Food Science and Engineering
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UNIVERSAL WISER PUBLISHER

Research Article

Assessing the Oxidative Stability of Extra Virgin Olive Oil from Different Regions Using Fluorescence Spectroscopy

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Received: 7 February 2025; **Revised:** 27 April 2025; **Accepted:** 21 May 2025

Abstract: To retain the quality of Extra Virgin Olive Oil (EVOO), it is critical to control oxidation during production and storage. It is a difficult task to prevent oxidation in EVOO since many physical and chemical factors must be controlled. In the current study, extra virgin olive oil was stored at room temperature for three months and monitored using quality changes (oxidation products, β-carotene, and chlorophyll content). Non-destructive fluorescence spectroscopy was used to evaluate oxidation changes in EVOO from several olive-growing regions in Pakistan and Al-Jouf, Kingdom of Saudi Arabia (KSA). Additionally, the impacts of geographic, climatic, and environmental factors on the oxidation of EVOOs were investigated. Three major changes in the fluorescence emission spectra of EVOO samples were observed: a decrease in intensities in the 500-600 nm and 650-690 nm regions, corresponding to the degradation of β-carotene and chlorophyll content, respectively, and an increase in the 365-500 nm region, associated with the formation of oxidation products. All EVOO samples were oxidized over time, with Al-Jouf EVOOs having a slower oxidation rate (3.6392) than Pakistani samples (7.029). This distinction can be linked to environmental and geographical considerations, as well as beneficial irrigation systems, harvesting processes, processing methods, and storage conditions. Fluorescence spectroscopy successfully monitored oxidation changes and antioxidant deterioration in EVOOs in a rapid, non-destructive manner.

***Keywords*:** EVOO, vitamin E, beta carotene, chlorophyll content, region of cultivation, fluorescence spectroscopy, quality assurance, rate of oxidation

1. Introduction

Extra Virgin Olive Oil (EVOO) is a well-known edible oil that is produced from the fruit of the olive tree [1]. It is rich in vitamin E, β-carotene, monounsaturated fatty acids, and phenolic antioxidants [2]. Additionally, it includes moderate quantities of vitamin K, vitamin E (tocopherols), provitamin A (β-carotene), and vitamin F components, particularly oleic and linoleic acids [3-5]. EVOO contains key pigments such as chlorophyll and carotenoids [6]. Olive oil color varies from light gold to deep green, depending on pigment content [7]. Green olives, rich in chlorophyll, contribute to the production of green oil, while ripe olives, containing carotenoids, result in the extraction of yellow oil [8]. Throughout the years, EVOO has been the topic of numerous scientific studies due to its well-known health-promoting benefits, and its ubiquitous position as an essential component of traditional diets worldwide [9, 10].

DOI: https://doi.org/10.37256/fse.6220256526

Recent research has focused on the relationship between monounsaturated fatty acids (particularly oleic acid) and minor constituents of Extra Virgin Olive Oil (EVOO), such as phenolic compounds, carotenoids, α-tocopherol, squalene, simple triterpenes (e.g., oleanolic and maslinic acids), and volatile compounds contributing to its aroma and flavor, and their associated health benefits, such as antihypertensive and chemopreventive, tumor-inhibitory, and anti-inflammatory activities. In addition to its nutritional significance, EVOO is widely acclaimed for its specific sensory properties, which include scent, taste, and texture [3, 11].

Several studies have found that oxygen, light, and temperature are important factors in accelerating deteriorative processes in EVOO via oxidative and hydrolytic reactions [12-17]. Oxidation reduces EVOO quality and shelf life. It is one of the major degradation factors during storage but can be mitigated by polyphenolic compounds and tocopherols [17-19]. After extraction, filtration, and packaging, Extra Virgin Olive Oil (EVOO) experiences slow chemical changes throughout storage, even under settings that minimize light, oxygen, and heat exposure. The most significant of these alterations is oxidative deterioration, also known as rancidity [20]. Autoxidation produces primary oxidation products such as hydroperoxides, which then break down into secondary compounds such as aldehydes, ketones, alcohols, and short-chain acids, resulting in off-flavors, lower nutritional quality, and, finally, customer rejection. Meanwhile, minor bioactive components are degraded [21]. Among them, tocopherols are lipid-soluble antioxidants that play an important role in protecting Extra Virgin Olive Oil (EVOO) from oxidative deterioration while also increasing its nutritional value [22]. Aside from their role in oil preservation, tocopherols are among the most powerful lipid-soluble antioxidants in the human body, protecting cell membranes from oxidative damage caused by peroxyl radicals and mutagenic nitrogen oxide species. These reactive species can be produced endogenously through metabolic processes such as lipid peroxidation, inflammatory responses, and mitochondrial activity, or exogenously through environmental sources such as air pollution, tobacco smoke, and the consumption of oxidized fats and processed foods. Their accumulation promotes oxidative stress and cellular malfunction [23].

Phenolic compounds are important for olive oil stability and flavour [7]. Furthermore, the phenolic components of EVOOs are frequently utilized to analyze their authenticity and prospective health impacts, including antioxidant, anti-inflammatory, and cardioprotective qualities [24].

For consumers, one of the most important characteristics of EVOO is freshness, as freshness is typically associated with high quality and ensures food safety. The term “shelf life” is commonly referred to when determining the freshness of EVOO, which is typically around 12 to 18 months under proper storage conditions. Contact with improper materials, such as metal containers and plastic bottles, should be avoided since they may promote oxidative degradation events, reducing the oil’s shelf life [12].

Oxidation stability is considered one of the most important quality indicators of edible vegetable oils. It determines their functional performance in food processing technologies and has a substantial impact on shelf life during storage [4].

In recent years, there has been an increased focus on developing analytical methods capable of detecting and quantifying oxidative changes in edible oils. Traditional techniques such as gas chromatography, High-Performance Size-Exclusion Chromatography (HPSEC), and Ultraviolet-Visible (UV-Vis) spectrophotometry have been frequently used for this purpose due to their great sensitivity and precision [25]. Despite their analytical strength, these methods are frequently linked with substantial restrictions, such as the use of toxic chemicals, labor-intensive procedures, lengthy analysis durations, high operational costs, and the necessity for thorough sample preparation. To overcome these limitations and get a better understanding of oil degradation mechanisms, a variety of other analytical methodologies have been investigated. Infrared and Raman spectroscopy, one- and two-Dimensional Nuclear Magnetic Resonance (1D and 2D NMR), differential scanning calorimetry, and Front-Face Fluorescence Spectroscopy (FFFS) have all been used to evaluate not only oxidation but also thermal stability [26], chemical composition, and overall quality of edible oils under various storage and processing conditions [27]. Among developing analytical techniques, fluorescence spectroscopy has received a lot of attention as a quick, non-destructive method with excellent specificity and sensitivity. Its use in food analysis has grown dramatically in recent years, notably for monitoring quality criteria such as oxidation, adulteration, and compositional changes, because of its minimal sample preparation needs and ability to provide precise molecular insight [21, 23, 28].

Fluorescence spectroscopy has emerged as a preferred technique to analyse and monitor olives and olive oil [16]. It has the advantage over the other conventional methods due to its speed of analysis and no sample preparation with the elimination of solvents and reagents. Moreover, fluorescence spectroscopy has been proposed to monitor olive oil

under varying storage conditions [14]. According to reports, fluorescence spectroscopy can accurately classify various types of edible oils. It has been used to assess the chlorophyll content of olive oil [21], track oxidative changes during frying, and distinguish between different types of edible oils. Furthermore, it has been used to distinguish olive oils prepared by various processes, such as virgin, refined, or blended oils [23]. This study presents a novel comparative analysis of oxidative degradation and antioxidant deterioration in Extra Virgin Olive Oil (EVOO) from Saudi Arabia and Pakistan during storage. While prior studies have examined EVOO stability, minimal emphasis has been given to oils from these locations, which vary in crop, climate, and production procedures. The primary region in Saudi Arabia for olive production, Al Jouf, is becoming more well-known worldwide, while Pakistan is becoming a producer thanks to domestic gardening programs. Investigating EVOO from these areas offers important insights into oil stability in a range of agricultural and environmental settings. What distinguishes this study from earlier fluorescence-based EVOO studies is its emphasis on regional diversity and compound-specific monitoring. It offers a quantitative, time-resolved fluorescence measurement of important antioxidants such as tocopherols, β-carotene, and chlorophyll during storage, allowing for a better understanding of oxidative degradation and antioxidant decline in oils from these underexplored regions.

2. Materials and methods

Five EVOO samples (A, B, C, D, and E) from the AL-Jouf region of Saudi Arabia and four local EVOO samples (F from Chaman Baluchistan, G from Morgha Biodiversity Park, Rawalpindi, H from Barani Agriculture Chakwal, and J from Loralai Baluchistan) were obtained for this study from various parts of Pakistan. All EVOO samples were maintained in amber glass bottles to reduce light exposure, kept at ambient temperature (22-25 °C), sealed tightly to limit air contact, and kept in a dry environment with controlled humidity. Fluorescence spectroscopy was used to test the stability of Extra Virgin Olive Oil (EVOO) samples throughout storage by monitoring the breakdown of important antioxidant components and the generation of oxidation products. EVOO samples from Saudi Arabia and Pakistan were stored at room temperature for three months before being evaluated at one-month intervals each. The degradation of vitamin E, β-carotene, and chlorophyll was assessed at three time points: the first, second, and third months. Following each examination, samples were returned to storage under the same circumstances for ongoing monitoring. Fluorescence spectra were acquired for each sample, with five spectra collected each time to assure consistency and accuracy. The fluorescence approach was utilized to measure changes in the relative intensity of fluorescence signals from antioxidants and oxidized products. The Area Under the Curve (AUC) method was used to quantify the degradation rates of individual substances. This approach allowed for the study of oxidation processes and antioxidant stability in EVOO over the storage period.

2.1 *Fluorescence spectra acquisition*

A right-angle configuration fluorescence spectrometer (FluoroMax-4, Horiba Scientific, Jobin Yvon, USA) was used to measure the fluorescence spectra. The excitation source was a continuous 150 W ozone-free xenon arc lamp, and the detector was a photomultiplier (R928P). To capture closely spaced emission spectra events, the excitation and emission monochromators slit sizes were fixed at 3 and 2 nm, respectively. Five spectra of each sample were obtained in order to validate the data for comparison between various measurements. The 350 nm excitation wavelength targets intrinsic fluorophores in EVOO, such as tocopherols, phenolic compounds, and primary oxidation products. The 365-690 nm emission range captures fluorescence from natural antioxidants and oxidative breakdown products, making it perfect for monitoring EVOO's oxidative condition.

