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Quantitative determination of olive oil adulteration with cheap oils using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR)

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Declaration

I certify that this thesis submitted for the degree of master in Applied and Industrial Technology Program, is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same material) has not been submitted for a higher degree to any other university or institution.

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Abstract

Extra virgin and virgin olive oils (EVOO and VOO respectively) have much higher nutritional and therapeutic values, pleasant taste and aroma, when compared with other oils. The consumption of EVOO and VOO are continuously increasing in all kitchens around the world. This explains why EVOO and VOO are relatively expensive and corrupted people, factories and traders tend to blend them with lower grade and cheap vegetable oils such as corn, sunflower and soybean oils. It is important to develop a fast, easy, cheap, without sample preparation, robust, accurate and precise method to recognize pure EVOO and VOO and to determine quantitatively the extent of adulteration with other oils. This method depends on using attenuated total reflection fourier transform infrared (ATR FTIR) to measure the absorbance of each oil sample at characteristic wavenumbers in the range 5000-500 cm^{-1}. Fifty fresh olive oil samples (EVOO) were collected from Palestinian farmers in the west bank. The area of collection covered all the Palestinian territories. The IR spectrum of each EVOO sample was taken and stored as data points of absorbance as function of wave number. Sample 22 of EVOO was chosen to be blended with other vegetable oils with different proportions in order to construct calibration curves for quantitative determination of olive oil adulteration with other cheap oils. This method reveals the presence of many values corresponding to pure EVOO and can be calculated by taking absorbance ratio at two certain wavenumbers. For example, absorbance at 583.9/2922.2 cm^{-1} is a constant value for all examined EVOO samples and equals 0.1136±0.0012. Also many equations were derived to measure quantitatively the extent of EVOO adulteration with other cheap oils, such as:

wt % corn oil = -779.92 **R** + 776.71, $R^2 = 0.997$, P=1.6E-11. Where **R** is the absorbance of the oil sample at wavenumber 2853.6 cm^{-1}/absorbance of the oil sample at 1160.6 cm^{-1}.

wt % of sunflower oil = 567.31 **R** - 610.16, $R^2 = 0.997$, P=2.1E-11. Where **R** is the absorbance of the oil sample at wavenumber 722.3 cm^{-1}/absorbance of the oil sample at 1463.2 cm^{-1}.

wt % soybean oil = 1641.8 **R** - 576.64 $R^2 = 0.998$, P=2.0E-11. Where **R** is the absorbance of the oil sample at wavenumber 1232.0 cm^{-1}/absorbance of the oil sample at 2922.2

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Chapter One: Introduction

1.1 background

The olive tree (Olea europaea L.) is one of the oldest fruit trees known to humans, with archaeological evidence indicating its domestication dates back more than 6,500 years. Analyses of fossilized pollen and botanical findings indicate that olive cultivation began in the southern Levant-present-day Palestine, Lebanon, and Syria-before spreading to Crete and the Aegean regions, and from there to the rest of the Mediterranean Basin (Langgut et al., 2019). This spread played a pivotal role in the development of the economic structure of Mediterranean villages and later contributed to the flourishing of urban civilizations in the region. Archaeological finds, such as ancient jars and oil presses, as well as paintings and inscriptions in the Minoan palaces of Knossos and Phaistos in Crete, highlight the importance of olive oil as an economic and commercial resource in ancient times. By the 6th century BC, olive cultivation had spread to Italy and North Africa, and then spread throughout the Roman Empire and Mediterranean communities (Kostellinos and Kyritsakis, 2017). Today, the worldwide olive tree populace exceeds 805 million trees, with about 98% located inside the Mediterranean region. Spain, Italy, Greece, Turkey, and Tunisia are the leading manufacturers of olive oil globally, accounting for greater than 90% of total manufacturing. Despite global expansion to the Americas, Australia, and Asia, the Mediterranean Basin stays the center of world olive cultivation and oil manufacturing, both culturally and economically (Barazani, Dag and Dunseth, 2023).

Olive tree was planted in the Mediterranean countries thousand years ago (Meenu et al 2019). According to the Palestinian Olive Council (2022), olive tree was planted in Palestine five thousand years ago. In the West Bank (Palestinian Authorities regions) more than ten million olive trees are existing, and more than 100 thousand Palestinian families benefit from their production of olive oil. Olive tree has a prestigious value among the Palestinian people from: cultural, historical, nutritional, medicinal and sacred perspective, as it is mentioned repeatedly in the Holy Quran. Harvesting olive tree in Palestine started from the last third of October until the mid of December. To obtain the oil from the fruit, fruits are harvested manually or some harvesting tools like rotation rubbers then collected, and as fast as possible subjected to physical or mechanically techniques cleaning, mechanical crushing and pressing, turning them into a paste. Oil then is separated from the solid material by centrifugation, without affecting any of its components, neither its nutritional vales, taste or aroma (Kalaitzis and El-Zein 2016, Ok, S. 2017, Meenu et al 2019, Kakouri et al 2021).

1.2 Olive Oil Classification

Classification of olive oil is crucial to both purchaser consumer and industry regulation. According to both the European Union and the International Olive Council (IOC), olive oils are categorized primarily based on their production method, acidity level, peroxide values, UVspectrophotometric parameters and sensory evaluation. The most common and commercially precious classes are Extra Virgin Olive Oil (EVOO) and Virgin Olive Oil (VOO) (Peri, 2014).

Olive oil is classified into the following categories, depending on sensory, physical and chemical properties as well as purity and quality: Extra virgin olive oil (EVOO) with free acidity less than 0.8g/100g, virgin olive oil (VOO) with free acidity less than 2.0g/100g, Ordinary olive, olive oil (OO), refined olive oil (ROO) and olive pomace oil (OPO) (Kakouri et al 2021, Meenu et al 2019). According to the International Olive Oil Council., Madrid/ Spain; EVOO is obtained only cold pressing techniques without any chemical or thermal treatments, while olive pomace oil, a fully refined olive oil, is obtained by organic solvent extraction processes from olive pomace.

From a sensory and nutritional point of view, EVOO has a better value due to high concentrations of minor bioactive compounds such as polyphenols, tocopherols, and volatile aromatics. These components are greatly affected by factors such as olive cultivar, harvest time processing conditions and storage conitions, which is why not all EVOOs are made equal (Fregapane and Salvador, 2013). In fact, EVOO's volatile and phenolic profiles not only serve as an indicator of freshness and aroma, but also play a role in its classification, especially as analytical techniques advance (Karatti et al., 2024).

1.3 Chemical Composition of Olive Oil

The chemical composition of olive oil is a direct functional quality. The bulk of olive oil—up to 98–99%—includes triacylglycerols (TAGs), whilst the last fraction consists of free fatty acids, mono and diacylglycerols, sterols, hydrocarbons, tocopherols, pigments, phenolic compounds, and different minor components (Boskou et al 2006). The relative proportions of these elements define the oil's oxidative stability and health properties besides serving as crucial parameters in its classification and authenticity assessment.

The major component of fresh olive oil is the triglyceride of oleic acid. The average percent of fatty acids in the triglycerides of olive oil are: 69.63 ± 1.83 oleic acid, 13.79 ± 0.97 palmitic acid, 10.74 ± 1.46 linoleic acid, 3.04 ± 0.34 stearic acid and 0.76 ± 0.11 linolenic acid. Of the mentioned fatty acids 70.94 ± 2.10 % are mono unsaturated fatty acids (MUFA), 11.50 ± 1.57% poly unsaturated fatty acids (PUFA) and 17.56 ± 1.46% saturated fatty acids (SFA). The minor components of olive oils are: polyphenolic compounds, vitamins as vitamin E (α-tocopherol) and pigments as carotenoids, which are the precursors of vitamin A. The percentage of all components is variable due to aging and storage conditions (Oguz Uncu and Banu Ozen, 2019). In addition to major fatty acids and TAGs, olive oil has a complex array of minor compounds with powerful bioactive effects. These include polyphenols (eg, hydroxytyrosol, oleuropein), tocopherols (vitamin E), phytosterol and carotenoids. Polyphenols act as natural antioxidants and anti-inflammatory agents and are responsible for the bitter and sharp flavor notes of oil. Tocopherols increase the shelf life by protecting unsaturated fatty acids from oxidation (zeb and Murkovic, 2011). Although these compounds exist in small amounts - 1-2% of total oil - they have an inconsistent effect on health benefits and oxidative stability.

The chemical profile of olive oil is fairly sensitive to environmental and technological elements. Variables such as olive cultivar, degree of fruit ripeness, extraction technique, and storage conditions can substantially regulate fatty acid ratios and the concentration of minor components (Kesen et al., 2017; Boudour-Benrachou et al., 2017).

Although of its relatively high price, the consumption of EVOO and VOO is continuously increasing in all kitchens around the world, due to its highly nutritional values, pleasant taste and aroma, when compared with other oils (Aparicio and Harwood 2003, Hashempour-baltork et al 2024). EVOO does a high reputation when compared with other oils, due to its therapeutic potentiality against chronic diseases. It relieves heart diseases and arteriosclerosis, since it increases high-density lipoproteins (HDL) and decreases LDL-density lipoproteins in the blood stream. EVOO fights against cancer, since it contains antioxidants like vitamin E and other polyphenolic compounds. It relieves diabetes type 2. EVOO is also a main constituent in different cosmetic preparations (Yang and Irudayaraj 2001, Rohman and Che Man 2010, Bodurov et al 2013, Mendes et al 2015, Roselli et al 2017, Hashempour-Baltork et al 2022, Hashempour-Baltork et al 2023). For example, oils comprised of the Picual range are acknowledged for higher oleic acid content, whereas oils from Arbequina or Chemlal cultivars can also have elevated levels of linoleic acid and barely lower oxidative stability.

The chemical makeup of olive oil is primary to its authentication and detection of adulteration. The ratios of particular fatty acids, the profile of TAGs, and the presence of unique bioactive compounds provide a biochemical fingerprint that can distinguish authentic extra virgin olive oil from blends or lower- quality oils. Advanced analytical techniques such as gas chromatography and mass spectrometry are often used to evaluate those profiles and ensure regulatory compliance (Piravi-Vanak et al., 2009).

