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APPLICATION OF FLUORESCENCE EXCITATION–EMISSION SPECTROSCOPY FOR WALNUT OIL AUTHENTICATION

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Abstract: *Walnut oil is a high-value edible oil appreciated for its nutritional, sensory, and health-promoting properties. Due to its premium market value, it is particularly vulnerable to adulteration with lower-cost vegetable oils, creating the need for rapid and reliable authentication methods. In this study, a methodology based on three-dimensional excitation–emission matrix (EEM) fluorescence spectroscopy was investigated for the detection of walnut oil adulteration. Fluorescence excitation–emission data were acquired using a Shimadzu RF-6000 spectrofluorophotometer from authentic walnut oil samples and adulterated samples prepared with sunflower, rapeseed, and soybean oils at different concentration levels (5%, 10%, 20%, 30%, 40%, and 50%). The obtained EEM spectra revealed distinct fluorescence fingerprints for each oil type, reflecting differences in their chemical composition and fluorophore distribution. Authentic walnut oil exhibited characteristic fluorescence regions associated with natural pigments and antioxidant compounds, whereas adulterated samples showed notable changes in fluorescence intensity and peak distribution. The most pronounced spectral modifications were observed in the 470–550 nm region, indicating the influence of adulterant oils on the fluorescence profile of walnut oil. These results demonstrate that EEM fluorescence spectroscopy is a rapid, non-destructive, and sensitive technique for distinguishing authentic walnut oil from adulterated samples. The method shows strong potential for food authentication applications and could support quality control and fraud prevention in the edible oil industry.*

Keywords: *fluorescence spectroscopy, excitation–emission matrices, edible oil adulteration, food authentication, chemometric analysis*

1. Introduction

Walnut (*Juglans regia* L.) is one of the most widespread and valuable nut species worldwide, ranking among the top five nut crops by production volume. Globally, the main producing countries are China, followed by the United States (approx. 30%) and France (around 11%) [1,2]. Walnut oil (WNO) is obtained by pressing walnut kernels, valued for its high yield, which ranges between 52% and 72%, depending on the variety, maturity, cultivation conditions, and extraction technology [2,3]. Walnut oil is known not only for its nutritional value but also for its antioxidant properties, its beneficial potential for cardiovascular health, and its diverse uses in gastronomy, cosmetics, and the pharmaceutical industry. Due to its characteristic aroma and valuable composition, walnut oil is often considered a premium product, which also makes it vulnerable to adulteration practices in the food chain [2,4]. The high economic value of walnut oil makes it particularly susceptible to adulteration with lower-priced edible oils, such as soybean and sunflower oils [5,6]. A number of conventional analytical techniques have been used for the authentication and detection of walnut oil adulteration, including gas chromatography (GC) [5,7,8]

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and high-performance liquid chromatography (HPLC) [9,10]. These approaches are mainly based on the quantitative determination of fatty acids, triglycerides, and hydrocarbons. However, such methods are generally laborious and time-consuming.

Fluorescence spectroscopy has been widely applied for the characterization and differentiation of different edible oils [11,12].

Using synchronous fluorescence spectroscopy, [13] demonstrated the rapid detection of sunflower oil in virgin olive oil at concentrations up to 3.4% (w/v) in a measurement time of approximately two and a half minutes. In this context, the present study aims to evaluate the authenticity of walnut oil and its adulteration with sunflower, rapeseed, and soybean oil using fluorescence spectroscopy. In recent years, fluorescence spectroscopy has become increasingly common in food analysis due to its speed, ease of use, and relatively low cost [2]. At the same time, food fraud has become a major concern for the food industry, regulatory authorities, and consumers, as cases of intentional product substitution have steadily increased globally.

These practices, mainly driven by economic gain, can include the addition of inauthentic ingredients or the provision of misleading information, representing a serious threat to public health and consumer safety [14]. They can also affect national security, the economy, and trust in food systems. In this context, the need for quick and simple methods to identify fraudulent ingredients is essential to prevent risks. Among the products most frequently subject to adulteration are vegetable oils, including walnut oil, which is appreciated for its sensory and nutritional qualities [14,15].