2.2 *Fluorescence spectra data processing using MATLAB*

A set of independently developed MATLAB (MathWorks, Release 2014a) routines was used for vector normalization and pre-processing of each fluorescence spectrum to enhance data quality and remove unwanted noise or artifacts. This included baseline correction to eliminate background noise, and normalization to standardize signal intensities across samples. Principal Component Analysis (PCA) based statistics were also utilized to categorize various

EVOO samples according to variances in spectral properties. PCA was then applied to the processed spectral dataset to reduce dimensionality and extract critical characteristics that characterize variation in antioxidant degradation and oxidation product production during storage. The purpose was to visualize temporal patterns (month-by-month), as well as to determine whether samples clustered based on oxidative stability. The score plots of PC1 vs PC2 were examined to determine grouping behavior, with each principal component indicating a linear combination of spectral properties. The percentage of variance described by PC1 and PC2 was used to determine how much variation was captured in the first two dimensions. This graphical representation provided insight into which EVOO samples were more stable or prone to degradation over the three-month storage period.

3. Results and discussion

The fluorescence spectra of EVOO A from Saudi Arabia are shown in Figure 1a, which were obtained to monitor changes in the composition of EVOO stored at room temperature for three months on the shelf, for the subsequent assessment of its shelf life. Relative intensity variations in emission spectra were recorded with 350 nm excitation wavelength and 365-690 nm emission wavelength. Four prominent emission bands appeared at 442, 466, 518 and 676 nm. The emission bands at 442 and 466 nm were assigned to vitamin E contents particularly the α-tocopherol [29]. The emission band found in the 440-470 nm range can be related to the existence of conjugated dienes and trienes in the oil, as well as to oxidative degradation processes that occur during the course of the product's shelf life, as represented by the increasing emission intensities [25, 28]. EVOO contains carotenoids like β-carotene and lutein [8, 29] that prevent oxidation of oil by acting as an antioxidant. The emission band at 518 nm has been associated with carotenoids, especially the β-carotene [28, 30]. Chlorophyll and polyphenols, which are naturally present in Extra Virgin Olive Oil (EVOO), deteriorate with time and under various storage conditions. A relatively high-intensity fluorescence emission band, commonly detected between 600 and 700 nm, is connected with chlorophyll concentration and is responsible for the distinctive greenish tint of EVOO [6]. More specifically, emissions associated with chlorophyll pigments, notably chlorophyll a and b, are usually observed in the range of 660-700 nm [24, 29]. The intensity of a fluorescence emission band indicates the concentration of the emitting biomolecule, and its spectral location relates to the individual intrinsic fluorophore [31]. In lipids non-conjugated double and triple bonds are converted to conjugated bonds (diene and triene) upon oxidation [29]. It is well-established that β-carotene and chlorophyll are highly sensitive to thermal stress, deteriorating even shortly after EVOO extraction and packaging [32]. Therefore, as storage time increases, the relative intensity of bands in the 365-500 nm range increases compared to control EVOO samples, due to the production of primary oxidation products and the deterioration of sensitive antioxidants.

The oxidation products are frequently produced during storage from the auto-oxidation and photo-oxidation of oil [32]. During auto-oxidation, peroxides react with low molecular weight compounds present in the food matrix such as free fatty acids, aldehydes, and ketones resulting in further oxidative destruction [33]. In this study, oil oxidation is primarily affected by the presence of pigments, antioxidants, and oxidation products [34, 35]. Oxidation products arise in olive oils quickly after the production of hydroperoxides. These hydroperoxides break down in the presence of heat, light, metal ions, or enzymatic activity, resulting in secondary oxidation chemicals that contribute to off-flavors in the oxidized oil [36].

This study found that variations in the fluorescence spectra of Extra Virgin Olive Oil (EVOO) throughout storage were predominantly caused by the presence and degradation of intrinsic fluorophores such as tocopherols, chlorophylls, and carotenoids. These molecules, which are crucial to the oil's oxidative stability, were detected using distinctive emission bands. Tocopherols serve as antioxidants, however, they deteriorate over time, resulting in diminished fluorescence at specific wavelengths. Photo-oxidation degrades chlorophylls, which lead to intense fluorescence between 660 and 700 nm. Carotenoids, while effective in quenching singlet oxygen and shielding against photo-oxidation, may contribute to autoxidation over protracted storage because their oxidation products can react with lipid substrates, facilitating additional oxidative degradation [17, 37].

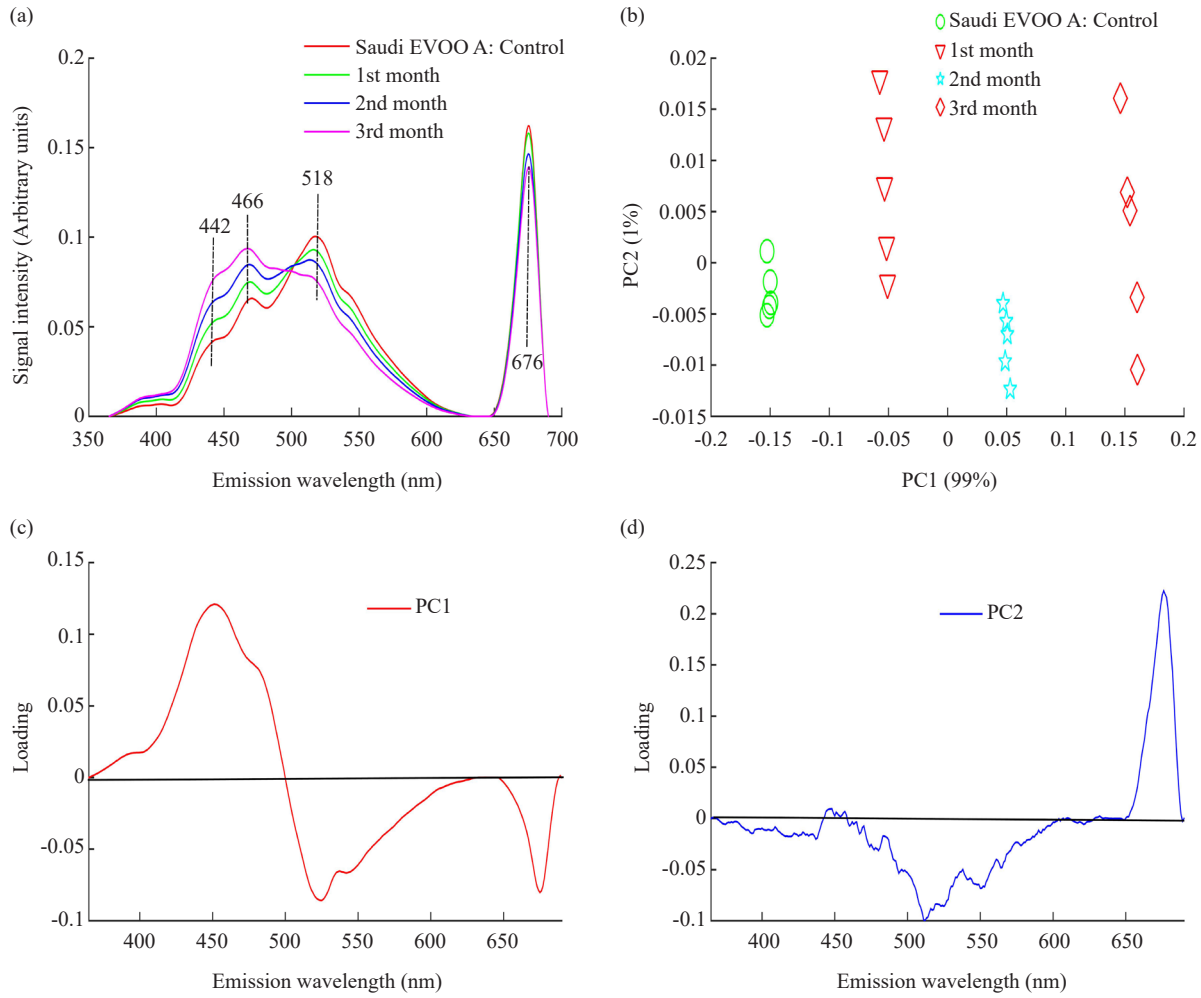

Figure 1. (a) Fluorescence spectra of Saudi EVOO A with the first, second and third months in shelf life (b) PCA scatter plot of Saudi EVOO A in shelf life (c) Loading vector for PC1 (d) Loading vector for PC2

Changes in fluorescence intensity reflect differences in the concentration of fluorophoric chemicals. A decrease in antioxidant-related bands indicates their breakdown, whereas the development of new bands suggests the production of oxidation products [34]. The variation in any particular peak was calculated using the Area Under the Curve (AUC) method will be discussed later with respect to control observations.

To examine spectral changes in Extra Virgin Olive Oil (EVOO) over storage, Principal Component Analysis (PCA) was used. The score plot (Figure 1b) shows a clear temporal trend along PC1, mostly capturing differences related to shelf life. Control and first-month samples cluster on the negative side of PC1, whereas second- and third-month samples gradually shift to the positive side, showing oxidation-induced chemical changes. Spectral changes are further supported by increased fluorescence intensity between 415-470 nm over time, attributed to the degradation of vitamin E and the formation of fatty acid oxidation products. Concurrently, a decrease in intensity between 500-535 nm suggests the breakdown of β-carotene due to prolonged storage. The PC1 loading plot (Figure 1c) shows strong positive contributions from 442-466 nm, which corresponds to oxidation products such as conjugated dienes and trienes, indicating their accumulation over time. At 518 and 676 nm, there are negative loadings for β-carotene and chlorophyll a, indicating a decrease in antioxidants and pigments. Despite accounting for only 1% of the variance, PC2 shows a strong positive loading at 676 nm (Figure 1d), indicating an independent variation in chlorophyll degradation that PC1 does not capture. Overall, PC1 reflects the primary oxidative degradation processes in EVOO, while PC2 captures subtler pigment-related changes. These findings underscore the effectiveness of combining fluorescence spectroscopy

with PCA to monitor oil quality during storage.

