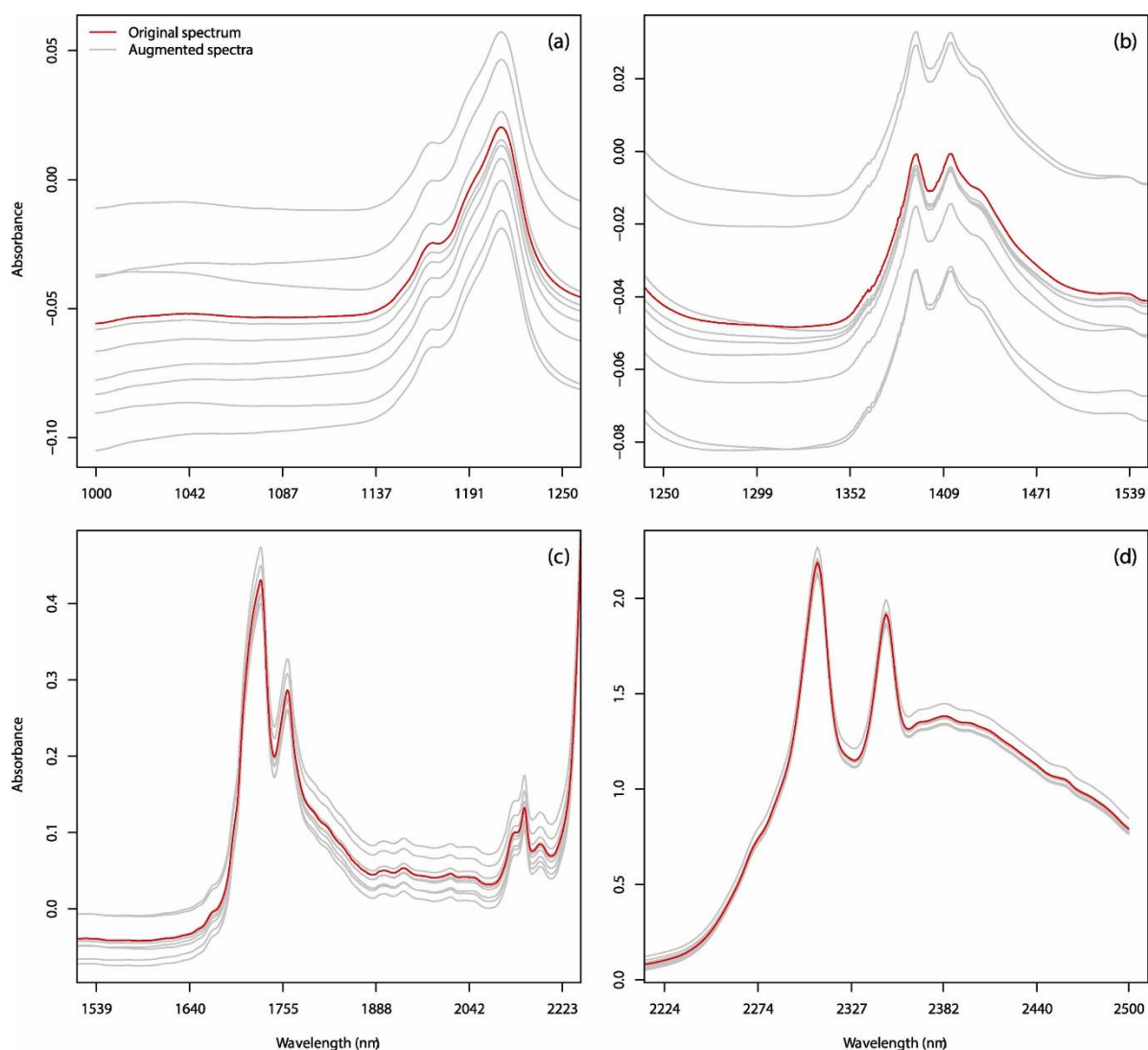


Figure supplementary 1. Nine augmented spectra obtained with data augmentation for each NIR spectrum.