Excitation-emission matrix (EEM) fluorescence spectroscopy, integrated with advanced chemometric methods, is recognized as a powerful and promising analytical technique for the characterization and identification of edible oils. This approach provides multidimensional information on the chemical composition of samples, facilitating both the discrimination of different types of oils and the rapid and reliable detection of adulteration. Furthermore, by utilizing statistical models and classification algorithms, the EEM method can contribute to the development of robust predictive tools capable of providing qualitative and semi-quantitative assessments of the authenticity of vegetable oils [2].

Food adulteration remains a significant problem in the edible oil sector, mainly due to the economic incentives associated with replacing high-quality oils with cheaper alternatives. Among edible oils, walnut oil is particularly vulnerable to adulteration, with lower-quality oils being used as a cost-saving additive, which can negatively impact both product quality and consumer confidence.

The aim of this study was to use emission-excitation matrices for the authentication of walnut oil and the detection of adulteration with sunflower oil, rapeseed oil, and soybean oil in various percentages (5%, 10%, 20%, 30%, 40%, and 50%).

2. Materials and methods

2.1. Materials

Four samples of walnut oil were obtained in 2024 through cold pressing from different walnut sources in Suceava County. The samples were stored in glass bottles and kept at a temperature between 4 and 8 °C. To adulterate the walnut oil, mixtures of walnut oil with sunflower oil, rapeseed oil, and soybean oil were prepared in various concentrations. Two types of commercial sunflower oil (Type I and Type II), as well as rapeseed oil and soybean oil (Type I and Type II) from different manufacturers, were used.

Four samples of walnut oil were adulterated with Type I and Type II sunflower oil, rapeseed oil, and Type I and Type II soybean oil in concentrations of 5%, 10%, 20%, 30%, 40%, and 50%, resulting in a

total of 144 adulterated samples that were analyzed, and 10 authentic samples.

2.2. Fluorescence measurements

To analyze the fluorescence spectra of adulterated oils, the Shimadzu RF-6000 Spectro Fluorophotometer equipped with a xenon lamp as excitation source was used. The oil samples were placed in quartz cuvettes (usually 1x10x45 cm) without chemical pretreatment.

The fluorescence spectrum was obtained by measuring the excitation-emission matrix (EEM), with a typical excitation range (EX) between 250 and 550 nm and emission range (EM) between 260 and 750 nm.

The bandwidth was set to 5 nm for both monochromators, and the scanning speed was about 6000 nm/min. These conditions make it possible to obtain a detailed spectral profile reflecting the chemical composition of the oil.

3. Results and discussion

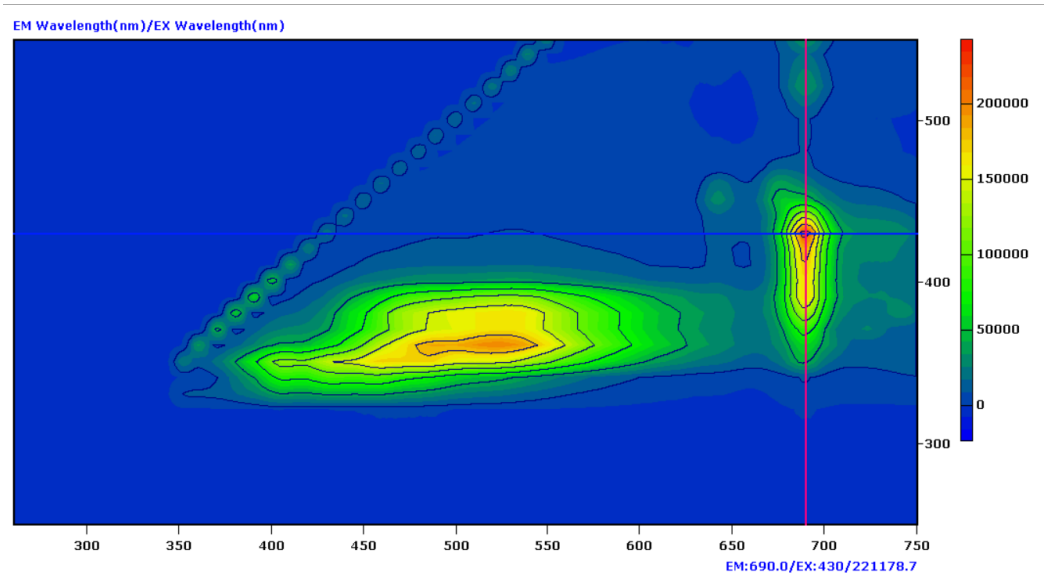
3.1. Three-dimensional fluorescence spectra of walnut oil