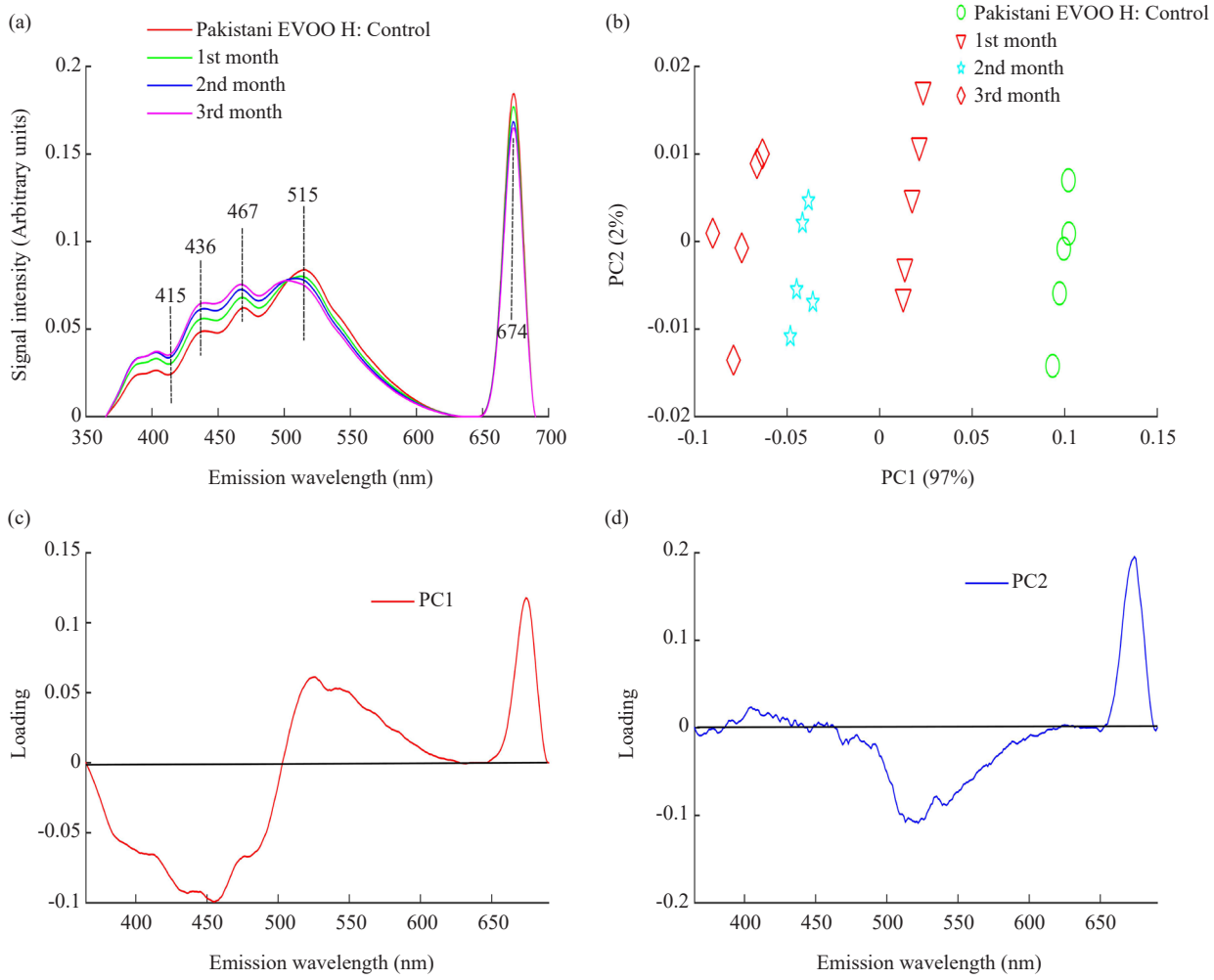

Figure 2. (a) Fluorescence spectra of Pakistani EVOO H with the first, second and third months in shelf life (b) PCA scatter plot of Saudi EVOO H in shelf life (c) Loading vector for PC1 (d) Loading vector for PC2

The Principal Component Analysis (PCA) and fluorescence spectrum analysis utilized to assess the oxidative stability of Pakistani Extra Virgin Olive Oil (EVOO) over a three-month storage period are shown in Figure 2. The following wavelengths exhibit distinctive fluorescence bands: 415, 436, 467, 515, and 674 nm. These bands indicate the presence of tocopherols, carotenoids, and chlorophyll derivatives all of which are recognized indicators of oil quality. The gradual deterioration of natural antioxidants and the beginning of oxidation are indicated by minute variations in the intensity of these bands over time, especially an increase at 674 nm. Based on spectral properties, the PCA score plot shows how the EVOO samples are clustered. 97% of the variance is captured by Principal Component 1 (PC1), with PC2 accounting for the remaining 2%. A significant temporal change in the fluorescence profile as a result of oxidation is suggested by the distinct separation between samples from various months along PC1. The method's sensitivity to chemical changes over time is demonstrated by the clear clustering of the control samples from those kept for longer periods of time. The emission wavelengths that most significantly contribute to the variance seen in the score plot are identified by the loading plot for PC1. The spectral characteristics associated with antioxidants degradation and the formation of oxidative products are reflected in positive and negative loadings at important wavelengths (about 430-540 nm and 670 nm). Its function as a crucial indicator of chlorophyll breakdown during oxidation is confirmed by the

peak at 674 nm, which exhibits an exceptionally strong positive loading. PC2 sheds light on subtle spectrum differences even though it only accounts for 2% of the variance. The loading figure displays variations throughout the emission spectrum, especially between 400 and 550 nm. These variations could be sample-specific or reflect minute secondary oxidation changes. PC2 aids in improving the ability to distinguish between closely comparable spectral profiles, despite being less dominating than PC1.

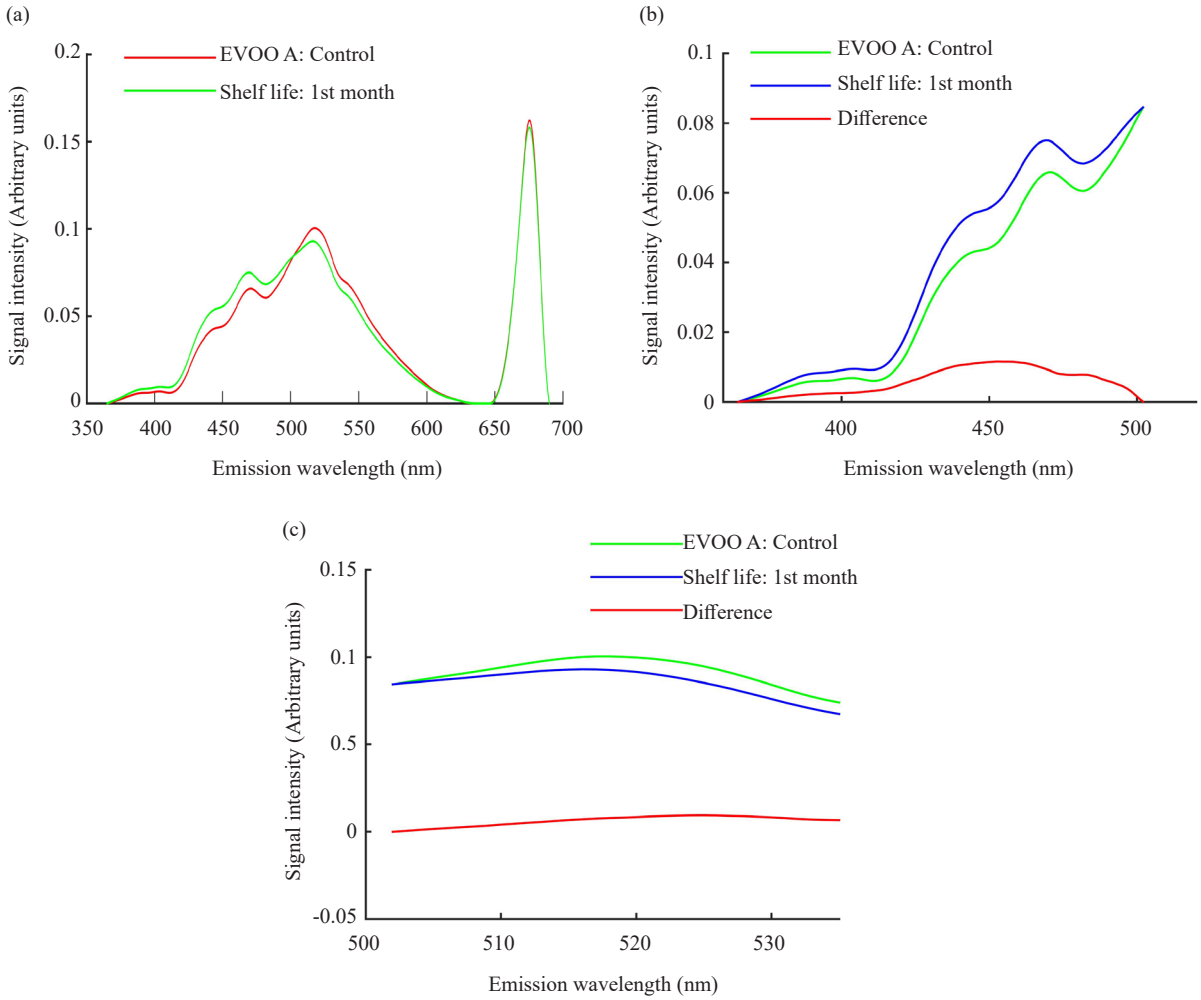

Figure 3. (a) Fluorescence spectra of Saudi EVOO A with first month in shelf life (b) Difference plot of Saudi EVOO A with first month for oxidation products (c) Difference plot of Saudi EVOO A with first month for beta carotene

The spectra of Saudi EVOO A are shown in Figure 3a both in its control state and following the first month of storage. Since quality degradation first appears during the first month, the data from that period were selected for further analysis. Early chemical and fluorescence changes start to appear at this phase, which is crucial for identifying minute variations linked to the development of shelf life. By concentrating on this stage, the analytical approach's sensitivity in detecting early quality markers is increased and important insights into the early causes of deterioration are gained.

Firstly, the analysis focused on the spectral region of oxidation products, followed by the spectral region of beta-carotene. The evolution of the fluorescence in the 365-500 nm region corresponds to oxidation products, as discussed earlier.

The analysis was first directed at the spectral region associated with oxidation products, followed by the region corresponding to β-carotene. As previously discussed, fluorescence evolution in the 365-500 nm range indicates the development of oxidation products. To quantify differences between the control and first-month shelf-life samples, the

spectral range 400-440 nm was evaluated using the Area Under the Curve (AUC) method, as shown in Figure 3b. This method identifies changes in the spectrum fingerprints of oxidation products. The difference between control and first-month samples was determined by analyzing the spectral region from 500-535 nm, which is related to β-carotene, as shown in Figure 3c. Origin software was used to compute numerical values for the difference plots of oxidized products, β-carotene, and chlorophyll concentration. Table 1 contains information for EVOO A during the second and third months. These components were quantified for each EVOO sample using the Area Under the Curve (AUC) method; the findings are also included in Table 1. The information related to the shelf life of all EVOO samples during the first month in shelf life is provided in supplementary figures (Figures S1 to S7) except EVOO A and H.