1.4 Chemical Quality Indices

The quality of olive oil, especially extra virgin and virgin grade, is tightly regulated through a series of standardized chemical parameters that evaluate its freshness, purity and degree of oxidation. The most important indices used internationally are peroxide value (PV), acid value (also known as free acidity or free fatty acid content), and the less commonly referred to acetyl value. These indices serve as a major diagnostic indicator for oil degradation due to oxidation or hydrolysis and play an important role in grading oil in the form of extra virgin, virgin or lampante .

Peroxide value measures primary oxidation products - i.e., hydroperoxides- formed when unsaturated fatty acids react with atmospheric oxygen and light. This parameter reflects the early stages of Rancidity, with higher values indicating more advanced lipid oxidation. The International Olive Council (IOC) sets a maximum PV of 20 milliequivalents (meq) O_2/kg for both extra virgin and virgin olive oil (Houshia et al., 2014). However, premium quality EVOOs usually have very low values, often below 10 meq O_2/kg shortly after production (Tuberoso et al., 2016). The peroxide value is usually determined through iodometric titration, although new methods, such as spectrophotometry and chemiluminescence, have been discovered to increase sensitivity and reduce environmental impact (Stepanyan et al., 2005; Longobardi and Paradiso, 2022).

Acid value, or free acidity, present in oil as a result of triglyceride hydrolysis, determines the amount of free fatty acids, especially oleic acid. This parameter is an important indicator of oil handling, fruit condition and storage. According to IOC standards, EVOO should not exceed 0.8% free oleic acid, while VOO is allowed up to 2.0% (Houshia et al., 2014). High acidity oils are usually the result of poor processing or delayed extraction after olive harvest. Free acidity is usually measured via titration with potassium hydroxide and is highly sensitive to microbial activity, fruit bruising or prolonged storage (Grossi et al., 2013). Recent progress such as electrical impedance spectroscopy is being simplified and adopted for rapid acidity measurement with comparable accuracy (Grossi et al., 2013).

Acetyl value is another parameter that offers insights into the presence of hydroxyl-containing compounds consisting of monoglycerides and partial esters. This value is normally used to assess esterification products in business programs or to detect adulteration with synthetic esters or low-grade oils. While IOC presently does not now specify acetyl value threshold for VOO or EVOO, deviations from not usual values can also lead to concerns about purity or mistaken refining. Therefore, it plays a supplementary role in advanced oil authentication studies (Janković et al., 2016). Monitoring those quality indices are important for consumer safety and for industrial integrity. For instance, Houshia and colleagues (2014) established how adulterating olive oil with sunflower, soybean, or corn oil significantly altered both peroxide and acidity values, helping to detect fraudulent practices. Similarly, variations in those chemical signs had been used to minor geographical origin, cultivar influence, and oil aging during storage (Awan et al., 2020).

1.5 Factors Affecting Oil Stability and Quality

The stability and quality of olive oil are determined with the aid of a complex interaction of chemical composition and external environmental factors. These include temperature, light exposure, oxygen, humidity, olive cultivar, storage conditions, and packaging material. Understanding the impact of these elements is important for maintaining the nutritional value, sensory profile, and shelf life of extra virgin and virgin olive oils.

Heat is one of the most important destabilizing factors. When olive oil is subjected to excessive temperatures, especially throughout cooking or poor storage, triglycerides can break into free fatty acids and glycerol. Prolonged heating may cause the isomerization of cis-unsaturated fatty acids into their trans isomers, affecting both health and physical properties. Trans isomers have greater stability but less beneficial nutritionally and are not clearly abundant in fresh olive oil. Additionally, heating leads to oxidation of double bonds in polyunsaturated fatty acids, resulting in forming off-flavors and poisonous toxic (Lanza and Ninfali, 2020). Light, especially UV radiation, accelerates photo-oxidation reactions in unsaturated lipids. Olive oil stored in transparent containers exposed to light rapidly deteriorates due to the formation of singlet oxygen, which reacts with double bonds in oleic and linolic acid. Studies have shown that oxidative stability decreases significantly under light exposure compared to dark storage, even at the same temperature (Škevin et al., 2020). This is why high quality olive oil is packed in dark glass bottles or opaque tin.

Oxygen is another major contributor to oxidative degradation. During storage or processing, oxygen can react with fatty acids, produce hydroperoxides, aldehydes and ketones that not only deteriorates oil quality, but also provides rancid flavors. Oil rich in polyphenols and tocopherols display more oxidative resistance, but this natural protection gradually decreases during storage (Stefanoudaki-Katzouraki, 2004). Modern practices often include nitrogen flushing in headspace packaging to displace oxygen and prolong shelf life.

Humidity affects oxidation indirectly through its effect on enzymatic activity and microbial contamination. If the olive oil comes in contact with a moist environment, especially after processing, it becomes more susceptible to hydrolytic rancidity, increases its free acidity and accelerates deterioration. High humidity can also promote mold growth on olive residues, which can leach in oil (Gucci et al., 2012). The olive cultivar is any other important indicator of oil quality. Different sorts include various proportions of oleic, linoleic, palmitic acids, and polyphenols, which all affect flavor, nutritional content, and oxidative balance. For example, oils from the Picual cultivar are regarded for high oleic acid and phenolic content, imparting superb resistance to oxidation, while Arbequina oils are more delicate and much less stable (Caratti et al., 2025; Franco et al., 2015).

Storage time necessarily impacts oil quality. Over time, even under optimal conditions, olive oil undergoes slow oxidative and hydrolytic degradation. Prolonged storage reasons a decline in polyphenols and aromatic compounds, leading to faded sensory quality and nutritional value. Research indicates that extra virgin olive oil can hold acceptable quality for 12–15 months if saved in cool, dark conditions with minimum air exposure (Pereira et al., 2002).

Packaging materials play an important role in keeping oil stability. Materials which are impermeable to oxygen and light, along with dark-colored glass or coated metal containers, provide superior protection as compared to plastic bottles, which are regularly semi-permeable to gases and vulnerable to degradation under UV light. The choice of packaging is particularly critical during long-distance transportation and commercial distribution (Yaşar et al 2012).

1.6 Physical and Chemical Changes During Storage and Use

During storage and regular use, olive oil - especially extra virgin olive oil - faces a range of physical and chemical changes that affect its nutritional quality, stability and sensory characteristics. These changes are mainly powered by two processes: oxidation and hydrolysis. Both reactions are extended by exposure to oxygen, light, elevated temperatures and long storage periods. They carry the formation of off-flavors, increase acidity, and degradation of triacylglycerols into mono- and diacylglycerides, which change the integrity of oil over time.

Oxidation begins with an attack of oxygen on unsaturated fatty acids, especially linolic and linolenic acids, forming hydroperoxides in the primary phase. These compounds then turn into aldehydes, ketones, and other volatile substances responsible for stale odor and taste. The rate of oxidation is greatly affected by storage conditions. According to Kolodiaznaia et al. (2021), virgin olive oil samples stored at high temperatures such as beta-carotene show rapid growth in peroxide values and reduce triacylglycerol content, reflecting quick oxidative degradation. In contrast, samples stored at 4 ° C or supplemented with beta-carotene, maintained oxidative stability for seven months, performing the protective role of the antioxidant and the temperature control (Kolodiaznaia et al., 2021).

Hydrolysis, the second major route, occurs when the water interacts with triacylglycerols, clearing ester bonds and releases free fatty acids and partial glycerides (mono- and diacylglycerols). This reaction increases oil acidity and reduces its freshness. Alonso- Salces et al. (2021) reported that storage in hot and oxygen-rich conditions resulted in high concentrations of 1,3- diacylglycerides, which are distinctive indicators of old or poorly stored olive oil. These were formed at the expense of 1,2- diacylglycerides, dominating in fresh oils, highlighting hydrolytic progression over time (Alonso-Salas et al., 2021).

Over time, physical changes also occur. Fresh olive oil is a clear, green liquid at room temperature, due to its unsaturated fat content. However, during extended storage, especially under cold conditions or after oxidation and hydrolysis, oil can be cloudy or semi-solid because the ratio of saturated molecules and partial glycerides increases. Li et al. (2014) said that storage at low temperatures (- 27 ° C) led to a slow degradation, but promoted physical separation, indicating possible solidification from crystallized TAGs and partial glycerides. They also observed that the concentration of α-tocopherol and polyphenols declined significantly, decreasing oxidative resistance over time (Li et al., 2014).

Supporting these findings, González-Hedström et al. (2020) explored the protective role of extra virgin olive oil when blended with polyunsaturated-rich oils such as algae oil they found that monounsaturated fatty acids and polyphenols in EVOO reduced hydrolysis and oxidation of triglycerides during prolonged storage. A low rate of PUFA degradation and limited formation of secondary oxidation products was seen in mixtures with higher EVOO ratios (González-Hedström et al., 2020).

These chemical changes not only change the nutritional value of olive oil but also have sensory contributions. Rancidity and mustiness arise from the formation of aldehydes and ketones, empowering fruity and grassy notes. Proper storage procedures are important in reducing these deteriorating changes such as refrigeration, dark and airtight containers, and short shelf durations.

1.7 Sensory Characteristics of Olive Oil

The sensory profile of olive oil is one of its most distinctive and valued characteristics, especially in the case of extra virgin olive oil (EVO), where taste and aroma are associated with quality. The sensory experience of olive oil consists of a combination of gustatory and olfactory perceptions, characterized by attributes such as fruitiness, bitterness and pungency. These attributes appear from complex interaction between volatile and non- volatile compounds, many of which can be formed by mechanical extraction of oil from olives (Bendini et al., 2012). EVOO includes bitterness, astringency, sweetness and pungency in taste features. Bitterness is particularly related to phenolic compounds including oleuropein and ligstroside aglycones, while pungency -described as a stinging sensation in the throat-which is attributed to oleocanthal, which has anti-inflammatory properties (Santis and Frangipane, 2015). These characteristics are considered differently by individuals and are affected by cultivar, maturity levels and extract conditions. For example, arbequina oils are milder and sweeter, while Picual oils are more pungent and bitter caused by high polyphenol content (Caratti et al., 2025).