Figure 1 shows the excitation–emission spectra of authentic walnut oil and the substances used for adulteration. Fluorescence spectroscopy offers high selectivity, allowing for the identification of fluorescent molecules, and can be applied in a two-dimensional manner through the simultaneous analysis of excitation and emission. According to the information presented in Fig. 1 and Fig. 2, walnut oil and each adulterant exhibit a unique spectrum.

Table 1.

Peak location where the maximum excitation/emission is recorded for the analyzed sample

Sample	Peak location λex/λem (nm)		
	I	II	III
Walnut oil (WO)	690/430	520/360	
Sunflower oil (SFO)	688/430	468/380	
Rapeseed oil (RO)	690/430	514/380	460/350
Soybean oil (SO)	542/380	690/390	
50% WO 50% SFO	518/370	690/430	
50% WO 50% RO	502/380	690/430	
50% WO 50% SO	538/380	690/390	690/530



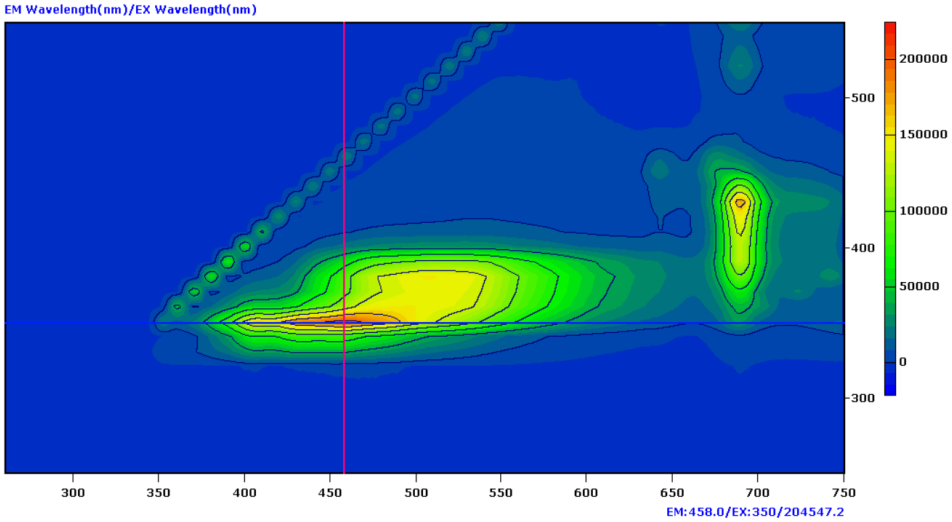
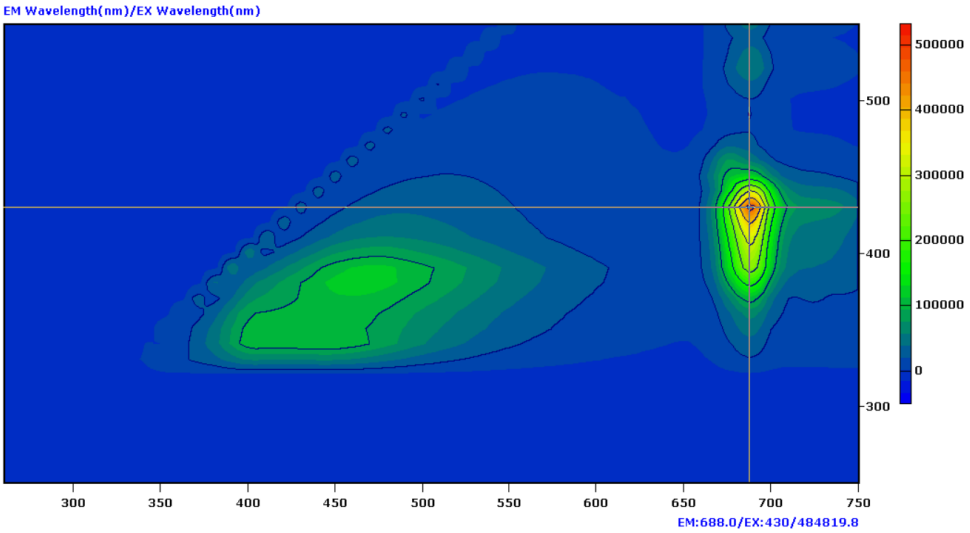
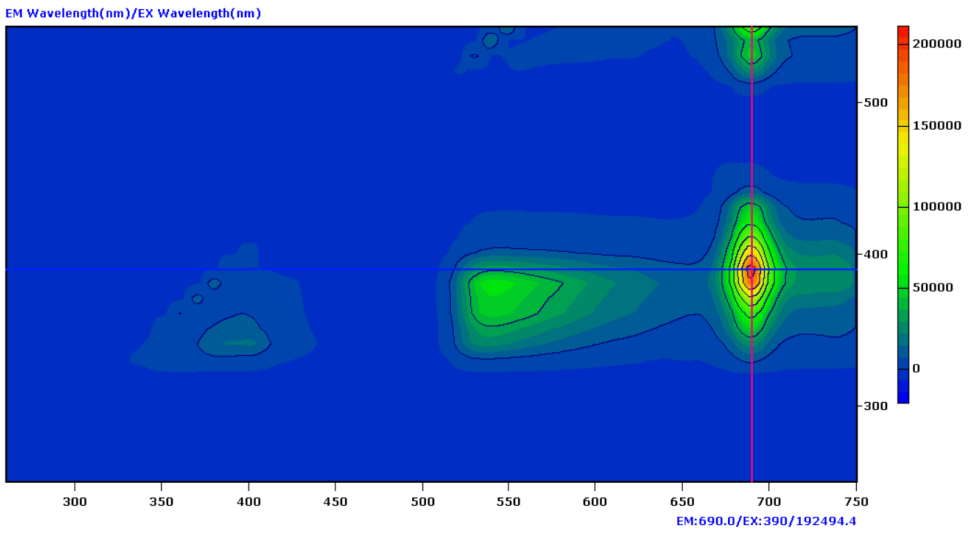


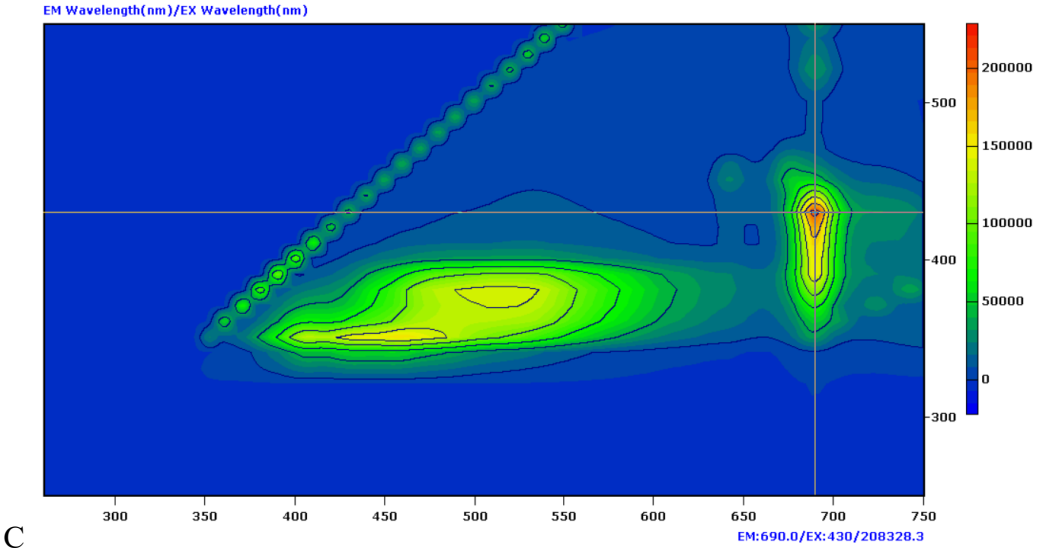
Fig. 1. EEM spectra of authentic walnut oils (2 random samples)