Table 1. Area under the curve values of oxidation products, beta carotene and chlorophyll of all EVOO samples obtained from Al-Jouf Olive Farms, Saudi Arabia and various parts of Pakistan

Country	Origin details	Control EVOO (Fridge)	Room temperature (22-25 °C)								
			Area under curve values								
			1st month-control			2nd month-control			3rd month-control		
			Oxidized products	Beta Carotene	Chlorophyll	Oxidized products	Beta Carotene	Chlorophyll	Oxidized products	Beta Carotene	Chlorophyll
AL-Jouf region, Kingdom of Saudi Arabia	Zone 1	A	0.804	0.206	0.063	1.618	0.383	0.261	2.281	0.666	0.397
	Zone 2	B	0.516	0.014	0.113	1.138	0.013	0.248	1.681	0.075	0.343
	Zone 3	C	0.527	0.238	0.036	0.732	0.268	0.054	1.091	0.483	0.086
	Zone 4	D	0.605	0.181	0.017	1.117	0.281	0.158	1.736	0.343	0.234
	Zone 5	E	0.604	0.174	0.015	1.271	0.277	0.136	1.794	0.451	0.225
Pakistan	Chaman, Baluchistan	F	0.865	0.038	0.525	1.195	0.067	0.808	1.285	0.094	0.842
	Morgha Biodiversity Park, Rawalpindi	G	0.733	0.007	0.171	1.730	0.049	0.438	3.340	0.195	1.006
	Barani Agriculture Research Institute, Chakwal	H	0.762	0.119	0.129	1.290	0.184	0.278	1.536	0.264	0.341
	Loralai Baluchistan	J	0.273	0.061	0.0192	0.561	0.151	0.031	2.381	0.279	0.621

Figure 4a shows the spectra of Pakistani EVOO H control and its first month shelf life. Figure 4b shows the difference between the control and the first month of the spectral region of oxidation products and Figure 4c shows the difference in the spectral region of β-carotene over the same period of time. It is evident that these fluorescent compounds, such as β-carotene and tocopherols, undergo changes during the one-month period, leading to alterations in their fluorescence properties. The fluorescence emission intensities of oxidation-sensitive components such as tocopherols, phenolic compounds, and conjugated dienes indicate the oil's oxidative status. A decrease in their fluorescence intensity corresponds to a higher degree of oxidation, demonstrating a gradual breakdown of antioxidants and accumulation of oxidation products over time [33]. The fluorescence intensity of β-carotene decreased throughout the first month of its shelf life, most likely as a result of its content and structural integrity degrading, which changes its concentration and fluorescence properties [38]. During the first month of shelf life, a minor increase in the fluorescence intensity of oxidized products shown in Figures 3 and 4, is attributed to the formation of certain fluorescent compounds

during the oxidation process. As the oxidation process progresses, the concentration of oxidation products within the EVOO increases. As oxidation progresses during the first month of shelf life, the concentration of oxidized products, such as peroxides, aldehydes, and ketones, also increases [32]. These compounds can exhibit fluorescence properties, thereby leading to higher fluorescence intensity.

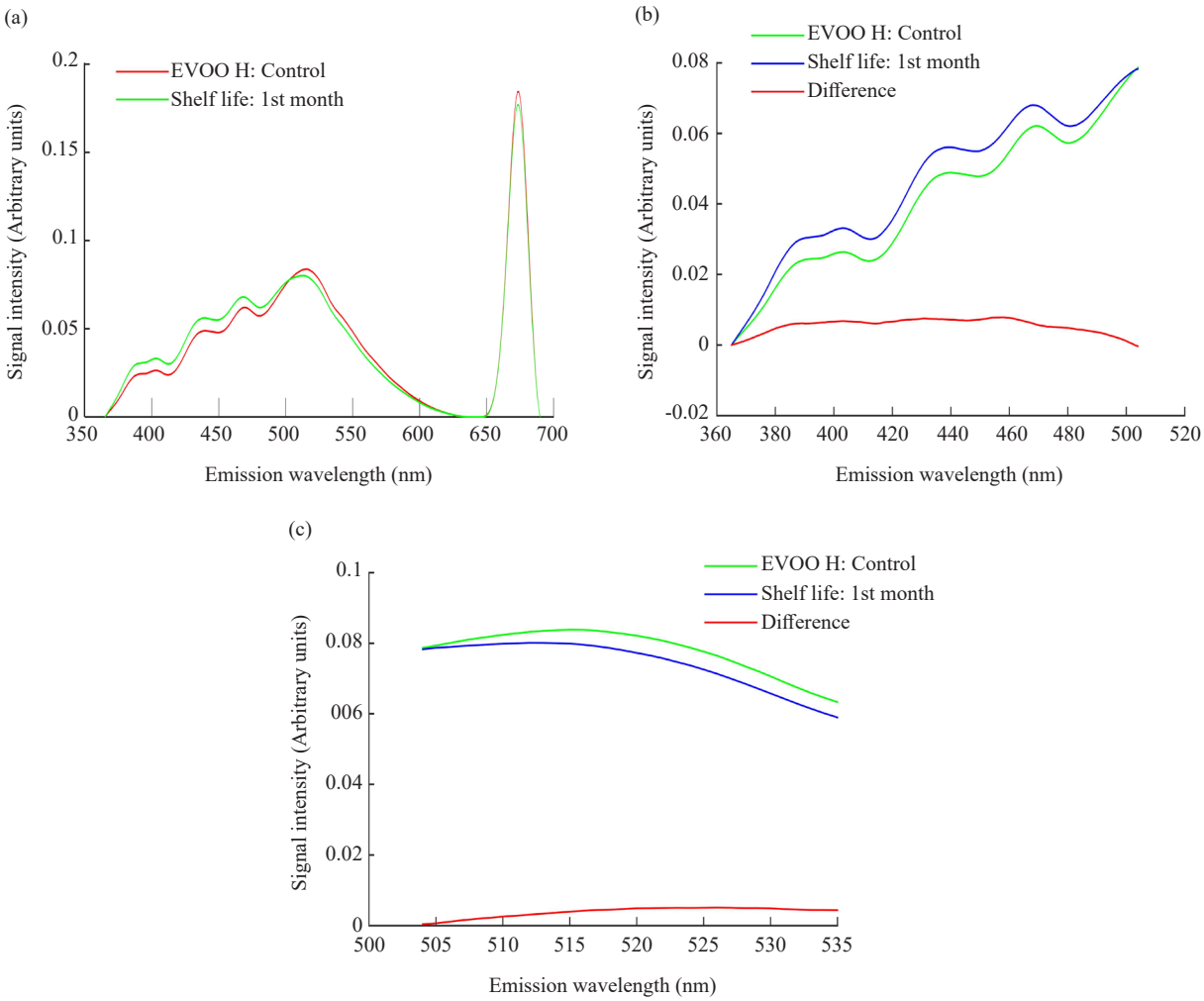

Figure 4. (a) Fluorescence spectra of Pakistani EVOO H with first month in shelf life (b) Difference plot of Pakistani EVOO H with control and first month for oxidation products (c) Difference plot of Pakistani EVOO H with control and first month for beta carotene

The chlorophyll band of both Saudi and Pakistani EVOO samples exhibits a decrease in relative intensity with storage time. This shows the deterioration of the chlorophyll compound in the form of a relative decrease in intensity similar to the case of beta carotene discussed earlier. Figure 5a depicts the fluorescence spectra of the Saudi EVOO A sample in the control stage and after one month of shelf life, revealing a significant drop in chlorophyll fluorescence intensity near 675 nm, indicating early oxidative degradation. Similarly, Figure 5c shows the spectra for the Pakistani EVOO H sample, which exhibits a similar trend, but with variable intensity shifts due to variances in pigment stability. Figures 5b and 5d show that the chlorophyll emission bands for Saudi EVOO A and Pakistani EVOO H decrease between the control and first-month samples. These decreases in fluorescence intensity indicate that photo-oxidation and chlorophyll breakdown occur gradually during storage. Saudi EVOO A and Pakistani EVOO H were chosen for extensive research due to their different geographical origins and distinct starting pigment profiles, allowing for a fair comparison of oxidative stability and fluorescence behaviour across EVOO variants. For sample A, the values calculated

from the difference plot using the area under the curve method for the first month of shelf life is 0.063 for chlorophyll. Similarly, the value for the sample H is 0.129. These numerical values were calculated using Origin software for each set of difference plots of chlorophyll. Similarly, the numerical values of oxidized products and beta carotene for the second and third months of EVOO A have been calculated and are shown in Table 1. The greater chlorophyll degradation in the Pakistani Extra Virgin Olive Oil (EVOO) sample compared to the Saudi sample may be due to various factors, including harvesting time, processing conditions, varietal variances, and geographical and environmental conditions. Harvesting time is important because olives taken earlier in the ripening process maintain more chlorophyll content but are also more vulnerable to photo-oxidation due to the presence of more photosensitizers [39]. Processing conditions, such as crushing, malaxation, and extraction temperatures, can hasten pigment degradation if not carefully controlled; elevated temperatures and prolonged malaxation, for example, have been linked to increased oxidative stress and rapid chlorophyll breakdown [40]. Varietal differences are also important, as different olive cultivars have varying quantities of chlorophyll and associated pigments, as well as varying oxidative stability due to genetic features. Additionally, geographical and environmental factors such as sunshine exposure, soil composition, altitude, and irrigation techniques influence chlorophyll biosynthesis and stability during fruit development and storage [41]. Nonetheless, oxygen availability is likely a factor in the oxidation of chlorophylls during the storage of olive oil, which would impact the color of the oil [42]. Collectively, these elements interact in various ways to determine the initial pigment level and subsequent rate of degradation, as seen in fluorescence behaviour during EVOO shelf life experiments.