The aroma profiles are equally crucial and are specially determined by the volatile organic compounds (VOCs) produced by enzymatic reactions within the lipoxygenase pathway. (E)-2-hexenal, hexanal, (Z)-3-hexen-1-ol, and α-farnesene contribute to the characteristic "green," "grassy," or "fruity" aroma of EVOO. A study reviewing five Turkish EVOOs mentioned that (E)-2- hexenal had a major aroma-active compound in many cultivars, which contributes to fresh, cut-grass notes of high-quality oils (Sevim et al, 2023). Other notes which include tomato, almond, apple and artichoke can emerge primarily based on varietal and harvest stage, imparting a rich aromatic complexity (Essid et al., 2016). The relationship between chemical markers and sensory characteristics has become a crucial aspect in quality control. Gas chromatography- olfactometry and headspace solid- phase microextraction (HS-SPME) techniques have been employed to correlate the presence of some VOCs with sensory impressions. For example, hexanal and (Z) -3 -hexenyl acetate are strongly paired with green and fruity notes, while undesirable compounds such as (E, E)-2,4-decadienal are associated with rancidity and Signal Oxidation or Poor Storage (Neugebauer et al 2020). Official sensory analysis methods, such as those developed by the International Olives Council (IOC), evaluate olive oil through trained tasting panels that assess positive and negative characteristics using structured parameters. Positive indicators include fruity, bitter and pungent, while negative traits such as fusty, musty, winey, and rancid can disqualify an oil from the EVOO category, even if he fulfills chemical standards (çakraj et al 2014). Therefore, sensory testing is essential in authentication and regulation, ensuring that the product consumers align with expectations and health claims.

Consumer preferences often differ from expert sensory evaluation. While trained panels favor complex and strong taste profiles, many consumers prefer milder oil with neutral aromas and minimal bitterness or pungency. Research in Italy using the hedonic pricing models showed a market priority for the low-intense sensory profile despite the industry's efforts to enhance the complexity and terroir-specific expression (Cicia et al., 2013; Cavalo et al., 2018).

1.8 Food and Industry-Related Applicatiions of Olive Oil

Extra virgin olive oil (EVOO) is broadly appreciated not only as a staple in Mediterranean cuisine but also for its therapeutic, cosmetic, and cultural significance. In the food sector, EVOO is basically used for dressing, sautéing, baking, and marinating. Its high content of monounsaturated fatty acids and bioactive compounds such as phenols, tocopherols, and squalene make it flavorful and health-supportive component (Dini and Laneri, 2021). Cooking with EVOO, mainly at moderate temperatures, keeps a good amount of its antioxidants and complements the absorption of fat-soluble nutrients.

In the pharmaceutical and scientific sectors, EVOO is applied for its anti-inflammatory, antimicrobial, and wound- healing properties. Recent studies have explored ozonated olive oil, which mixes the oxidative strength of ozone with the stability and absorption properties of olive oil, showing ability in dermatological applications and infection control (Carata et al 2019). Its phenolic components have also been connected to gastrointestinal protection and cardiovascular benefits, regularly making it a functional ingredient in nutraceuticals (Farhan et al., 2023). Olive oil has a long history in skin and hair care. Its emollient and antioxidant properties make it effective in treating dry skin, preventing premature aging, and maintaining skin barrier function. Hippocrates referred to as "the great healer," and its application in conventional ointments and soaps continues to this day (Clodoveo et al., 2014).

Chapter two: Aim of this work

In this work we are developing a direct method, without sample preparation, simple, cheap, fast and without complicated mathematical and statistical operations, to determine quantitative adulteration of EVOO and VOO with cheap oils as sunflowers, soybean and corn oils. This can be achieved by using Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR). The method depending on finding a linear equation by which % of olive oil in an oil sample can be calculated accurately. This equation is derived from a calibration curve between the % of olive oil in an oil sample mixture to the relative absorbance of olive oil at two different wave numbers at which olive oil absorb specifically in the mid IR region.

Chapter three: Literature review

Due to the relatively high price of EVOO and VOO, corrupted people, factories and traders tend blend them with lower grade and cheap OO (old OO), ROO, OPO, or with other cheap oils as: sunflowers, corn, palm, peanut and rapeseed oils and others. Adulteration of EVOO and VOO will decrease their quality and nutritional values. It also will harm the economy and the public health (Ok, S., 2017, Hashempour-baltork et al 2024, Andrikopoulos et al 2001).

Detecting adulteration in EVOO and VOO is still a big challenge, for chemists and olive oil councils as: European commission, international olive council and codex committee. This is due to the complex structure of EVOO and VOO and their tendency to change with different environmental conditions (Meenu et al 2019, Hashempour-baltork et al 2024).

Numerous methods for detecting adulteration of EVOO and VOO with either cheap oils or with low grade olive oil were found in the literature. Chromatography is one of these methods. However, this method needs sample preparation, consuming time and its running cost is relatively high (Andrikopoulos et al 2001, Lorenzo et al 2002, Dourtoglou et al 2003, Christopoulou et al 2004, Peña et al 2005, Mildner-Szkudlarz and Jeleń 2008, Yang et al 2013, Jabeur et al 2014, Garrido-Delgado et al 2018). Other methods based on differential scanning calorimeter (DSC) (Bodurov et al 2013), and on DNA-based approaches (Kalaitzis, and El-Zein 2016). Spectroscopic methods for quantitative determination of EVOO adulteration are the most common. These methods are fast and don't destruct the samples. Each oil has its unique composition and chemical structures of its components. So each oil has its unique absorption spectrum at certain radiation frequencies. Each absorption spectrum of an oil sample acts as a finger print of that oil, which can be used for qualitative and quantitative analysis of it. A combination of more than one spectroscopic method was sometimes applied for quantitative analysis of oil samples. However, the following spectroscopic methods were able to analyze the adulteration of oil samples when a complex statistical model was applied, as partial least squared-discriminant analysis (PLS-DA). The spectroscopic methods have been applied for food quality and safety issues and the detection of olive oil adulteration include: Nuclear magnetic resonance (NMR) (Fragaki et al 2005, Agiomyrgianaki et al 2010, Ok, S. 2017). UV-visible spectroscopy (Torrecilla et al 2010). Laser-Induced Fluorescence spectroscopy (LIF) (Poulli et al 2007, Mu et al 2016, Nanou et al 2023). Raman spectroscopy (Baeten et al 1996, Yang and Irudayaraj 2001, Zou et al 2009, Philippidis et al 2017, de Lima et al 2020). Near-Infrared (NIR) spectroscopy (Christy et al 2004, Vanstone et al 2018). Fourier Transform Infrared spectroscopy (FTIR) (Tay et al 2002, Gurdeniz and Ozen 2009, Maggio et al 2010, Hirri et al 2015, Georgouli et al 2017, Oguz Uncu and Banu Ozen, 2019).

3.1. Detection of Ternary Mixtures of Virgin Olive Oil Using ATR-FTIR and Chemometrics

Summary of Results: One study explored a novel approach to detecting ternary adulteration in virgin olive oil using ATR-FTIR spectroscopy paired with advanced chemometric models. Virgin olive oil samples were intentionally adulterated with a blend containing 5% canola oil and 15% hazelnut oil (totaling 20% v/v), while safflower oil was also considered due to its high unsaturation and similarity to olive oil. The researchers used second-derivative ATR-FTIR spectra in combination with two classification models: Partial Least Squares Discriminant Analysis (PLS-DA) and Soft Independent Modeling of Class Analogy (SIMCA). Both models achieved high accuracy, with detection sensitivity reaching 100% in all tested adulteration scenarios. Importantly, even with the natural variability in EVOO due to cultivar and harvest year, the predictive power of the models remained above 92%.

Recommendations and Implications: The study recommends the application of non-targeted fingerprinting using ATR-FTIR for routine authenticity screening in regulatory and industrial laboratories. It demonstrated that ATR-FTIR, especially when combined with chemometric modeling, is sensitive enough to detect subtle adulteration patterns, even in complex ternary mixtures. This marks a significant advancement over traditional single-adulterant detection methods, offering a robust approach that reflects real-world fraudulent practices. The authors advocate for expanding this methodology to lower adulteration levels (below 10% v/v) and integrating it into broader food fraud surveillance systems.

3.2. Comparative Evaluation of FTIR and Fluorescence Hyperspectral Imaging in Detecting Olive Oil Adulteration

Jiao and colleagues (2024) designed an elegant head-to-head trial to decide which of two contemporary optical platforms—attenuated-total-reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) or fluorescence hyperspectral imaging (FHSI)—delivers the sharper eye for fraud when extra-virgin olive oil (EVOO) is secretly blended with cheaper seed oils such as soybean, sunflower or corn. Authentic EVOO was titrated with those oils in fine steps from 2.5 % up to 25 % (v/v); every mixture was then interrogated on a benchtop FTIR spectrometer and, in parallel, on an FHSI camera system that collects a three-dimensional cube of spatial and spectral fluorescence data.

The mid-infrared fingerprints generated by FTIR, once parsed with a support-vector-machine algorithm, proved remarkably discriminating: the model flagged adulteration with 99.1 % accuracy and sounded the alarm at concentrations as low as 2.5 % (Jiao et al. 2024). FHSI, by contrast, needed roughly double that threshold—around five per cent—before its convolutional neural network reached its optimum accuracy of 94.2 %. The authors attribute FTIR's finer sensitivity to the pronounced shifts it records in the C–H and C=O stretching bands when foreign triglycerides infiltrate the native olive-oil matrix, whereas fluorescence signatures, though rich in spatial detail, are intrinsically less responsive at very low dilution levels.

Despite that gap, FHSI holds a trump card: its ability to scan large batches rapidly, making it attractive for industrial through-put screening. Jiao and colleagues therefore champion a layered strategy: deploy FHSI on the factory floor for swift triage, then confirm any suspect lots with the chemically incisive FTIR platform. Such a tandem approach, they argue, would weave speed and forensic certainty into a single authenticity net capable of outpacing increasingly sophisticated oil adulteration tactics.

3.3. Review of some adulteration detection techniques of edible oils

Salah and Nofal (2020) open their mini review by underlining the huge price gap between premium extra-virgin olive oil (EVOO) and mainstream seed oils, a gap that routinely tempts producers to dilute the former for economic gain. Their declared purpose is therefore “to provide a brief review of different methods and techniques used to detect adulteration in edible oils, especially olive oil, with the aim of promoting consumer awareness of the purity of edible oils” (Salah and Nofal 2020) . Rather than producing fresh experimental data, the authors interrogate and synthesize the recent analytical literature, filtering it through three practical lenses: analytical sensitivity, operational speed/cost and ease of routine deployment in regulatory or industrial laboratories. This narrative approach allows them to construct a comparative map of the instrumental landscape available for authenticity control.

