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UV-VIS methodology for the quantification of vegetable oil in adulterated olive oil

Metodologia UV-VIS visando a quantificação de óleo vegetal em azeite adulterado

Metodología UV-VIS para la cuantificación de aceite vegetal en aceite de oliva adulterado

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Abstract
Olive oil is a high-value product aggregated the obtained simply by pressing the olives. Known for being a functional and preventive food for several diseases such as cancer, coronary heart disease, and lowering of bad cholesterol; its adulteration by the addition of vegetable oil of less commercial value becomes a practice in the industry. To facilitate the verification of adulteration, methods such as UV-Vis spectroscopy were used to quantify the vegetable oil present in virgin olive oil. This study aimed to understand the UV-Vis technique in a previous reading in the evaluation and investigation of adulteration in extra virgin olive oils available on the market through quantitative research. F pray laboratory samples tampered with 4 types of vegetable oil for evaluation of the reading spectrum responses. Calibration curves have been traces of and not there was a difference between the results of the oil were obtained from 0 to 70% substitution of olive oil by vegetable oil. Also, compound oils were analyzed to verify that the labeled percentage was being respected, obtaining results of up to 96% soy oil in a product that should contain a maximum of 88%. Therefore, it was possible to verify that the 456 nm region can be used to check adulterations with efficiency and agility and that UV-Vis spectroscopy can be considered a fast and low-cost method for previous analyzes.
Keywords: Adulteration; Spectroscopy; Sweep.

Resumo
O azeite de oliva é um produto de elevado valor agregado, obtido pela simples prensagem da azeitona. Conhecido por ser um alimento funcional e preventivo de diversas doenças como câncer, doenças coronárias e abaixamento do colesterol ruim; sua adulteração pela adição de óleo vegetal de menor valor comercial torna-se uma prática na indústria. Para facilitar a verificação da adulteração, métodos como a espectroscopia UV-Vis foram usados para quantificar o óleo vegetal presente no azeite virgem. Este estudo objetivou evidenciar a técnica UV-Vis em leitura prévia na avaliação e investigação de adulteração em azeites de oliva extra virgem disponíveis no mercado por meio de uma pesquisa quantitativa. Foram adulteradas em laboratório amostras com 4 tipos de óleo vegetal para avaliação das respostas do espectro de leitura. Curvas de calibração foram traçadas e não houve diferença entre os óleos, obteve-se resultados de 0 a 70% de substituição do óleo de oliva por óleo vegetal. Além disso, foram analisados óleos compostos para verificar se o percentual rotulado estava sendo respeitado, obtendo-se resultados de até 96% de óleo de soja em um produto que deveria conter no máximo 88%. Assim sendo, foi possível verificar que a região de 456 nm pode ser utilizada para verificar adulterações com eficiência e agilidade, e ainda, que a espectroscopia UV-Vis pode ser considerada um método rápido e de baixo custo para análises prévias.
Palavras-chave: Adulteração; Espectroscopia; Varredura.

Resumen
El aceite de oliva es un producto de alto valor agregado al obtenido simplemente presionando las aceitunas. Conocido por ser un alimento funcional y preventivo para diversas enfermedades como el cáncer, las enfermedades coronarias y la disminución del colesterol malo; su adulteración mediante la adición de aceite vegetal de menor valor comercial se convierte en una práctica en la industria. Para facilitar la verificación de la adulteración, se utilizaron métodos como la

espectroscopia UV-Vis para cuantificar el aceite vegetal presente en el aceite de oliva virgen. Este estudio tuvo como objetivo comprender la técnica UV-Vis en una lectura previa en la evaluación e investigación de la adulteración en aceites de oliva virgen extra disponibles en el mercado através de una investigación cuantitativa . Por favor, muestras de laboratorio manipuladas con 4 tipos de aceite vegetal para evaluar las respuestas del espectro de lectura. Las curvas de calibración han sido trazas y no hubo diferencia entre los aceites, obteniendo resultados de 0 a 70% de sustitución de aceite de oliva por aceite vegetal. Además, se analizaron aceites compuestos para verificar que se estaba respetando el porcentaje etiquetado, obteniendo resultados de hasta 96% de aceite de soja en un producto que debía contener un máximo de 88%. Por tanto, se pudo comprobar que la región de 456 nm puede utilizarse para comprobar adulteraciones con eficacia y agilidad, y que la espectroscopia UV-Vis puede considerarse un método rápido y de bajo coste para análisis previos.
Palabras clave: Adulteración; Espectroscopia; Barrer.

1. Introduction

Olive oil (OO) is the product extracted by cold pressing of the ripe fruit of the olive tree (Olea europaea), followed by centrifugation, without going through any stage of extraction by solvent or refining (Da Silveira et al., 2017; Meenu & Xu, 2019). It is known worldwide as a functional food, as it helps in the prevention of cancer, coronary heart disease, and the reduction of bad cholesterol when its consumption is carried out in parallel with healthy habits (Meenu & Xu, 2019; Porcari et al., 2016). In addition, it is rich in oleic acid (18: 1n-9), antioxidants, and phytosterols (Lopez et al., 2021).