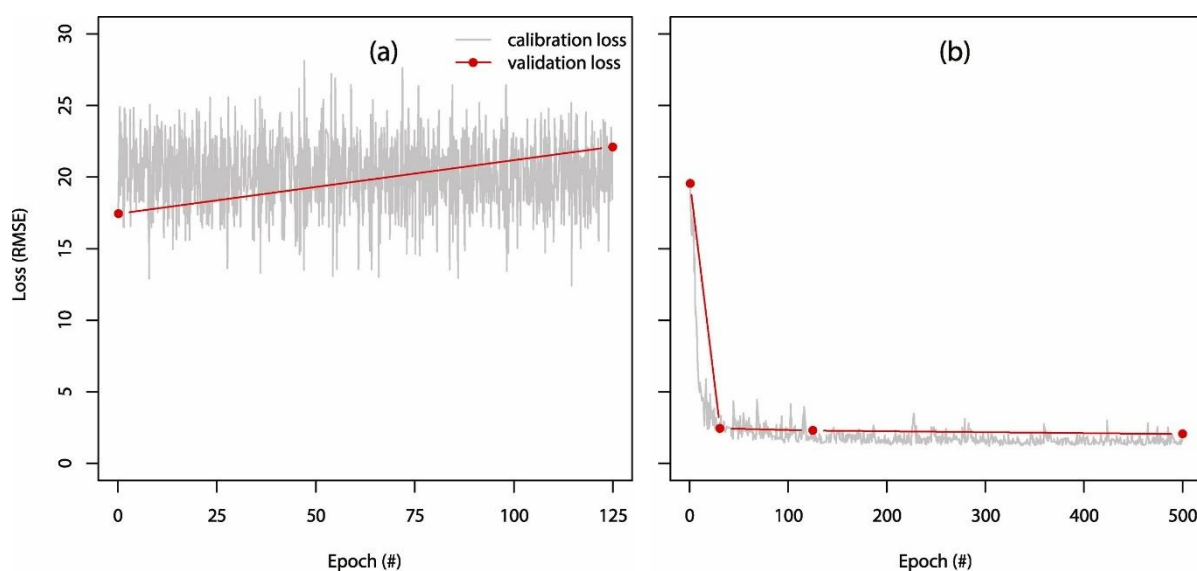


Figure supplementary 2. Peanut oil adulteration in EVOO predicted using CNN-Ap (a) and CNN-Ap (b) in FT-NIR spectroscopy.

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OTHER SCIENTIFIC PUBLICATIONS 6

During the PhD , I participated in several research activities, the details of which are listed below. These activities enhanced my experience and background on various topics related to my area of study.

Bandiera, A., Bedini, G., Chakravartula, S. S. N., Massantini, R., & Moschetti, R. (2022). Computer vision for the development of smart drying processes | Impiego della visione artificiale per lo sviluppo di processi di disidratazione smart. *Industrie Alimentari*, 61(633), 22–29.

Bandiera, A., Rosati, V., Capone, D., Benelli, A., Taormina, E., Massantini, R., & Moschetti, R. (2025). Deep Learning Versus Chemometrics: A Comparative Study in EVOO Adulteration Detection. In L. Sartori, P. Tarolli, L. Guerrini, G. Zuecco, & A. Pezzuolo (Eds.), *Biosystems Engineering Promoting Resilience to Climate Change - AIIA 2024 - Mid-Term Conference* (pp. 542–549). Springer Nature Switzerland.

Bedini, G., Chakravartula, S. S. N., Nardella, M., **Bandiera, A.**, Massantini, R., & Moschetti, R. (2023). Prediction of potato dry matter content by FT-NIR spectroscopy: Impact of tuber tissue on model performance. *Future Foods*,

Bedini, G., Haff, R. P., Benelli, A., **Bandiera, A.**, Taormina, E., Massantini, R., & Moschetti, R. (2024). Potatoes (*Solanum tuberosum* L.) grown at “Patata dell’alto Viterbese” PGI have different quality characteristics and storage responses. *Postharvest Biology and Technology*, 214

Benelli, A., Pandolfi, F., **Bandiera, A.**, Taormina, E., Russo, P., Adiletta, G., Massantini, R., & Moschetti, R. (2025). Smart Drying Technologies: A Case Study on Red Flesh Apple (Kissabel®) Using Computer Vision and Deep Learning. In L. Sartori, P. Tarolli, L. Guerrini, G. Zuecco, & A. Pezzuolo (Eds.), *Biosystems Engineering Promoting Resilience to Climate Change - AIIA 2024 - Mid-Term Conference* (pp. 513–520). Springer Nature Switzerland.

Chakravartula, S. S. N., **Bandiera, A.**, Nardella, M., Bedini, G., Ibba, P., Massantini, R., & Moschetti, R. (2023). Computer vision-based smart monitoring and control system for food drying: A study on carrot slices. *Computers and Electronics in Agriculture*, 206

Moschetti, R., Zambelli, S., Taormina, E., Bandiera, A., Benelli, A., & Riccardo, M. (2025).