A



B



C

Fig. 2. EEM spectra of adulteration agents: A- sunflower oil, B- rapeseed oil, C- soybean oil

The results showed that sunflower oil exhibits maxima at 430/688 nm and 380/468 nm, consistent with its broad excitation (320–540 nm) and emission (340–750 nm) ranges. Rapeseed oil exhibits characteristic bands at 430/690 nm, 380/514 nm, and 350/460 nm, reflecting a complex distribution of fluorophores in the excitation range of 330–520 nm and the emission range of 380–702 nm. In the case of soybean oil, fluorescence maxima were observed at 380/542 nm and 390/690 nm, corresponding to excitation ranges of 320–550 nm and emission ranges of 330–750 nm, attributed to pigment components such as lutein, carotene, carotenoids, chlorophyll, and other compounds [19,20]. The differences observed in the position and intensity of the fluorescence bands between walnut oil and the oils used as adulterants reflect variations in the chemical composition and distribution of fluorophores. These spectral characteristics can serve as unique fingerprints for each type of oil, making them essential for the identification and subsequent differentiation of authentic samples from adulterated ones.

In the case of the adulterated walnut oil samples containing 50% adulterant, obvious changes in the fluorescence profile were observed compared to the authentic sample. The authentic oil exhibited characteristic peaks at approximately 430/690 nm and 360/520 nm, associated primarily with chlorophyll pigments and phenolic compounds. Following adulteration with sunflower oil, the spectrum showed bands at 370/518 nm and 430/690 nm, suggesting an increased contribution of fluorescent compounds in the 400–550 nm region, which is characteristic ofoxidation products and lipid constituents. For the mixture with rapeseed oil, fluorescence peaks were identified at 380/502 nm and 430/690 nm, indicating a shift of the bands and a redistribution of fluorescence intensity due to the overlap of the fluorophores in the two oils. In the case of soybean oil, the spectral profile became more complex, with bands appearing at 380/538 nm, 390/690 nm, and 530/690 nm, reflecting a significant change in the lipid microenvironment and the distribution of fluorophores.

These spectral variations, particularly in the 400–550 nm region, are attributed to compounds such as fatty acids, oxidation products, and phenolic compounds, while the constant band around 690 nm is associated with chlorophyll pigments. Therefore, changes in the position and

intensity of the fluorescence bands can be used as sensitive indicators of adulteration, constituting specific spectral fingerprints for distinguishing authentic walnut oil from adulterated samples [18].