A focal point of the review is the voltametric electronic tongue, portrayed as an emerging frontline screening tool. The device consists of a small array of carbon-paste electrodes interrogated by cyclic voltammetry; each botanical oil generates a characteristic pattern of anodic and cathodic peaks that reflects its polyphenol and tocopherol content. When sunflower, soybean or corn oils exceed roughly ten per cent in EVOO the fingerprint shifts measurably, enabling straightforward multivariate discrimination (Salah and Nofal 2020). Because the e-tongue delivers results within minutes, consumes almost no reagents and can be miniaturized, the authors position it as an attractive first-tier sensor that can flag suspicious lots before more elaborate tests are invoked.

Optical methods occupy the next tier in their hierarchy. A simple UV–Vis spectrophotometer operating between 200 and 400 nm registers a pronounced absorbance at 268 nm whenever refined or highly poly-unsaturated oils are introduced, pushing the detection threshold down to 0.5 % in olive oil (Salah and Nofal 2020). Broader spectral windows accessed by near-infra-red, mid-infra-red or FT-Raman instruments capture vibrational fingerprints whose subtle changes betray foreign triglyceride structures. Coupled with chemometric models such as partial least squares, these spectroscopic platforms can quantify both nature and the proportion of adulterants in a single, non-destructive measurement cycle (ibid.). Their non-contact nature also makes them ideal for on-line quality assurance.

For confirmatory analysis the review turns to mass-spectrometric and chromatographic technologies. Static headspace–MS isolates filbertone, a volatile absent from olive oil, enabling the unequivocal detection of hazelnut oil at levels as low as seven per cent, while MALDI-TOF/MS exploits shifts in the triacylglycerol (TAG) distribution to reveal sunflower-oil or pomace-oil additions at only one per cent. Ultra-high-pressure LC combined with accurate-mass TOF/MS resolves the distinctive “hand-shaped” TAG profile of argan oil and identifies adulteration down to 0.03 % (Salah and Nofal 2020). Conventional GC or HPLC of fatty-acid methyl esters and TAGs, together with the diagnostic parameter ΔECN_{42}, still play an important role when sophisticated MS is unavailable. Complementing the chemical approaches, a fibre-optic long-period grating sensor monitors tiny refractive-index changes and already differentiates paraffin-in-coconut oil or sunflower-in-olive oil at three to four per cent, offering a solvent-free, field-deployable option (Salah and Nofal 2020). Finally, the authors document how real-time PCR and capillary electrophoresis single-strand-conformation-polymorphism kits amplify chloroplast or nuclear DNA markers unique to each crop; these molecular signatures expose adulteration even when the chemical profile has been masked by refining (ibid.).

Across all these platforms adulteration is modelled in the primary literature as a straightforward binary blending exercise: authentic EVOO, argan or coconut oil is titrated with cheaper seed oils (sunflower, soybean, corn, rapeseed, hazelnut) or, in extreme cases, non-edible hydrocarbons such as paraffin. Calibration sets typically span one to fifty per cent substitution so that detection limits and chemometric boundaries can be established rigorously before unknown commercial samples are screened (Salah and Nofal 2020) . By weaving these individual studies into a coherent framework, Salah and Nofal argue that no single instrument suffices on its own; robust defense against fraud is achieved by layering rapid spectroscopic or electrochemical tests with selective chromatographic or DNA-based confirmation. Their review therefore furnishes researchers and regulators with a practical decision tree that balances cost, speed and legal defensibility.

3.4. Comparative Spectroscopic Analysis for Detecting Soybean Oil Adulteration in Olive Oil

Meng and colleagues (2022) assembled a rigorous side-by-side trial to judge how well three contemporary optical platforms—Fourier-transform infrared spectroscopy (FTIR), visible–near-infra-red spectroscopy (Vis-NIR) and excitation–emission matrix fluorescence spectroscopy (EEMs)—could unmask soybean oil concealed in extra-virgin olive oil. Authentic EVOO was titrated with soybean oil in incremental steps from five to thirty per cent v/v and each blend was measured on every instrument. The resulting spectra were then funneled through multivariate chemometric engines, chiefly partial-least-squares discriminant analysis (PLS-DA) and

principal-component analysis (PCA), to see whether the algorithms could reliably sort pure from doctored samples.

The outcome was strikingly clear-cut. Both FTIR and Vis-NIR delivered perfect discrimination: once the mid-infra-red or broad-band Vis-NIR fingerprints were modelled with PLS-DA, classification accuracy rose to a full 100 %, leaving no ambiguities between authentic and adulterated oils (Meng et al. 2022). Fluorescence mapping told a more nuanced story. When the raw EEM data were interpreted with conventional chemometrics, accuracy stalled at around 73 %. However, replacing linear modelling with a back-propagation neural network lifted the fluorescence route to parity with the other two techniques, again achieving flawless separation.

Even so, the authors single out FTIR as the most pragmatic first-line weapon for routine surveillance. The attenuated-total-reflectance accessory on a benchtop FTIR spectrometer permits direct, solvent-free scanning of a few microliters of oil, compressing analysis time to seconds and sidestepping the heavier calibration effort that fluorescence imaging demands. Coupled with the fact that FTIR spectra already encapsulate those C–H and C=O stretching zones most sensitive to foreign triglycerides, the study argues that FTIR-PLS-DA offers the best compromise between speed, cost and forensic reliability for on-site policing of olive-oil integrity. Nevertheless, Meng et al. emphasize that layering complementary optical signatures-especially where fraudsters may attempt more elaborate blends-will fortify quality-control laboratories against ever more sophisticated adulteration strategies.

3.5. Evaluation of Extra Virgin Olive Oil Adulteration with Edible Oils Using ATR-FTIR Spectroscopy

Mashodi et al. (2020) set out to confirm whether attenuated-total-reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy could serve as a quick, solvent-free gatekeeper against the recurrent practice of stretching extra-virgin olive oil (EVOO) with lower-cost seed oils. To put the instrument through its paces, the team prepared a suite of calibration blends in which canola, corn, sunflower and soybean oils were dosed into authentic EVOO at fairly hefty levels—20, 40, 60 and 80 % (v/v)—and scanned each liquid directly on the diamond crystal of a benchtop ATR-FTIR spectrometer. Particular attention centered on the mid-IR band at 3006 cm^{-1}, which reflects the C=C stretch of olefinic triglycerides, and the neighboring aliphatic C–H stretch at 2925 cm^{-1} that remains comparatively constant across all oils.

Systematic dilution produced a clean, monotonic rise in absorbance at 3006 cm^{-1}, while the 2925 cm^{-1} peak acted as an internal reference. Plotting the absorbance ratio A_{3008}/A_{2925} against the percentage of foreign oil yielded a near-perfect linear regression ($R^2 > 0.98$), and the corresponding limit of detection proved low enough for day-to-day policing of authenticity claims. EVOO itself registered the weakest unsaturated C=C signal, so any upward drift in the ratio immediately flagged adulteration, regardless of which of the four seed oils had been used. Because spectra were captured in seconds without chemical work-up, the analytical throughput comfortably matched the demands of routine batch inspection.

On the strength of those metrics, the authors argue that ATR-FTIR, deployed with a simple regression model anchored on well-defined functional-group bands, offers regulatory laboratories a cost-effective first barrier against economically motivated fraud. They note that the current protocol is already sensitive enough to expose admixtures at the 20 % level and suggest that further refinement—perhaps finer wavenumber resolution or chemometric pre-treatments—could drive the threshold lower still. Crucially, the method generates no solvent waste, requires only microliter sample volumes and can be mastered by operators with modest spectroscopic training, making it an attractive addition to both official surveillance programs and in-house quality-control suites.

Chapter four: Materials and methods

4.1. Oil samples

Fifty fresh olive oil samples were collected from Palestinian farmers in the west bank. The area of collection covered all the Palestinian territories, from the most north (Jenin) to the most south (Hebron). The collection time was during the olive harvest and pressing season, from late October to late November. One fresh sample was obtained from the Palestinian olive oil council. This sample was given arbitrary a code number **22**, and calibration curves for quantitative analysis of olive oil adulteration with other vegetable oils was depending on it, since it is the most trustworthy and its absorbance at different wavenumbers in the IR spectrum as well as absorbance ratios are fall in the mean values of the 50 olive oil samples in this study as seen in table 1 and 2. All olive oil samples were put in dark glass bottles and analyzed within one month after collection. The other vegetable oils ("Safi" corn oil, "Safi" sunflower oil and "Al-khayyal" soybean oil) were purchased from a domestic market. Oil mixtures from olive oil sample **22** with other vegetable oils were accurately prepared as weight % using analytical balance.

4.2. Acid value analysis

Acid value or free fatty acids content was determined according to ISO 660:2009 (Mahboubifar et al 2016) by the acid–base titration of a solution of oil in hot neutral ethanol (3g/10 mL) with 0.1 M KOH using phenolphthalein indicator. The KOH solution was standardized against potassium hydrogen phthalate. All chemicals were purchased from Sigma Aldrich. The titration was conducted by a micro titration apparatus Metrohm 665 Dosimat equipped with a titration vessel of 7 cm.

4.3. FTIR instrument and method specifications

The instrument used in this study is Bruker Tensor II with Platinum ATR. The method specifications applied was: resolution 1 cm^{-1}, 120 scan per each run in 120 seconds, data points of absorption were saved from 5000-500 cm^{-1}. Each oil sample was measured three times while it was located on the ATR. The data points collected for each sample from the three runs were completely identical.

Chapter five: Results and discussion

5.1. Free fatty acid content

The free fatty acid content in the tested olive oil samples range between 0.7-0.14%. This means that all the tested olive oil samples in this work are classified as extra virgin olive oil (EVOO).