Optimization on Banana Maturation Classification for Logistics Efficiency Using Computer Vision. In L. Sartori, P. Tarolli, L. Guerrini, G. Zuecco, & A. Pezzuolo (Eds.), *Biosystems Engineering Promoting Resilience to Climate Change - AIIA 2024 - Mid-Term Conference* (pp. 505–512). Springer Nature Switzerland.

CONCLUSIONS AND FUTURE PROSPECTS 7

The objective of this thesis was to compare classical and deep chemometrics for the analysis of FT-NIR and Vis spectral data to predict olive oil quality. To this end, several research activities were conducted to develop models for predicting the chemical composition, detecting adulterants, and determining the shelf life of extra virgin olive oil. In fulfilling this objective, the research demonstrated that deep chemometrics generally offers comparable or superior performance, while also revealing specific contexts where traditional methods remain competitive and highlighting key limitations of the newer approach.

Monitoring chemical parameters during storage indicated the efficacy of Convolutional Neural Networks (CNNs) in predicting total phenol and peroxide content, regardless of the spectroscopic technique used. However, the predictive models for acidity and K_{232} values were generally unsuccessful, a finding that points to a limitation related to the narrow range of data available for these parameters.

The optimal CNN configuration (i.e., type II) for shelf-life studies demonstrated a noteworthy application in characterizing chemical profiles. In general, deep chemometrics outperformed Partial Least Squares (PLS) for nearly all chemical parameters analyzed (with the exception of oleic and linoleic acids), provided the data range was adequate for model development. The performance of these models appeared unaffected by the number of available samples, whether the sample size was large (e.g., acidity) or small (e.g., peroxides, extinction coefficients K).

Regarding the key objective of adulteration, the detection of adulterants in EVOO was generally successful regardless of the spectroscopic and chemometric analysis performed. The main exception was the detection of peanut oil in EVOO with FT-NIR, which presented a strong spectral overlap. Quantitatively, model development showed comparable results between CNN and PLS for FT-NIR spectroscopy. For Vis spectroscopy, however, deep chemometric algorithms consistently performed better.

In general, the application of near-infrared spectroscopy demonstrated promising results for discriminating oils from diverse vegetable sources. Furthermore, FT-NIR models could effectively predict various compounds when a sufficient dataset was available. However, spectroscopic analysis in the Vis-NIR range generally exhibited superior performance, likely due to the presence of specific chromophores like carotenoids and chlorophyll. Data Augmentation proved to be indispensable for training the neural networks and also generally improved the performance of classical models. However, as the adulteration study suggested,

the same Data Augmentation configuration may not be optimal for both spectral ranges, due to their different physical origins.

The results consistently showed that neural network models had comparable or superior performance to the PLS models. Specifically, CNNs yielded the best performance, while other architectures like Artificial Neural Networks (ANNs) showed poorer results, likely due to the absence of convolutional layers needed to process raw spectral data.

Despite these promising results, a critical evaluation of the thesis reveals several significant limitations that must be acknowledged. A primary constraint was the data itself; the restricted range of values for certain parameters, as seen with acidity and K_{232} , led to unreliable predictions. Furthermore, a clear performance threshold was identified, as the models were unable to identify adulteration percentages below 1.5 %, which represents a practical drawback of the proposed technique. While CNNs are powerful, they are subject to inherent constraints, including the necessity of large datasets and significant computational power. A more fundamental challenge is their “black box” nature. Unlike the PLS technique, where feature contribution can be evaluated, the difficulty in interpreting NN models complicates the identification of factors driving a result. Finally, the research highlighted the need for more detailed studies on the interaction between preprocessing and neural networks, as its application was shown to enhance model predictions in some cases.

Future studies could investigate the application of deep chemometrics to other spectral ranges, such as ultraviolet, mid-infrared (MIR), and far-infrared (FIR). Model development should be performed using larger and more varied datasets to validate and improve the robustness of the models obtained. The Data Augmentation strategy could be customized to optimize performance. A systematic study on optimal preprocessing techniques in combination with neural networks is also needed. Furthermore, research should be conducted on alternative network architectures (e.g., transformers). Finally, future work should involve more sophisticated hyperparameter tuning and the application of Explainable Artificial Intelligence (XAI) techniques to better understand the internal workings of the network models.