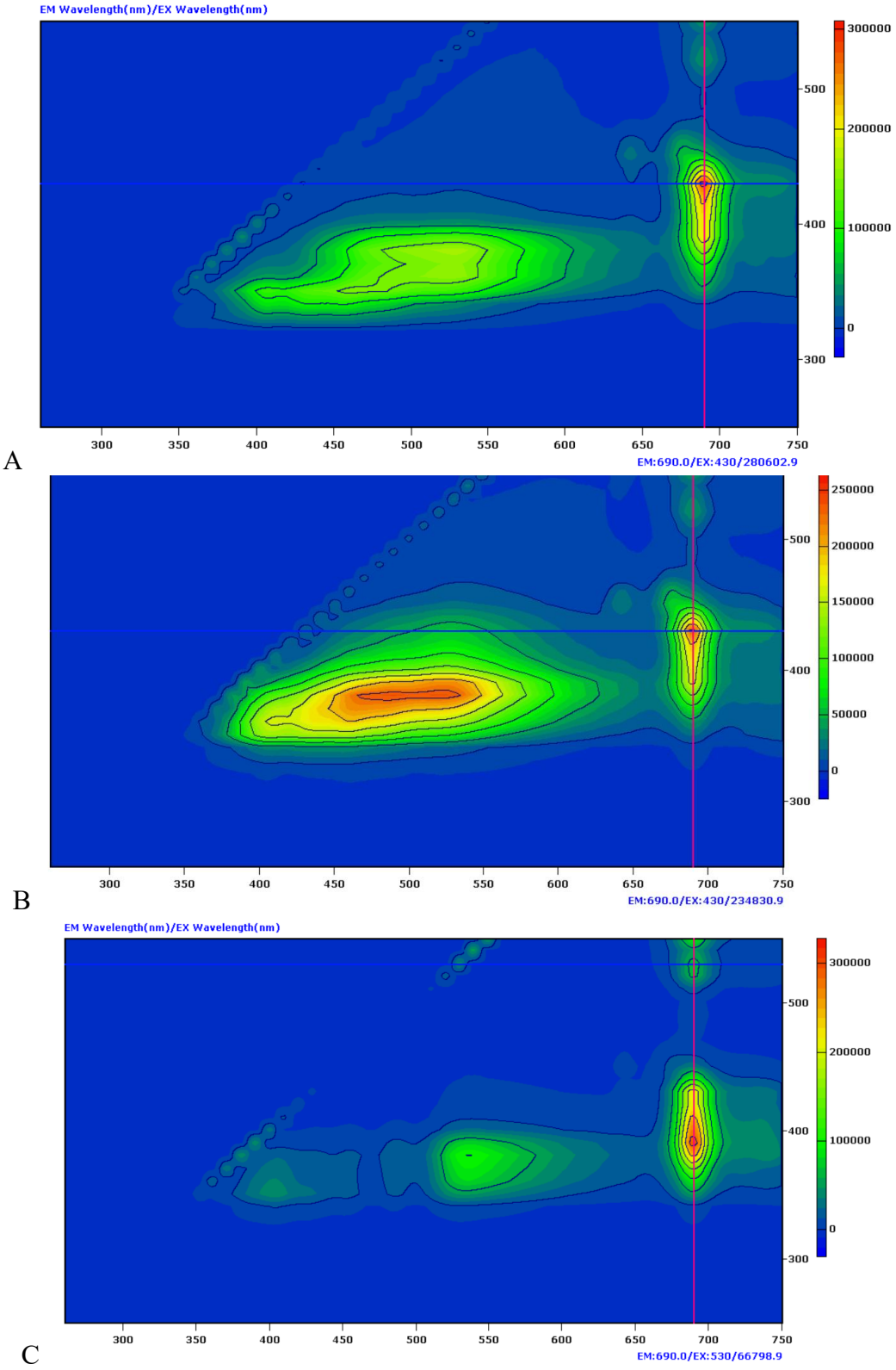


Fig. 3. EEM spectra of walnut oil adulterated with A. 50% sunflower oil, B. 50% rapeseed oil, C. 50% soybean oil

4. Conclusions

The authenticity of the samples was assessed using fluorescence spectroscopy, comparing authentic walnut oil with samples adulterated by the addition of 50% sunflower oil, rapeseed oil, and soybean oil. The results revealed significant changes in the fluorescence profile, demonstrating the sensitivity of the method to changes in oil composition. Authentic walnut oil exhibited two characteristic fluorescent regions, while rapeseed oil was characterized by three distinct spectral regions. However, in the adulterated sample, the appearance of a third fluorescent region specific to rapeseed oil was not observed. Instead, significant changes in the distribution of fluorescence intensity were noted in the existing regions. The most obvious change was observed in the second spectral region, located between 470 and 550 nm, where the signal became more intense and concentrated, suggesting a change in the composition of the fluorophores present in the sample. These results indicate that adulteration with rapeseed oil does not necessarily lead to the appearance of new fluorescent regions, but may cause changes in the intensity and distribution of the signal within the regions characteristic of walnut oil. In conclusion, the variations observed in the 470–550 nm range may serve as a useful marker for detecting adulteration and assessing the authenticity of walnut oil.

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