5.2. IR spectra of olive oil samples and other vegetable oils

The IR spectra of all oils in this work are identical in terms of the wavenumbers of the absorption peaks as shown in figure 1. This matching is due to the fact that the main component of these oils is a triglyceride of the same fatty acids. The main fatty acids are: oleic, linoleic, linolenic, palmitic and stearic acids. The relative abundance of these fatty acids is different from oil to another. The major fatty acid in olive oil is oleic acid ((Oguz Uncu and Banu Ozen, 2019), but in the other vegetable oils is linoleic acid (Lofland et al 1954, Laiara et al 1990, Ivanov and Sredanović 2010). The difference in the relative abundance of the fatty acids is responsible for the different absorption extent at each wavenumber of each oil. This difference was the basic principle on which quantitative analysis was based to estimate the extent of olive oil adulteration with other vegetable oils. Absence of a broad absorption peak between 3300-3000 cm^{-1} indicates the absence of O-H bond stretching of carboxylic acid (Shriner et al 2003), this means the absence of free carboxylic acid in oil samples. This is consistent with the fact that all olive oil samples are extra virgin olive oils (the quantity of the free fatty acids in oils is negligible). The most important absorption peaks are shown in Fig.1and table 1. The C-H alkene stretching absorbing peak can be seen as shoulder peak in figure 1 at 3005 cm^{-1}. As can be seen from table1 , the absorption extent of olive oil at this location is less than that of any other examined vegetable oil. This is expected because the percentage of linoleic acid (two carbon-carbon double bonds) is lower in olive oil compared to other oils. The two sharp strong absorption peaks at 2922.2 and 2853.6 cm^{-1} refer to C-H alkane stretching absorbing peak. Olive oil absorbance at these two wavenumbers is higher than that of any other oil sample as shown in table 1. This is also expected, since the oleic acid (less unsaturation and has more alkane carbon atoms) content in olive oil is higher. The sharp strong absorption peak at 1743.0 cm^{-1} refers to the stretching of the carbonyl group (carbon double bond oxygen) of the ester group of the triglyceride. The C-H of $–CH_2-$ alkane bending has absorption peak at 1463.2. At this wavenumber also olive oil has more absorbance than other vegetable oils. The C-O ester group bending has absorption peaks at 1232.0, 1160.6 and 1117.6 cm^{-1}. The absorption peaks at 583.9 and 722.3 cm^{-1} are in the finger print region of oil samples (Shriner et al 2003).

Table 1: The wavenumbers of the absorption peaks of different oil samples and their corresponding absorption values. (note: SD is the standard deviation).

Wavenumber /cm^{-1}	Absorbance of the olive oil sample 22	Average absorbance of 50 olive oil samples	SD of 50 olive oil samples	Absorbance of corn oil sample	Absorbance of soybean oil sample	Absorbance of sunflower oil sample
3005.0	0.0333	0.0322	0.0042	0.0609	0.0573	0.0502
2922.2	0.2534	0.2528	0.0025	0.2330	0.2238	0.2370
2853.6	0.1775	0.1767	0.0028	0.1587	0.1536	0.1658
1743.0	0.2539	0.2540	0.0029	0.2397	0.2448	0.2658
1463.2	0.0889	0.0889	0.0021	0.0849	0.0835	0.0874
1232.0	0.0899	0.0893	0.0012	0.0935	0.0914	0.0937
1160.6	0.1794	0.1799	0.0013	0.1703	0.1717	0.1833
1117.6	0.1124	0.1127	0.0022	0.1053	0.1034	0.1095
722.3	0.0961	0.0961	0.0004	0.1026	0.1049	0.1067
583.9	0.0288	0.0288	0.0003	0.0322	0.0331	0.0338

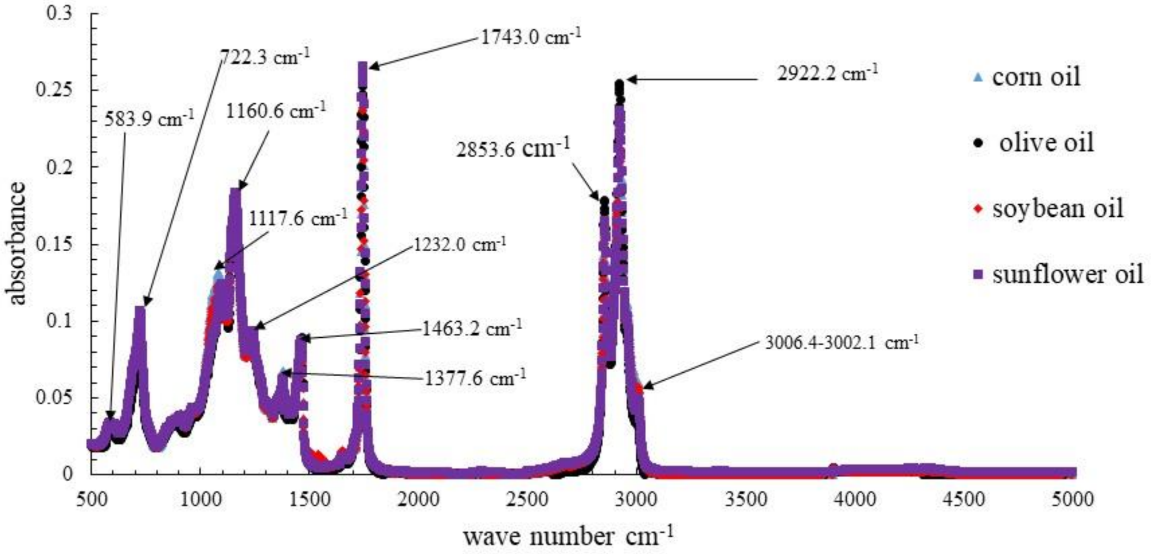

Fig. 1: IR spectra of olive oil and other vegetable oils used in this study.

5.3. The Criteria for determining the purity of the extra virgin olive oil.

In this work absorbance ratios at two characteristic wavenumbers in the IR spectrum is used as a tool for checking the purity of extra virgin olive oil. Since absorbance at certain wavenumber for any same sample will change slightly from one time of measuring to another and from one instrument to another. It also will definitely change depending on the method used for measuring IR spectrum (ATR, liquid film, CCl_4 solution, KBr,….etc). That's why absorbance ratio and not the absolute absorbance at single wavenumber is adopted in this work as sort of normalization to eliminate any factor that can produce differences in reading absorbance at certain wavenumber. Table 2 shows the absorbance ratios at certain wavenumbers of oil sample 22 as well as the average ratio values for the fifty studied olive oil samples. It can be seen that these ratios are constants for all examined olive oils with relatively small standard deviations. Since ratios are constant for extra virgin olive oils and definitely different from those of other vegetable olive oils, these absorbance ratios, altogether, can be used as a criteria verifying the purity of virgin olive oil and a principal for constructing calibration curves for quantitative analysis of adulteration of extra virgin olive oil with other vegetable oils as can be seen in the next section.

Table 2: The absorbance ratios at certain wavenumbers of oil sample 22 as well as the average ratio values for the fifty studied olive oil samples.

Wavenumber ratio	Absorbance ratio of oil sample 22	Average absorbance ratio of 50 olive oil samples	SD
583.9/2922.2	0.1136	0.1127	0.0012
583.9/2853.6	0.1622	0.1625	0.0014
583.9/1463.2	0.3238	0.3229	0.0013
583.9/1160.6	0.1605	0.1617	0.0013
583.9/1117.8	0.2560	0.2576	0.0016
2853.6/1743.0	0.6992	0.6978	0.0018
1743.0/1117.8	2.2585	2.2563	0.0037
722.3/2853.6	0.5382	0.5416	0.0014
722.3/2922.2	0.3778	0.3795	0.0026
722.3/1463.2	1.0804	1.0811	0.0025
722.3/1160.6	0.5356	0.5327	0.0013
722.3/1117.8	0.8545	0.8525	0.0015
2853.6/1160.6	0.9950	0.9973	0.0014
1463.2/2853.6	0.5008	0.5033	0.0012
1232.0/2922.2	0.3533	0.3529	0.0012

5.4. Quantitative analysis of olive oil adulteration with other vegetable oils

The quantitative analysis of olive oil adulteration with other vegetable oils is constructed based on the ratio of absorbance of oil mixtures at two certain wavenumbers, as function of the weight percentage of the vegetable oil in the olive oil mixture. Large numbers of calibration curves were obtained, but only calibration curves with a coefficient of determination (R^2) higher than 0.977 were adopted.

5.4.1. Quantitative analysis of corn oil in olive oil

The absorbance of each corn oil mixtures with olive oil, of weight % ranging from 0.0 to 90% of corn oil, at characteristic wavenumbers as well as the absorbance ratios are shown in table 3. These absorbance ratios as function of weight % corn oil were plotted for constructing the calibration curves, by which extent of adulteration of olive oil with corn oil can be calculated as shown in fig. 2. The calibration curves obtained from fig.2. are summarized in table 4. These calibration curves together can be used as a robust tool for quantitative determination of corn oil in olive oil samples.