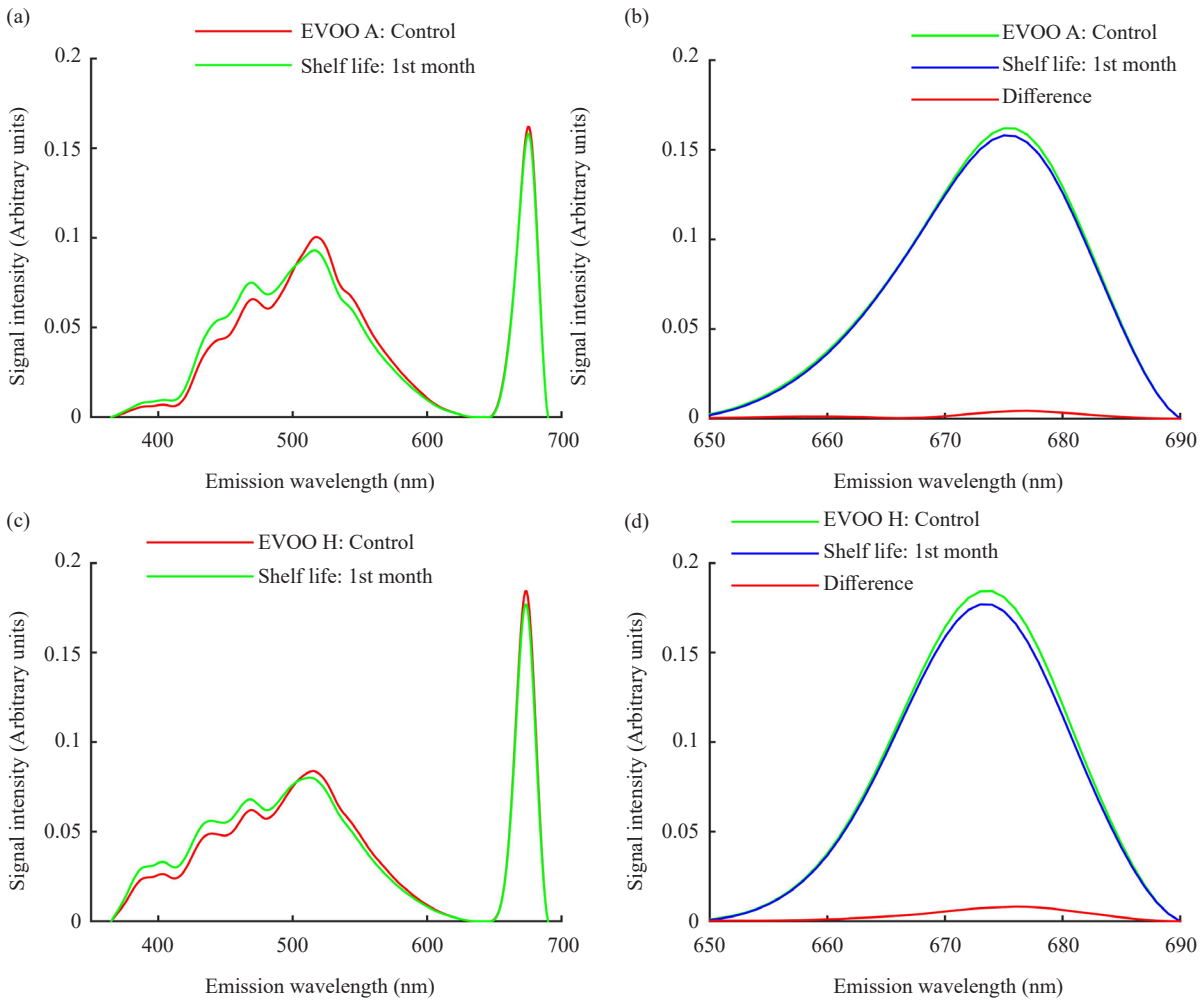

Figure 5. (a) Fluorescence spectra of Saudi EVOO A with first month in shelf life (b) Difference plot of Saudi EVOO A with control and first month for chlorophyll (c) Fluorescence spectra of Pakistani EVOO H with first month in shelf life (d) Difference plot of Pakistani EVOO H with control and first month for chlorophyll

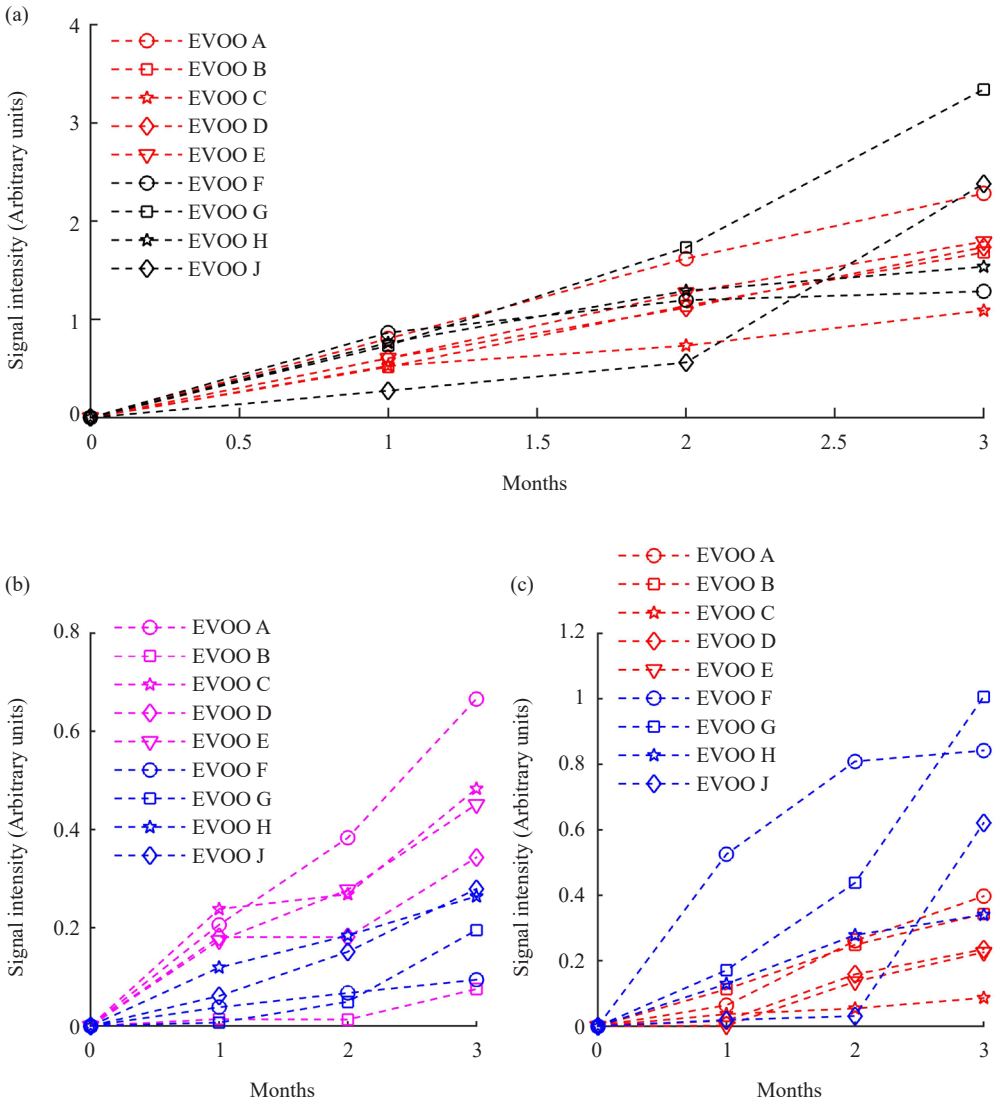

Figure 6. (a) Oxidation of vitamin E/Fatty acids with storage time (b) Oxidation of beta carotene with the storage time (c) Oxidation of chlorophyll content with storage time

Figure 6a illustrates the rate of oxidation of fatty acids and vitamin E in various foreign and Pakistani Extra Virgin Olive Oil (EVOO) samples over time, calculated using the area under the curve. A clear trend of increasing oxidation is observed for all EVOO samples from the first to the third time period of storage. Notably, EVOO samples from different regions of Pakistan exhibit a higher rate of oxidation (7.029) compared to those from Saudi Arabia (3.6392). The oxidation trend in Saudi EVOO samples is more gradual compared to the Pakistani ones.

Among the Saudi samples, EVOO A shows the highest relative intensity of oxidation from the first to the third month of storage, compared to the other Saudi samples. A similar oxidation pattern is seen in EVOO samples B, D, and E. However, EVOO C has a reduced oxidation rate throughout storage, indicating improved oxidative stability. This improved stability could be related to a higher initial concentration of natural antioxidants, such as polyphenols and tocopherols, or to cultivar-specific features that provide greater resistance to oxidative destruction. The smoother oxidation pattern found in all Saudi EVOO samples could be attributable to interconnected factors, including harvesting procedures and regional environmental conditions. Early harvesting and little fruit damage retain olive quality and prevent enzymatic oxidation before oil extraction. Climatic conditions specific to Saudi Arabia, such as strong sunlight exposure and low humidity, may alter olive composition by increasing the accumulation of antioxidant chemicals such

as polyphenols and carotenoids, resulting in improved oxidative stability during storage [43]. These combined causes most likely lead to the comparatively constant oxidation patterns observed in the Saudi EVOO samples.

In contrast, among the Pakistani samples, EVOO G from Morgha Biodiversity Park, Rawalpindi, remains stable during the first month but shows an accelerated rate of oxidation in the second and third months compared to other Pakistani EVOO samples. EVOO J from Loralai, Baluchistan, exhibits a lower oxidation rate in the first and second months but shows a higher rate after the third month. The relatively stable behavior of EVOO J and F, both sourced from the Baluchistan region, may be linked to the region's diverse weather and climatic conditions. Baluchistan has an arid to semi-arid climate, with little humidity, plenty of sunlight, and cold evenings [44]. These circumstances are ideal for olive production because they promote the accumulation of beneficial substances such as polyphenols and carotenoids, which lead to increased oxidative stability in olive oil.

Chaman (Baluchistan) has an arid environment and sandy soils with minimal rainfall, which may increase early oxidation but eventually improve stability due to adaptive phenolic profiles. In contrast, Chakwal (Punjab) has a semi-arid environment with moderate precipitation and clay-loam soils, which may support more stable oxidative properties. The observed variations between EVOO F (early oxidation, later stability) and EVOO H (moderate, constant stability) can be attributed to crop and terroir heterogeneity. The findings presented in Figure 6a are also dependent on the botanical variety of the olives, the locale of olive harvesting, and the environmental conditions affecting each sample.

Figure 6b shows the variations or deterioration of β-carotene also calculated by area under the curve. The unusual characteristics of carotenoid performance could be linked to factors such as the specific species, methods of extraction, transportation procedures, and packaging techniques applied to the EVOO samples. The presence of carotenoids in olive oil depends not only on olive fruits, but also on genetic factors (olive cultivar), the stage of fruit ripening, and environmental factors [8]. Oxidation of β-carotene is generally higher in Saudi-based EVOO samples as compared to Pakistani EVOO samples except EVOO B. Maximum deterioration of beta carotene can be seen in EVOO A as compared to all foreign and Pakistani samples so it shows much higher trend. There is a prominent change in the trend of EVOO C after the first month and most stable during the second and shows a higher trend after third month. EVOO E also shows maximum deterioration of β-carotene in shelf life. In contrast, EVOO F shows stability while EVOO G shows an increase in oxidation rate during the third month. Temperature fluctuations affect beta-carotene degradation [45], which could be related to the high temperatures in the Al-Jouf region. Thus, the warmer climate in the region may be the cause of the overall greater degradation seen in Saudi EVOO samples.

Figure 6c shows the rate of oxidation of chlorophyll content of different foreign and Pakistani EVOO samples with storage time, also based on the area under the curve. The method employed successfully calculated the numerical values of deterioration of the chlorophyll contents. The results show that all Extra Virgin Olive Oil (EVOO) samples from Al-Jouf, KSA, had moderate to slow rates of chlorophyll degradation when compared to Pakistani samples. Among the Al-Jouf samples, EVOO A showed the fastest degradation rate, while EVOO C showed the slowest. Similarly, Pakistani samples exhibited abrupt change in trends and the deterioration of chlorophyll after second month was higher. It is evident that chlorophyll contents in EVOO F underwent much faster deterioration until the second month which can be associated with its storage in the plastic bottle when purchased from the farmer. Similarly, EVOO J oxidized slowly for the first two months of shelf life, then accelerated in the third month. This shift can be linked to higher ambient temperatures during production and storage, which speed up lipid peroxidation [2]. Environmental elements such as climate, soil type, precipitation, and altitude all have an impact on the oil's oxidative stability. Higher elevations and steady weather patterns often increase phenolic content, delaying oxidation. Given that Loralai, Baluchistan experiences semi-arid circumstances with large temperature swings, it is possible that initial phenolic richness slowed oxidation in EVOO J, but the introduction of high ambient temperatures during the third month may have spurred rapid degradation. Furthermore, the Pakistani EVOO G sample showed the second-fastest rate of chlorophyll contents deterioration in three months shelf life time. The behaviour of Saudi Arabian EVOO samples showed less degradation of chlorophyll thus they proved to be more stable as compared to Pakistani EVOO samples.