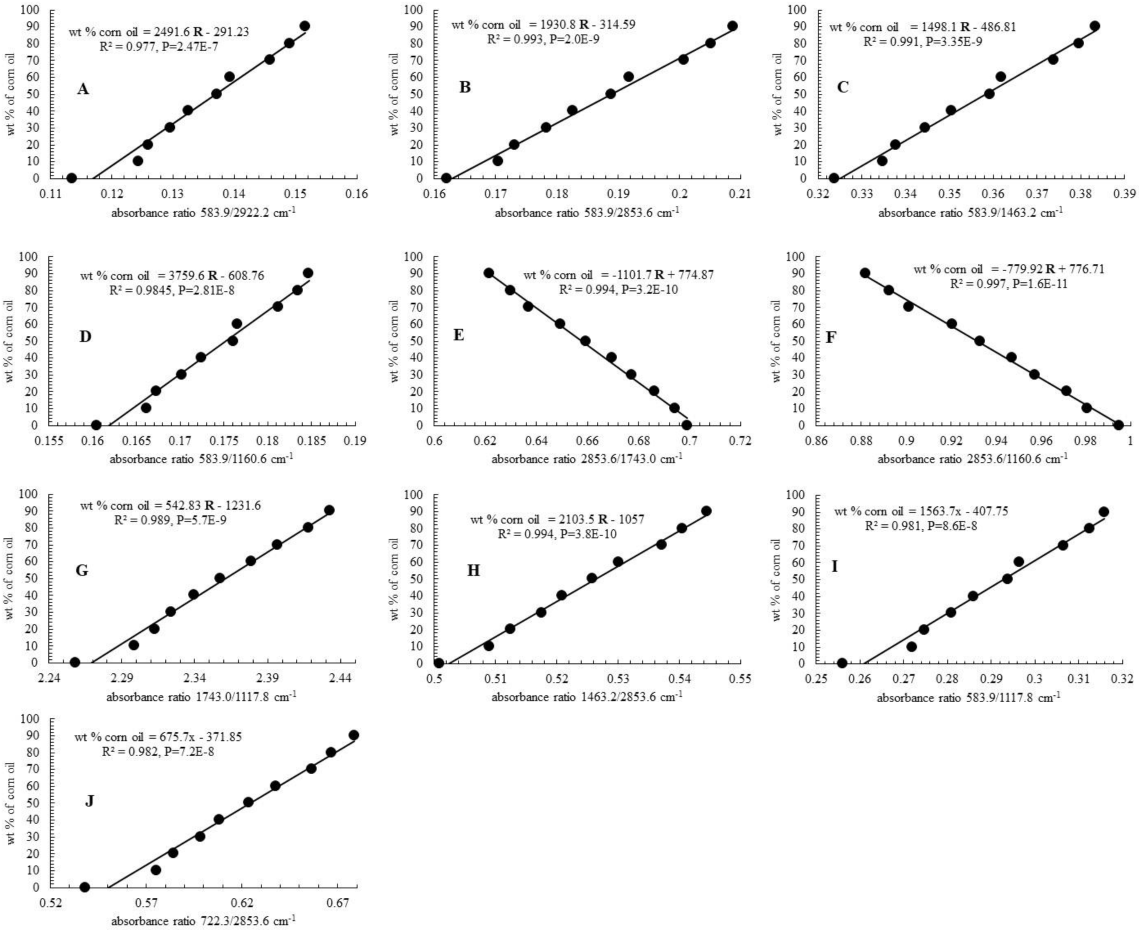

Fig. 2.: Calibration curves for quantitative determination of olive oil adulteration with corn oil.

Table 3: The absorbance of corn oil mixture with olive oil, ranging from 0.0 to 90% of corn oil, at characteristic wavenumbers as well as the absorbance ratios of certain couple wave numbers.

Wt % corn oil	Absorbance at 583.9 cm^{-1}	Absorbance at 722.3cm^{-1}	Absorbance at 1117.8 cm^{-1}	Absorbance at 1160.6 cm^{-1}	Absorbance at 1463.2 cm^{-1}	Absorbance at 1743.0 cm^{-1}	Absorbance at 2853.6 cm^{-1}	Absorbance at 2922.2 cm^{-1}	Absorbance ratio 583.9/2922.2	Absorbance ratio 583.9/2853.6	Absorbance ratio 583.9/1463.2	Absorbance ratio 583.9/1160.6	Absorbance ratio 2853.6/1743.0	Absorbance ratio 2853.6/1160.6	Absorbance ratio 1743.0/1117.8	Absorbance ratio 1463.2/2853.6	Absorbance ratio 583.9/1117.8	Absorbance ratio 722.3/2853.6
0	0.0288	0.0961	0.1124	0.1794	0.0889	0.2539	0.1775	0.2534	0.1136	0.1622	0.3238	0.1605	0.6992	0.9950	2.2585	0.5008	0.2560	0.5382
10	0.0275	0.0928	0.1011	0.1655	0.0821	0.2323	0.1613	0.2209	0.1245	0.1705	0.3349	0.1662	0.6944	0.9808	2.2986	0.5090	0.2721	0.5754
20	0.0278	0.0938	0.1011	0.1660	0.0822	0.2338	0.1605	0.2205	0.1260	0.1731	0.3378	0.1674	0.6865	0.9715	2.3128	0.5124	0.2748	0.5846
30	0.0283	0.0951	0.1009	0.1665	0.0822	0.2345	0.1589	0.2185	0.1297	0.1784	0.3446	0.1702	0.6776	0.9575	2.3241	0.5176	0.2809	0.5986
40	0.0288	0.0959	0.1006	0.1668	0.0821	0.2353	0.1576	0.2170	0.1326	0.1826	0.3505	0.1725	0.6697	0.9471	2.3396	0.5209	0.2861	0.6086
50	0.0294	0.0970	0.1000	0.1668	0.0818	0.2357	0.1555	0.2140	0.1373	0.1890	0.3594	0.1761	0.6595	0.9331	2.3579	0.5258	0.2938	0.6239
60	0.0295	0.0982	0.0996	0.1673	0.0816	0.2370	0.1540	0.2120	0.1393	0.1919	0.3620	0.1766	0.6496	0.9206	2.3789	0.5302	0.2966	0.6377
70	0.0304	0.0995	0.0993	0.1680	0.0814	0.2380	0.1516	0.2088	0.1458	0.2008	0.3738	0.1812	0.6370	0.9015	2.3970	0.5372	0.3066	0.6565
80	0.0309	0.1004	0.0988	0.1682	0.0813	0.2389	0.1505	0.2071	0.1491	0.2052	0.3796	0.1835	0.6299	0.8926	2.4180	0.5405	0.3125	0.6669
90	0.0311	0.1011	0.0985	0.1684	0.0811	0.2396	0.1490	0.2052	0.1516	0.2088	0.3835	0.1847	0.6218	0.8820	2.4326	0.5446	0.3159	0.6787

Table 4: Summary of the calibration curves, by which the extent of adulteration of olive oil with corn oil can be calculated.

Equation No.	Equation	Details of equation
1	Wt % corn oil = 2491.6 **R** - 291.23 $R^2 = 0.977$, P=2.47E-7	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 583.9 cm^{-1}/absorbance of the oil sample at 2922.2 cm^{-1}). As shown in Fig. 2,A
2	wt % corn oil = 1930.8 **R** - 314.59 $R^2 = 0.993$, P=2.0E-9	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 583.9 cm^{-1}/absorbance of the oil sample at 2853.6 cm^{-1}). As shown in Fig. 2,B
3	wt % corn oil = 1498.1 **R** - 486.81 $R^2 = 0.991$, P =3.35E-9	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 583.9 cm^{-1}/absorbance of the oil sample at 1463.2 cm^{-1}). As shown in Fig. 2,C
4	wt % corn oil = 3759.6 **R** - 608.76 $R^2 = 0.9845$, P=2.81E-8	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 583.9 cm^{-1}/absorbance of the oil sample at 1160.6 cm^{-1}). As shown in Fig. 2, D
5	wt % corn oil = -1101.7 **R** + 774.87 $R^2 = 0.994$, P=3.2E-10	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 2853.6 cm^{-1}/absorbance of the oil sample at 1743.0 cm^{-1}). As shown in Fig. 2, E
6	wt % corn oil = -779.92 **R** + 776.71 $R^2 = 0.997$, P=1.6E-11	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 2853.6 cm^{-1}/absorbance of the oil sample at 1160.6 cm^{-1}). As shown in Fig. 2, F
7	wt % corn oil = 542.83 **R** - 1231.6 $R^2 = 0.989$, P=5.7E-9	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 1743.0 cm^{-1}/absorbance of the oil sample at 1117.8 cm^{-1}). As shown in Fig. 2, G
8	wt % corn oil = 2103.5 **R** - 1057 $R^2 = 0.994$, P=3.8E-10	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 1463.2 cm^{-1}/absorbance of the oil sample at 2853.6 cm^{-1}). As shown in Fig. 2, H.
9	wt % corn oil = 1563.7x - 407.75 $R^2 = 0.981$, P=8.6E-8	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 583.9 cm^{-1}/absorbance of the oil sample at 1117.8 cm^{-1}). As shown in Fig. 2, I.
10	wt % corn oil = 675.7x - 371.85 $R^2 = 0.982$, P=7.2E-8	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 722.3 cm^{-1}/absorbance of the oil sample at 2853.6 cm^{-1}). As shown in Fig. 2, J.

5.4.2. Quantitative analysis of sunflower oil in olive oil

The absorbance of each sunflower oil mixtures with olive oil, of weight % ranging from 0.0 to 90% of sunflower oil, at characteristic wavenumbers as well as the absorbance ratios are shown in table 5. These absorbance ratios as function of weight % sunflower oil were plotted for constructing the calibration curves, by which extent of adulteration of olive oil with sunflower oil can be calculated as shown in fig. 3. The calibration curves obtained from fig.3. are summarized in table 6. These calibration curves together can be used as a robust tool for quantitative determination of sunflower oil in olive oil samples.

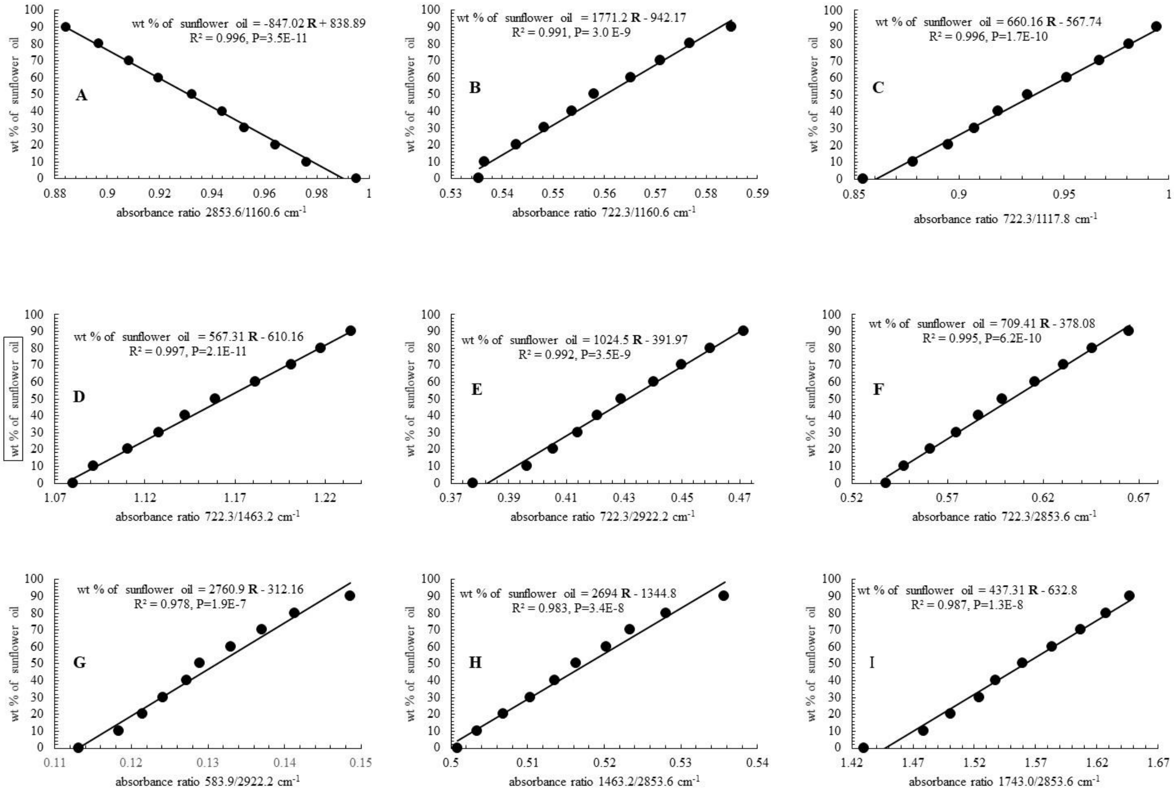

Fig. 3.: Calibration curves for quantitative determination of olive oil adulteration with sunflower oil.

Table 5: The absorbance of sunflower oil mixture with olive oil, ranging from 0.0 to 90% of sunflower oil, at characteristic wavenumbers as well as the absorbance ratios of certain couple wave numbers.

Wt% sunflower oil	**Absorbance at 583.9 cm^{-1}**	**Absorbance at 722.3cm^{-1}**	**Absorbance at 1117.8 cm^{-1}**	**Absorbance at 1160.6 cm^{-1}**	**Absorbance at 1463.2 cm^{-1}**	**Absorbance at 1743.0 cm^{-1}**	**Absorbance at 2853.6 cm^{-1}**	**Absorbance at 2922.2 cm^{-1}**	**Absorbance ratio 583.9/2922.2**	**Absorbance ratio 2853.6/1160.6**	**Absorbance ratio 1743.0/2853.6**	**Absorbance ratio 1463.2/2853.6**	**Absorbance ratio 722.3/2853.6**	**Absorbance ratio 722.3/1160.6**	**Absorbance ratio 722.3/1117.8**	**Absorbance ratio 722.3/1463.2**	**Absorbance ratio 722.3/2922.2**
0	0.0288	0.0961	0.1124	0.1794	0.0889	0.2539	0.1775	0.2534	0.1136	0.9950	1.4303	0.5008	0.5382	0.5356	0.8545	1.0804	0.3778
10	0.0285	0.0953	0.1085	0.1776	0.0873	0.2564	0.1733	0.2403	0.1184	0.9761	1.4793	0.5035	0.5474	0.5366	0.8784	1.0919	0.3965
20	0.0289	0.0966	0.1079	0.1779	0.0869	0.2575	0.1715	0.2381	0.1215	0.9642	1.5013	0.5068	0.5612	0.5428	0.8949	1.1107	0.4056
30	0.0294	0.0980	0.1080	0.1787	0.0869	0.2594	0.1702	0.2367	0.1242	0.9524	1.5242	0.5103	0.5747	0.5484	0.9074	1.1282	0.4141
40	0.0300	0.0992	0.1079	0.1791	0.0868	0.2600	0.1691	0.2357	0.1273	0.9438	1.5378	0.5136	0.5862	0.5537	0.9190	1.1424	0.4208
50	0.0301	0.1002	0.1074	0.1796	0.0865	0.2612	0.1674	0.2338	0.1290	0.9323	1.5599	0.5163	0.5988	0.5581	0.9330	1.1594	0.4289
60	0.0307	0.1017	0.1068	0.1799	0.0861	0.2620	0.1654	0.2310	0.1330	0.9196	1.5840	0.5203	0.6158	0.5653	0.9516	1.1816	0.4402
70	0.0313	0.1028	0.1062	0.1799	0.0855	0.2627	0.1634	0.2284	0.1371	0.9082	1.6073	0.5234	0.6307	0.5711	0.9672	1.2014	0.4500
80	0.0320	0.1041	0.1061	0.1804	0.0854	0.2633	0.1618	0.2262	0.1413	0.8967	1.6278	0.5281	0.6458	0.5768	0.9811	1.2180	0.4600
90	0.0333	0.1058	0.1064	0.1809	0.0857	0.2635	0.1600	0.2244	0.1486	0.8844	1.6474	0.5357	0.6650	0.5849	0.9946	1.2346	0.4715

Table 6: Summary of the calibration curves, by which the extent of adulteration of olive oil with sunflower oil can be calculated.

Equation No.	Equation	Details of equation
11	wt % of sunflower oil = -847.02 **R** + 838.89 $R^2 = 0.996$, P=3.5E-11	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 2853.6 cm^{-1}/absorbance of the oil sample at 1160.6 cm^{-1}). As shown in Fig. 3, A
12	wt % of sunflower oil = 1771.2 **R** - 942.17 $R^2 = 0.991$, P= 3.0 E-9	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 722.3 cm^{-1}/absorbance of the oil sample at 1160.6 cm^{-1}). As shown in Fig. 3, B
13	wt % of sunflower oil = 660.16 **R** - 567.74 $R^2 = 0.996$, P=1.7E-10	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 722.3 cm^{-1}/absorbance of the oil sample at 1117.8 cm^{-1}). As shown in Fig. 3, C
14	wt % of sunflower oil = 567.31 **R** - 610.16 $R^2 = 0.997$, P=2.1E-11	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 722.3 cm^{-1}/absorbance of the oil sample at 1463.2 cm^{-1}). As shown in Fig. 3, D
15	wt % of sunflower oil = 1024.5 **R** - 391.97 $R^2 = 0.992$, P=3.5E-9	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 722.3 cm^{-1}/absorbance of the oil sample at 2922.2 cm^{-1}). As shown in Fig. 3, E
16	wt % of sunflower oil = 709.41 **R** - 378.08 $R^2 = 0.995$, P=6.2E-10	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 722.3 cm^{-1}/absorbance of the oil sample at 2853.6 cm^{-1}). As shown in Fig. 3, F
17	wt % of sunflower oil = 2760.9 **R** - 312.16 $R^2 = 0.978$, P=1.9E-7	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 583.9 cm^{-1}/absorbance of the oil sample at 2922.2 cm^{-1}). As shown in Fig. 3, G
18	wt % of sunflower oil = 2694 **R** - 1344.8 $R^2 = 0.983$, P=3.4E-8	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 1463.2 cm^{-1}/absorbance of the oil sample at 2853.6 cm^{-1}). As shown in Fig. 3, H
19	wt % of sunflower oil = 437.31 **R** - 632.8 $R^2 = 0.987$, P=1.3E-8	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 1743.0 cm^{-1}/absorbance of the oil sample at 2853.6 cm^{-1}). As shown in Fig. 3, I

5.4.3. Quantitative analysis of soybean oil in olive oil

The absorbance of each soybean oil mixtures with olive oil, of weight % ranging from 0.0 to 90% of soybean oil, at characteristic wavenumbers as well as the absorbance ratios are shown in table 7. These absorbance ratios as function of weight % soybean oil were plotted for constructing the calibration curves, by which extent of adulteration of olive oil with soybean oil can be calculated as shown in fig. 4. The calibration curves obtained from fig.4. are summarized in table 8. These calibration curves together can be used as a robust tool for quantitative determination of soybean oil in olive oil samples.

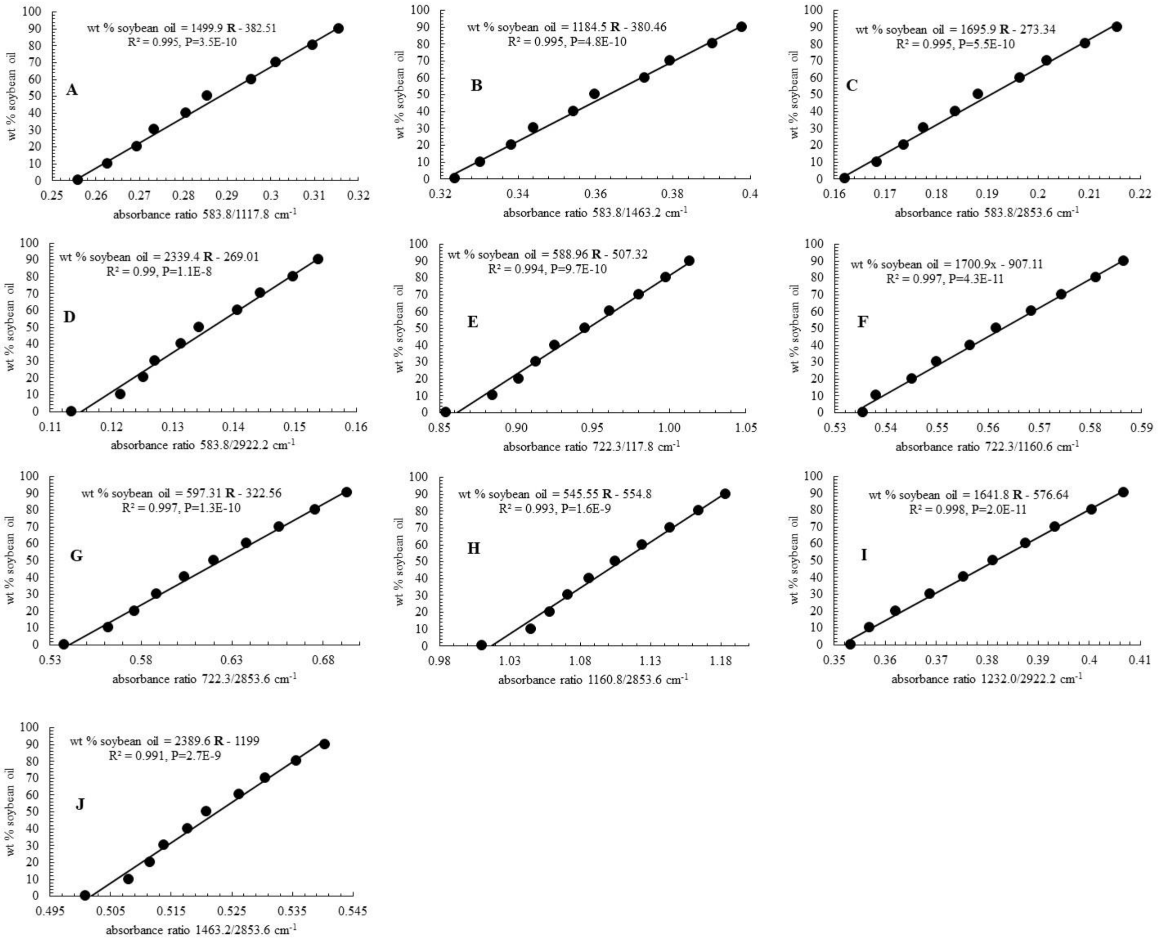

Fig. 4.: Calibration curves for quantitative determination of olive oil adulteration with sunflower oil.