As indicated from the present study, significant differences among olive oil samples from the Al-Jouf region of KSA and from different regions of Pakistan were observed. The Al-Jouf region has appropriate soil properties. Summers in Al-Jouf are long and extremely hot, while winters are short with average temperatures of 26 which throughout the olive oil production process is crucial to preserve the quality and freshness of the oil and consequently show more stability [18, 43].

It is reported that the Loralai and Chaman regions, located in the northern part of Baluchistan, Pakistan, undergo a climate characterized by brief, mild summers and extended, chilly winters, with an average temperature of 21 °C [44]. This climatic variation may contribute to the relatively lower stability observed in the Extra Virgin Olive Oil (EVOO) produced in these areas when compared to EVOO samples from Al-Jouf, KSA. The results here showed a rapid oxidation rate of Pakistani sample EVOO F from Chaman Baluchistan due to the fact that in the market, it was sold in plastic bottles with direct exposure to sunlight which played a role in the production and acceleration of oxidized products. Samples from the Loralai region of Baluchistan (province of Pakistan) showed less stability, possibly due to informal plantation, inappropriate irrigation system. As of June 2023, Pakistan has roughly five million newly planted olive trees spread across an estimated 42,000 acres (17,000 hectares). Muhammad Ramzan Anser, an agronomist and Deputy Project Director at the Centre of Excellence for Olive Research and Training (CEFORT), presented this information at Pakistan's third annual Olive Value Chain Conference [46]. Further analysis of additional EVOO samples from different regions of Pakistan is required to uncover the potential factors contributing to its superb quality. Even though the current study was only able to observe for three months, this period was specifically chosen to track early-stage oxidation trends. Although the results are preliminary, we recognize that they offer valuable information on when EVOO breakdown begins. For short-term shelf-life evaluation, early detection of such changes is crucial, and it can direct quality control measures throughout distribution and storage.

A comparative analysis was carried out with studies from top olive oil-producing nations like Spain, Italy, and Tunisia in order to put our findings in perspective. Several investigations have documented spectrum shifts and a decrease in fluorescence intensity as markers of oxidative degradation during storage, which is consistent with our findings. For example, under comparable storage circumstances, EVOO samples from Italy and Spain likewise showed early fluorescence alterations associated with the decomposition of phenolic compounds and chlorophyll [16, 23]. However, the rate and degree of oxidation may be influenced by geographical variations in olive varietals, processing methods, and climate. These comparisons support global studies on the deterioration behavior of EVOO and highlight the significance of localized quality monitoring.

4. Conclusion

Area Under the Curve (AUC) method was employed to investigate the oxidation of fatty acids/vitamin E, β-carotene, and chlorophyll content in different samples of EVOO during storage. Fluorescence emission was monitored at compound-specific wavelengths: approximately 365-500 nm for vitamin E, 500-600 nm for β-carotene, and 650-690 nm for chlorophyll, allowing selective tracking of their degradation over time. Numerical values expressing oxidation rate have been calculated and plotted against shelf life.

Principal Component Analysis (PCA) was also used to classify different sample groups based on spectral variations. Results have shown that EVOO samples collected from Al-Jouf, KSA are more stable than those from different regions of Pakistan. Furthermore, the oxidation rate of fatty acids/vitamin E was significantly faster in EVOO samples G and J. β-carotene showed a more accelerated oxidation rate in EVOO samples A and C, which are from Saudi Arabia. Similarly, degradation of chlorophyll content in EVOO samples G, F, and J has also been observed.

The oxidation rate of all EVOO samples may be associated with various climatic, geographic, and environmental factors such as average yearly temperature, rainfall, and irrigation systems, as well as harvesting, packing, and transport methods. Additionally, the quality and stability of the samples were influenced by factors including the state of olive ripeness, extraction methods, geographical origin, and conditions throughout the product's commercial life.

These results demonstrate that fluorescence spectroscopy has the potential to be a quick and cost-effective method for EVOO quality analysis. Although the application of PCA and AUC provided meaningful insights into EVOO oxidation behaviour, future studies should include complementary validation using established chemical methods such as peroxide value, TBARS, and GC-MS to further enhance the reliability and robustness of the findings.

Acknowledgments

Ms. Rimsha Hafeez is very grateful to the National Institute of Lasers and Optronics (NILOP) Islamabad for providing the necessary research facilities.

Ethical approval

Ethical approval letter from MUST University, Mirpur Azad Kashmir Pakistan has been uploaded in related file upload section.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection were performed by Areeba Ansar and Dr. Hina Ali. The first draft of the manuscript was written by Rimsha Hafeez and all authors commented on previous versions of the manuscript. Major editing and rewriting of the manuscript is done by Dr. Naveed Ahmad.

Conflict of interest

There are no competing interests of a financial or personal nature.

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Appendix

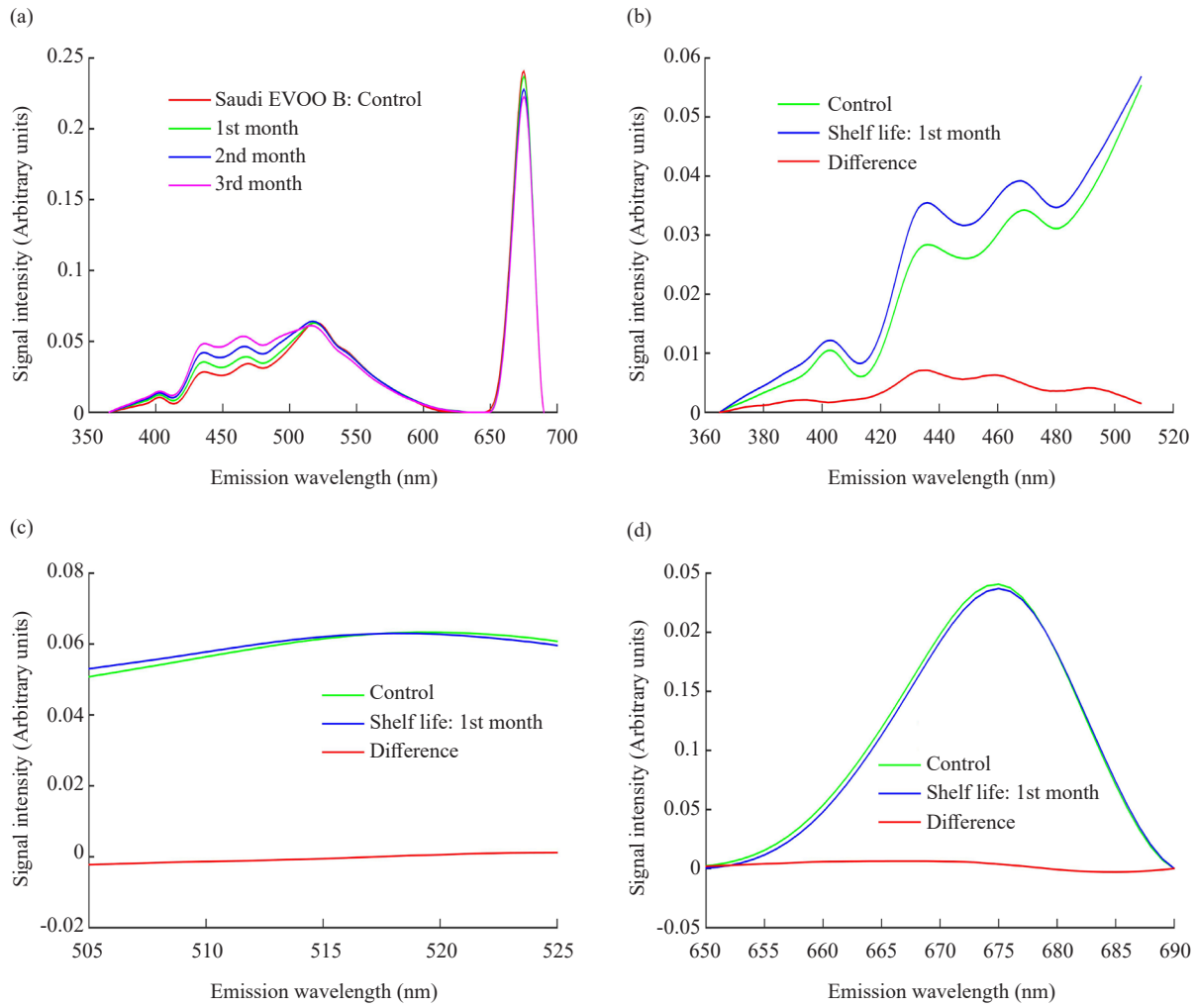

Figure S1. (a) Fluorescence spectra of Saudi EVOO B for three months in shelf life (b) Difference plot of Saudi EVOO B with first month for oxidation products (c) Difference plot of beta carotene with first month in shelf life (d) Difference plot of chlorophyll with first month in shelf life