Table 7: The absorbance of soybean oil mixture with olive oil, ranging from 0.0 to 90% of soybean oil, at characteristic wavenumbers as well as the absorbance ratios of certain couple wave numbers.

Wt% soybean oil	**Absorbance at 583.9 cm^{-1}**	**Absorbance at 722.3cm^{-1}**	**Absorbance at 1117.8 cm^{-1}**	**Absorbance at 1160.6 cm^{-1}**	**Absorbance at 1232.0 cm^{-1}**	**Absorbance at 1463.2 cm^{-1}**	**Absorbance at 2853.6 cm^{-1}**	**Absorbance at 2922.2 cm^{-1}**	**Absorbance ratio 583.9/2922.2**	**Absorbance ratio 1463.2/2853.6**	**Absorbance ratio 722.3/2853.6**	**Absorbance ratio 722.3/1160.6**	**Absorbance ratio 722.3/1117.8**	**Absorbance ratio 583.9/1117.8**	**Absorbance ratio 583.9/1463.2**	**Absorbance ratio 583.9/2853.6**	**Absorbance ratio 1160.8/2853.6**	**Absorbance ratio 1232/2922.2**
0	0.0288	0.0961	0.1124	0.1794	0.0899	0.0889	0.1775	0.2534	0.1136	0.5008	0.5382	0.5356	0.8545	0.2560	0.3238	0.1622	1.0104	0.3533
10	0.0282	0.0950	0.1074	0.1765	0.0824	0.0854	0.1681	0.2320	0.1216	0.5081	0.5622	0.5381	0.8847	0.2628	0.3304	0.1684	1.0453	0.3571
20	0.0288	0.0963	0.1068	0.1767	0.0828	0.0851	0.1664	0.2298	0.1253	0.5116	0.5767	0.5451	0.9019	0.2696	0.3383	0.1737	1.0586	0.3622
30	0.0292	0.0977	0.1070	0.1776	0.0842	0.0850	0.1653	0.2299	0.1272	0.5139	0.5889	0.5500	0.9130	0.2734	0.3442	0.1776	1.0716	0.3689
40	0.0301	0.0992	0.1072	0.1783	0.0854	0.0849	0.1640	0.2289	0.1315	0.5177	0.6039	0.5565	0.9253	0.2807	0.3545	0.1839	1.0863	0.3754
50	0.0303	0.1003	0.1061	0.1785	0.0852	0.0842	0.1616	0.2254	0.1344	0.5209	0.6202	0.5617	0.9452	0.2856	0.3600	0.1883	1.1053	0.3811
60	0.0314	0.1020	0.1061	0.1795	0.0857	0.0841	0.1599	0.2231	0.1407	0.5262	0.6383	0.5685	0.9612	0.2957	0.3729	0.1965	1.1243	0.3875
70	0.0317	0.1032	0.1053	0.1797	0.0856	0.0836	0.1575	0.2196	0.1444	0.5306	0.6563	0.5744	0.9803	0.3012	0.3795	0.2018	1.1442	0.3934
80	0.0325	0.1047	0.1049	0.1802	0.0861	0.0832	0.1554	0.2170	0.1498	0.5357	0.6757	0.5812	0.9980	0.3097	0.3904	0.2093	1.1647	0.4005
90	0.0330	0.1061	0.1047	0.1809	0.0865	0.0830	0.1536	0.2148	0.1538	0.5404	0.6933	0.5866	1.0138	0.3156	0.3980	0.2155	1.1838	0.4068

Table 8: Summary of the calibration curves, by which the extent of adulteration of olive oil with soybean oil can be calculated.

Equation No.	Equation	Details of equation
20	wt % soybean oil = 1499.9 **R** - 382.51 R^2 = 0.995, P=3.5E-10	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 583.8 cm^{-1}/absorbance of the oil sample at 1117.8 cm^{-1}). As shown in Fig. 4, A
21	wt % soybean oil = 1184.5 **R** - 380.46 R^2 = 0.995, P=4.8E-10	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 583.8 cm^{-1}/absorbance of the oil sample at 1463.2cm^{-1}). As shown in Fig. 4, B
22	wt % soybean oil = 1695.9 **R** - 273.34 R^2 = 0.995, P=5.5E-10	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 583.8 cm^{-1}/absorbance of the oil sample at 2853.6cm^{-1}). As shown in Fig. 4, C
23	wt % soybean oil = 2339.4 **R** - 269.01 R^2 = 0.99, P=1.1E-8	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 583.8 cm^{-1}/absorbance of the oil sample at 2922.2cm^{-1}). As shown in Fig. 4, D
24	wt % soybean oil = 588.96 **R** - 507.32 R^2 = 0.994, P=9.7E-10	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 722.3 cm^{-1}/absorbance of the oil sample at 1117.8cm^{-1}). As shown in Fig. 4, E
25	wt % soybean oil = 1700.9x - 907.11 R^2 = 0.997, P=4.3E-11	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 722.3 cm^{-1}/absorbance of the oil sample at 1160.6cm^{-1}). As shown in Fig. 4, F
26	wt % soybean oil = 597.31 **R** - 322.56 R^2 = 0.997, P=1.3E-10	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 722.3 cm^{-1}/absorbance of the oil sample at 2853.6 cm^{-1}). As shown in Fig. 4, G
27	wt % soybean oil = 545.55 **R** - 554.8 R^2 = 0.993, P=1.6E-9	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 1160.8 cm^{-1}/absorbance of the oil sample at 2853.6 cm^{-1}). As shown in Fig. 4, H
28	wt % soybean oil = 1641.8 **R** - 576.64 R^2 = 0.998, P=2.0E-11	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 1232.0 cm^{-1}/absorbance of the oil sample at2922.2 cm^{-1}). As shown in Fig. 4, I
29	wt % soybean oil = 2389.6 **R** - 1199 R^2 = 0.991, P=2.7E-9	Obtained by plotting absorbance ratio **R** (absorbance of the oil sample at wavenumber 1463.2 cm^{-1}/absorbance of the oil sample at 2853.6 cm^{-1}). As shown in Fig. 4, J

Chapter six: Conclusions

The dilemma of olive oil adulteration with other cheaper oils still exists. According to our knowledge, there is no easy and simple way to solve it. In this research, we were able to find a solution using a direct simple method to detect the purity of virgin olive oil as well as the extent of its adulteration with other vegetable oils using ATR-FTIR. ATR FTIR spectroscopy can be used as a direct, robust, simple, easy, not expensive, fast method and without sample preparation for determining of olive oil purity. Appropriate equations were selectively derived for quantitative determination of olive oil adulteration with other vegetable oils (corn oil, sunflower oil and soybean oil). We are looking forward to apply this method as a tool for studying the extent and kinetics of olive oil rancidity.

There are still gaps to be bridged in terms of knowing the type of oils used in adulteration. There is an urgent need to develop a comprehensive model for this issue. We believe that other innovative and new methods can be used rather than ATR-FTIR, like isotope composition analysis of the atoms exist in olive oil compounds.

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التحليل الكمي لغش زيت الزيتون بزيوت رخيصة باستخدام جهاز قياس طيف الاشعة تحت الحمراء.

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الملخص:

زيت الزيتون البكر ذو قيم غذائية و علاجية عالية و طعم و نكهة مميزين مقارنة بالزيوت الاخرى. استهلاك زيت الزيتون البكر يزداد يوما بعد يوم في جميع مطابخ العالم و هذا يفسر غلاء هذا الزيت و كثرة محاولات غشه بمزجه مع زيوت نباتية منخفضة الجودة و رخيصة الثمن كزيت الذرة و دوار الشمس و الصويا. من الملح تطوير طريقة سريعة و سهلة و رخيصة و دون جهد في تحضير العينات للتحليل و أن تكون الطريقة دقيقة و موثوقة. تعتمد طريقة التحليل الحالية على استخدام جهاز الاشعة تحت الحمراء لقياس امتصاص عينات الزيوت عند اطوال امواج محددة. تم جمع 50 عينة من زيت الزيتون الطازج البكر من مزارعين فلسطينيين من الضفة الغربية غطت كامل مناطق الضفة من اقصى شمالها (جنين) الى اقصى جنوبها (الخليل) و تم اخذ طيف الاشعة تحت الحمراء لكل عينة من زيت الزيتون و الزيوت النباتية الاخرى ، بحيث تم تخزين مقدار الامتصاص كنقاط بيانية. تم اعتماد العينة رقم 22 و المأخوذة من مجلس الزيت الفلسطيني لخلطها مع الزيوت الاخرى بنسب مختلفة و ذلك لبناء منحنيات للمعايرة لتحديد كمية الغش بالزيوت الرخيصة. يمكن حسابه عن طريق اخذ نسبة الامتصاص الضوئي عند طولي موجتين محددتين. مثال نسبة امتصاص زيت الزيتون البكر(2922.2/583.9) هي قيمة ثابتة لكل عينة زيت زيتون بكر و تساوي 0.1136 و بهامش خطأ 0.0012. كما تم التوصل الى ايجاد منحنيات لمعايرة كمية الغش وفق المعادلات التالية

نسبة الغش يزيت الذرة

wt % corn oil = -779.92 **R** + 776.71, $R^2 = 0.997$, P=1.6E-11.

نسبة الغش بزيت دوار الشمس

wt % of sunflower oil = 567.31 **R** - 610.16, $R^2 = 0.997$, P=2.1E-11.

نسبة الغش بزيت الصويا

wt % soybean oil = 1641.8 **R** - 576.64 $R^2 = 0.998$, P=2.0E-11