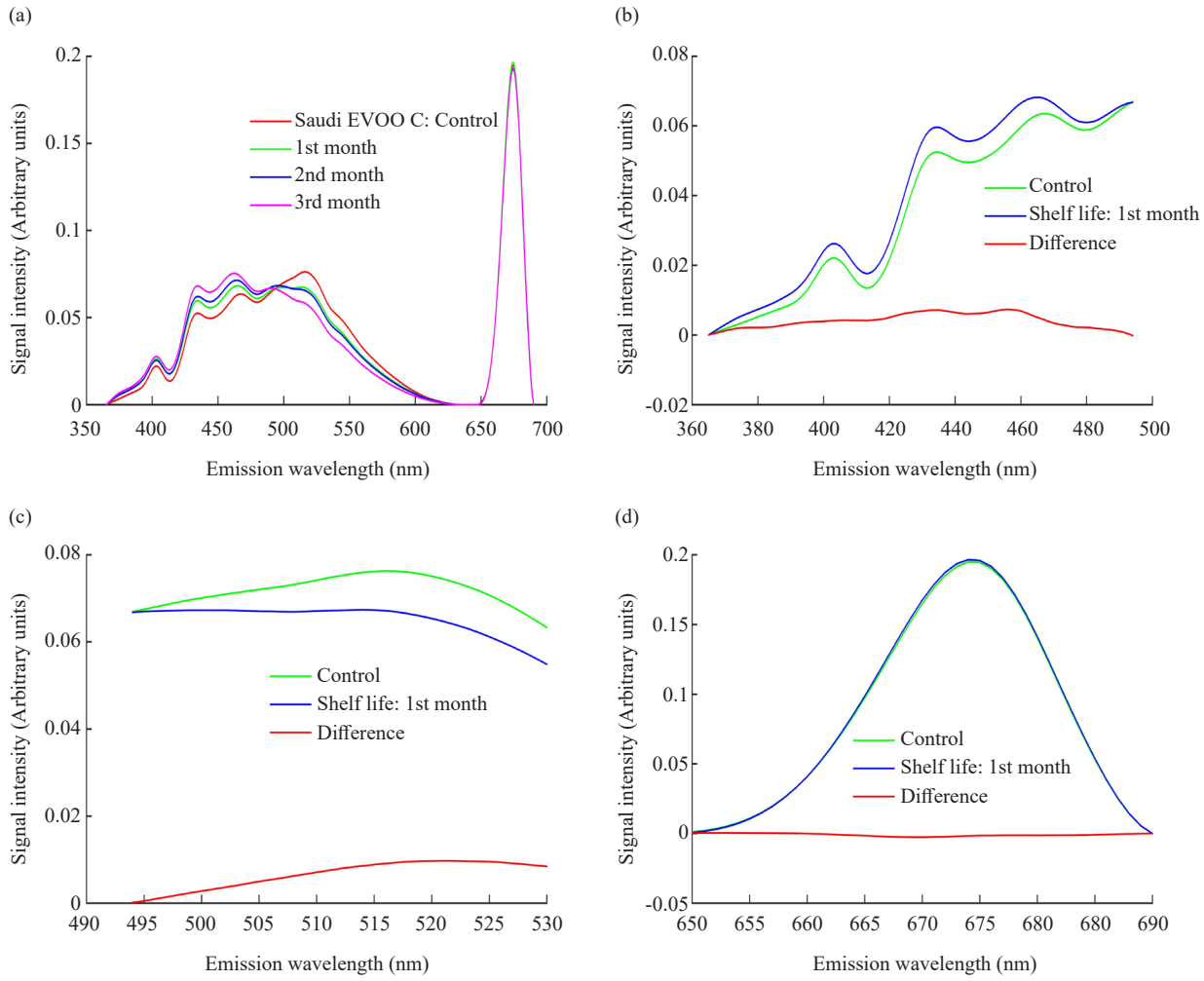

Figure S2. (a) Fluorescence spectra of Saudi EVOO C in three months shelf life (b) Difference plot of Saudi EVOO C with first month for oxidation products (c) Difference plot of beta carotene with first month in shelf life (d) Difference plot of chlorophyll with first month in shelf life

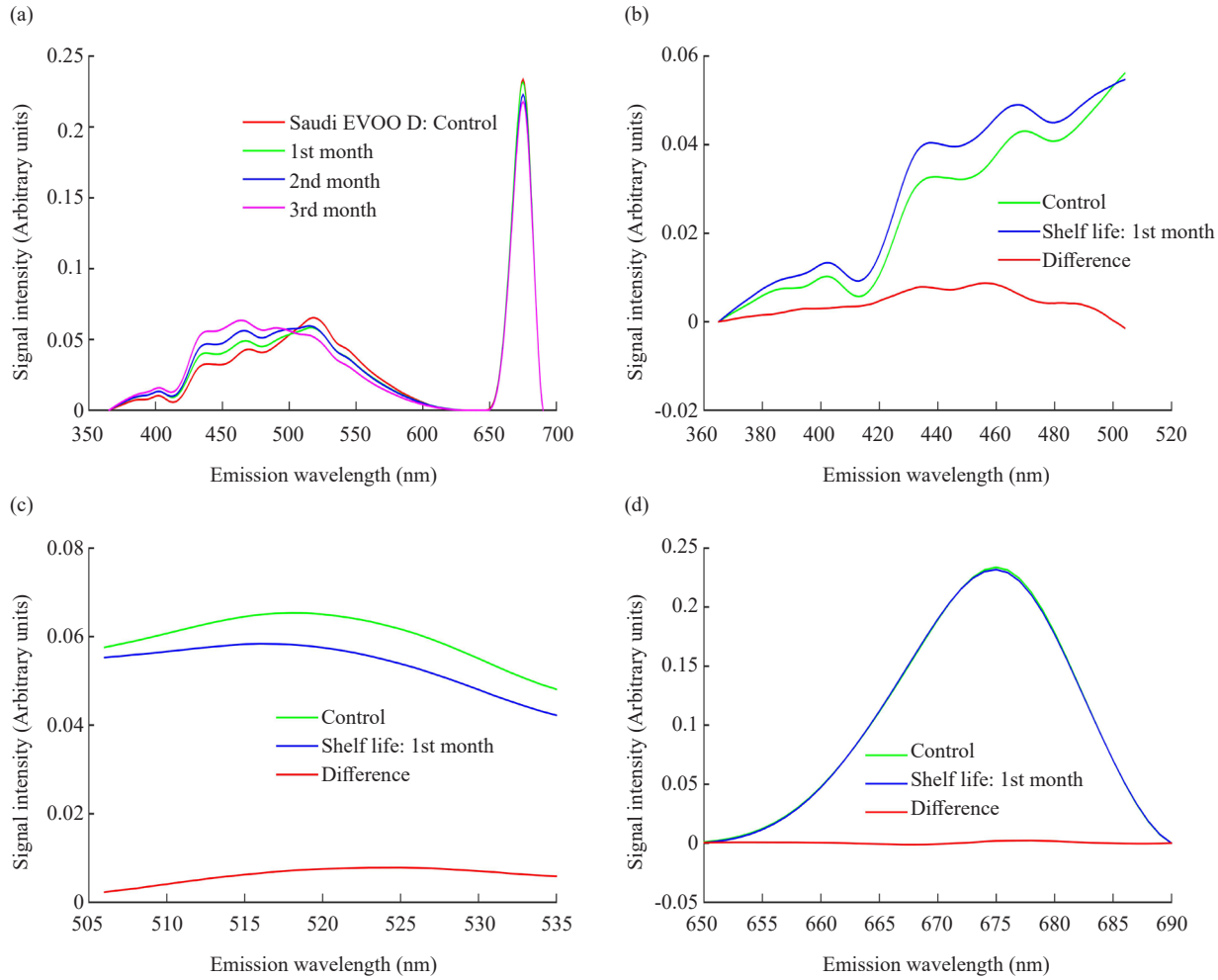

Figure S3. (a) Fluorescence spectra of Saudi EVOO D in three months shelf life (b) Difference plot of Saudi EVOO D with first month for oxidation products (c) Difference plot of beta carotene with first month in shelf life (d) Difference plot of chlorophyll with first month in shelf life

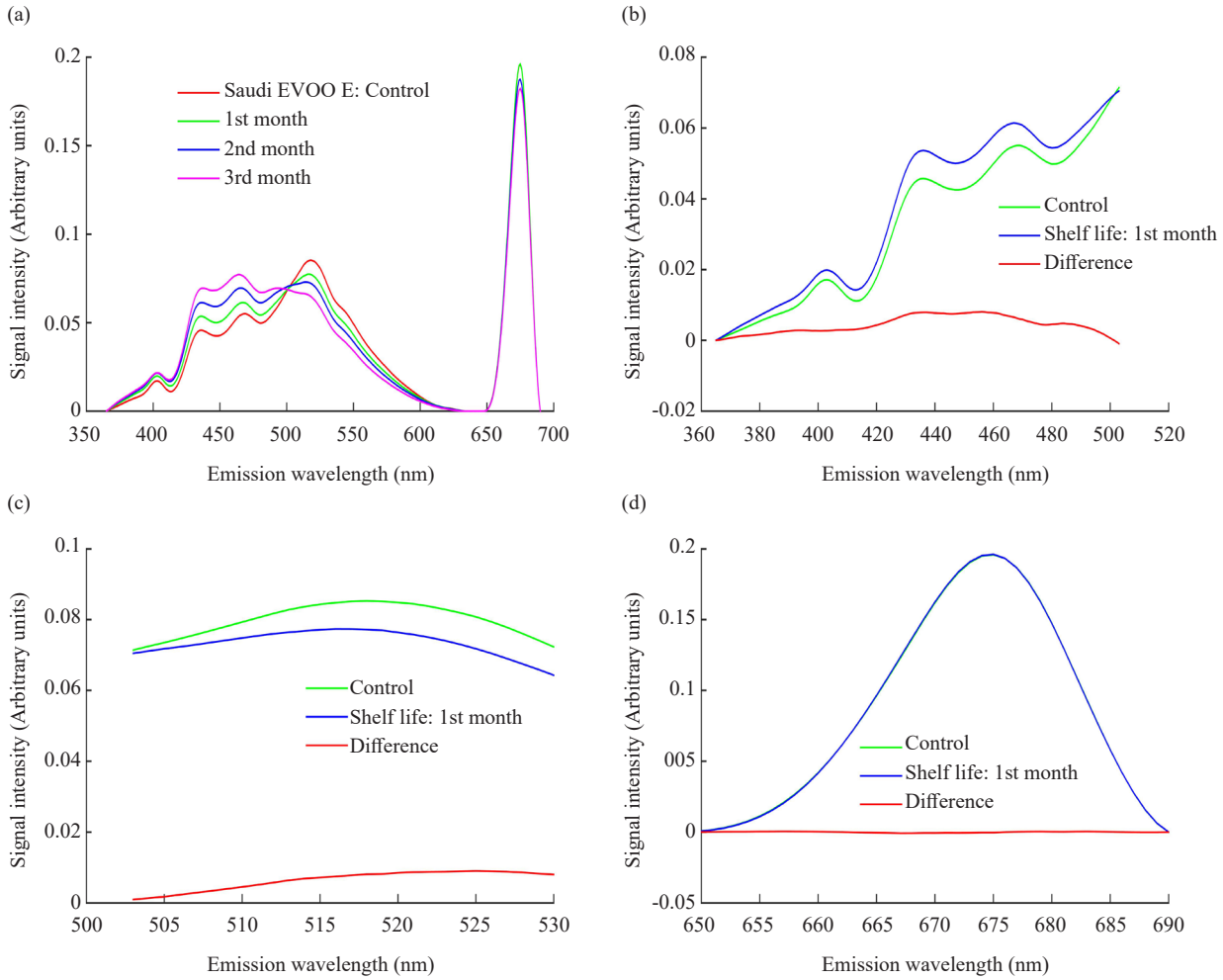

Figure S4. (a) Fluorescence spectra of Saudi EVOO E in three months shelf life (b) Difference plot of Saudi EVOO E with first month for oxidation products (c) Difference plot of beta carotene with first month in shelf life (d) Difference plot of chlorophyll with first month in shelf life

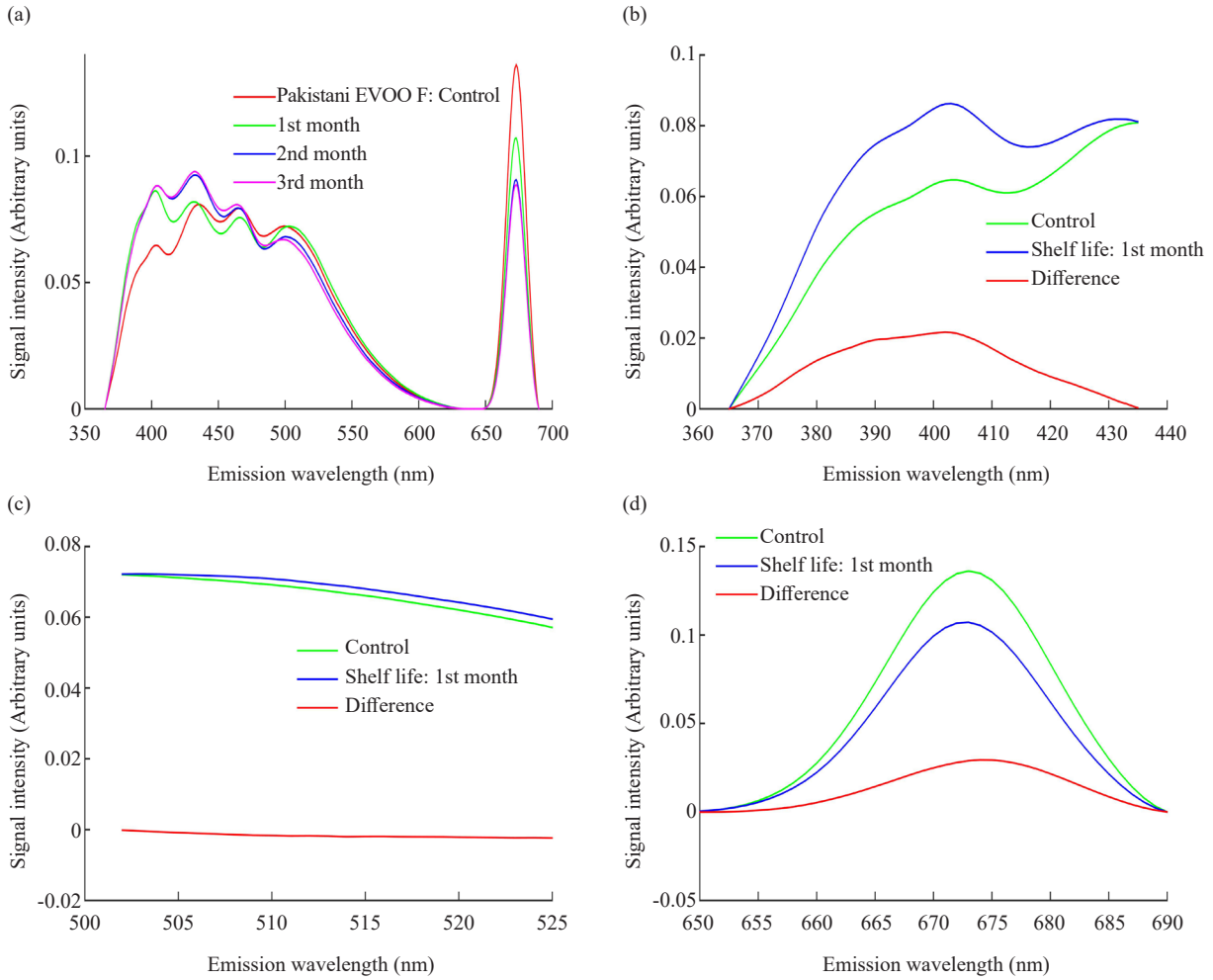

Figure S5. (a) Fluorescence spectra of Pakistani EVOO F in three months shelf life (b) Difference plot of Pakistani EVOO B with first month for oxidation products (c) Difference plot of beta carotene with first month in shelf life (d) Difference plot of chlorophyll with first month in shelf life

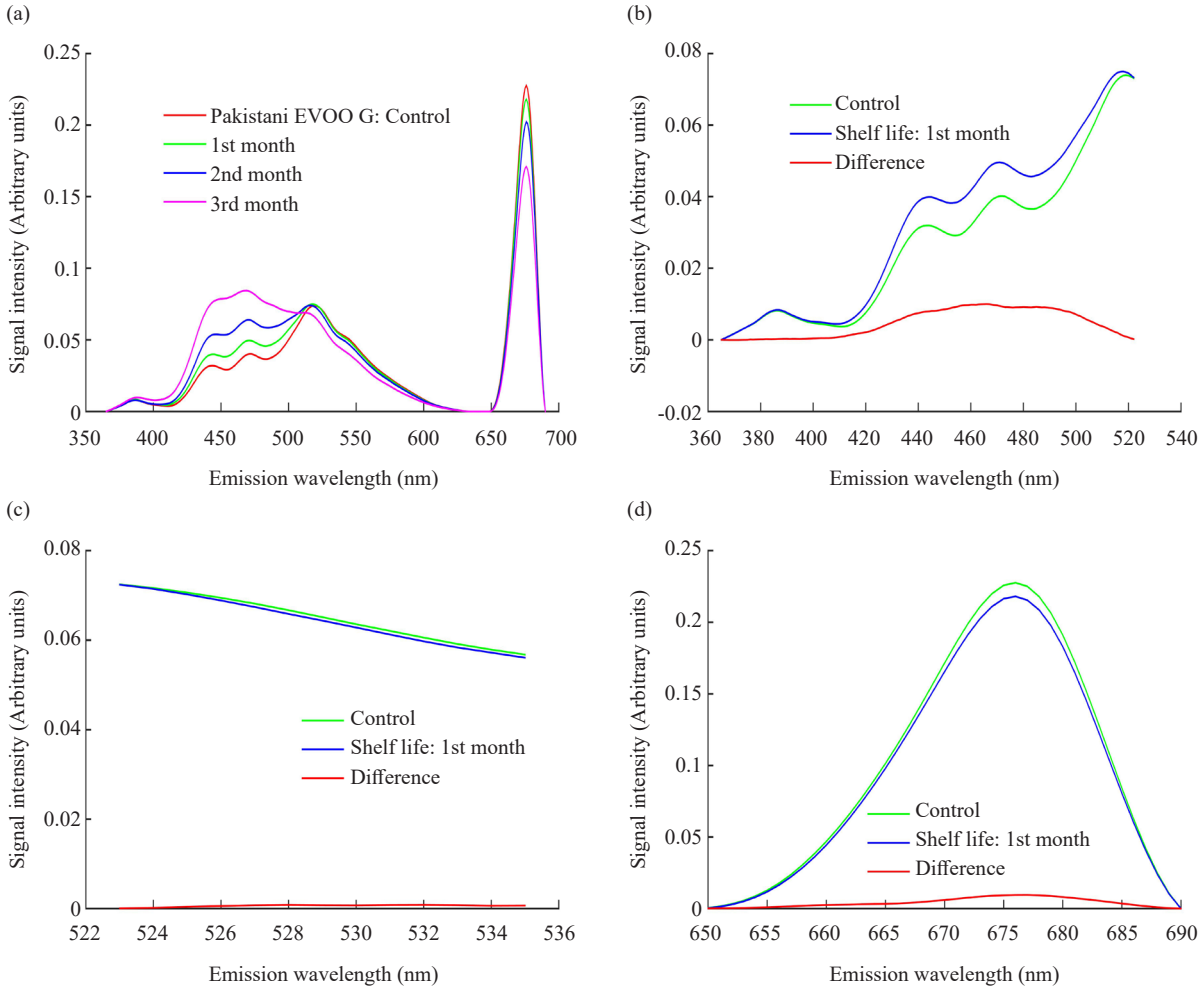

Figure S6. (a) Fluorescence spectra of Pakistani EVOO G in three months shelf life (b) Difference plot of Pakistani EVOO G with first month for oxidation products (c) Difference plot of beta carotene with first month in shelf life (d) Difference plot of chlorophyll with first month in shelf life

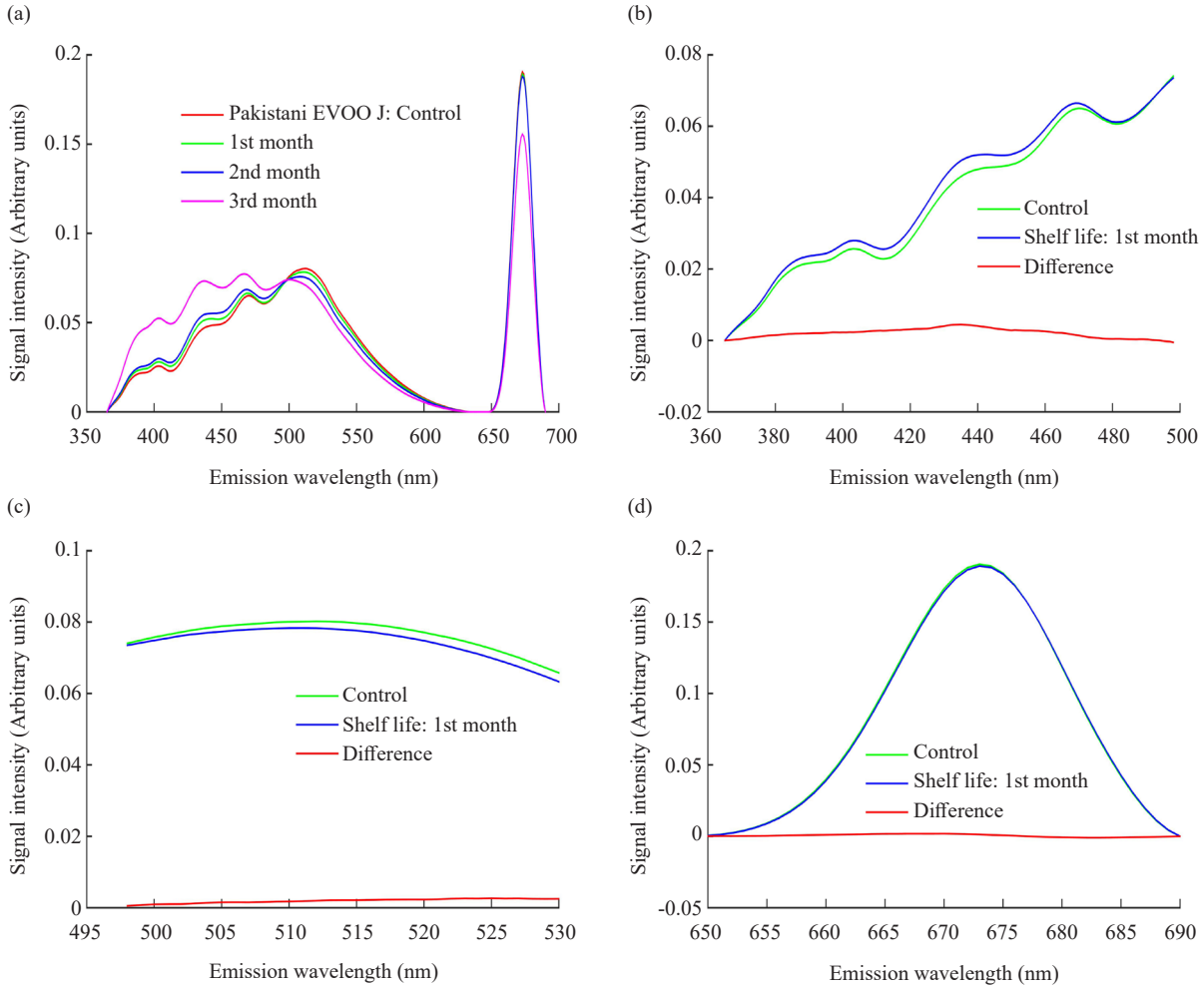

Figure S7. (a) Fluorescence spectra of Pakistani EVOO J in three months shelf life (b) Difference plot of Pakistani EVOO J with first month for oxidation products (c) Difference plot of beta carotene with first month in shelf life (d) Difference plot of chlorophyll with first month in shelf life