The benefits of olive oil, combined with its high commercial value, make it the target of several adulterations due to the addition of vegetable oil of less commercial value, with the addition of soy oil being the most common (Hartman et al., 2011). This, due to the great availability of Soy Oil, which is the second most-produced vegetable oil in the world, behind only palm oil (Da Silveira et al., 2017). Among the different types of food, olive oil is considered the healthiest option among edible oils and also, Brazil is considered one of the world's largest importers of olives and derivatives and the cultivation of olive trees in the country is a recent agricultural activity and in the expansion (dos Santos, 2017).

Fraud or adulteration is one of the major concerns of consumers, suppliers, and producers of the food sector (Van Ruth et al., 2018). That said, the adulteration of olive oil may not pose a major threat to public health, however, a major caveat about allergens is valid. Therefore, the fraud control for this product is essential (Garrido-Delgado et al., 2018).

The main techniques used in the analysis and control of food quality are: high-performance liquid chromatography (HPLC), gas chromatography (CG), ultraviolet spectroscopy (UV / VIS), spectroscopy in the near-infrared region, spectroscopy near-infrared (NIRS), medium-infrared spectroscopy, medium-infrared spectroscopy (MIRS), Raman spectroscopy, nuclear magnetic resonance (NMR) and electronic noses (Santos et al., 2020). Therefore, analytical techniques such as HPLC-MS / MS, UHPLC-MS / MS, CG-FID or CG-MS, fluorescence, and NMR are widely used to combat possible adulterations (Meenu et al., 2019). In addition to the high cost, such techniques require specific sample preparation time and relatively high to have a result.

In this sense, the analysis in UV-Vis spectrometer becomes a viable alternative due to easy accessibility, reduced cost, analytical facility, with quick and immediate response, in need of sample preparation, and the reading can be performed in the establishment using portable equipment. (Torrecilla et al., 2010). Besides, the spectroscopy technique has a high degree of band overlap and a lack of selectivity (Alves et al., 2018). The study in question is justified by the frequent occurrence of fraud are detected in the olive oil market in Brazil. In Brazil, the quality control of Extra Virgin Olive Oil (EVOO) is carried out by the agencies: ANVISA (National Health Surveillance Agency) and MAPA (Ministry of Agriculture, Livestock, and Food Supply). The regulation complies with the IOC (International Olive Council) and establishes four parameters: acidity, peroxide index, ultraviolet absorbance, and sensory analysis (Brasil, 2005).

However, there is that the analyzes used for the detection of these practices are expensive and need to be carried out in specialized laboratories, where those responsible receive the samples to be investigated.

On the other hand, it is worth noting that, even though the advances in conventional spectrometers, miniaturized and portable equipment are being developed to address the need for reliable non-destructive in situ analysis, reducing the time and cost of analysis (Borghi et al., 2020).

Therefore, this study has presented a technique that can be used by those responsible for making spot investigation which does not require the routing of all samples to specialized laboratories, but only the proposed analysis, which therefore allows for greater speed and lower cost in the investigation of fraud. Moreover, the research carried out was quantitative. Pereira et al. (2018) offer methodological support for the type of research in question.

In this context, the objective of the present work was to develop an analytical technique using UV-VIS in the previous proof of adulteration of extra virgin olive oils by edible vegetable oils through quantitative research.

2. Methodology

An experimental UV-Vis research was carried out, using a standard scan to verify the largest peak, which was carried out in a wavelength range between 300 and 800 nm. From the determined at a smooth, has been defined the fixed range of 456 nm for the analysis of oils in the laboratory tampered with.

2.1 Material

Six extra virgin olive oil brands classified according to the label specifications (EVOO) and two commercial blends of olive oil and soybean oil from three different lots were purchased in the local market Maringá-PR, Brazil (Table 1). In addition to a sample of olive oil with an international certificate identified as P and used as standard and soy, canola, corn, and sunflower oils. All the samples were stored at 6 ° C to 10 ° C in a refrigerator, sheltered from light when used were withdrawn 24 h duh before remain at 25 ° C ambient temperature.

Table 1. Specifications of the oils used.

Sample	Produced	Packaged	Specifications[a]
1	Portugal	São Paulo	EVOO
2	Spain	Spain	EVOO
3	São Paulo	São Paulo	EVOO
4	Chile	Chile	EVOO
5	Portugal	Portugal	EVOO
6	Brazil (Minas Gerais)	Brazil (Minas Gerais)	EVOO
7	Brazil (Paraná)	Brasil (Paraná)	A mixture of SO and EVOO (10%)
P[b]	Italy	Italy	EVOO
PI[c]	Brazil (São Paulo)	Brazil (São Paulo)	A mixture of SO and EVOO (12%)
SO	Brazil	Brazil	SO

[a]Classification according to label specifications; (EVOO): Extra Virgin Olive Oil.
[b] Sample of internationally certified olive oil
[c] Internal standard (compound olive oil)
Source: Authors themselves (2020).

Three lots containing three samples of each refined vegetable oil (soy (SO), canola (CO), corn (COO), and sunflower (SUO) were purchased in the local market in Maringá-PR, Brazil.

Standard (P), was intentionally adulterated with the refined oils SO, CO, COO, and SUO in the proportions (1, 5, 10,

20, 50, 70, and 90% (v / v)) for each oil, being carried out five replicates for each point according to Table 2.

Table 2. Intentional tampering with the Standard (P) with SO, CO, COO and SUO.

% of P[a]	% of oil[a]
100	0
99	1
95	5
90	10
80	20
50	50
30	70
10	90
0	100

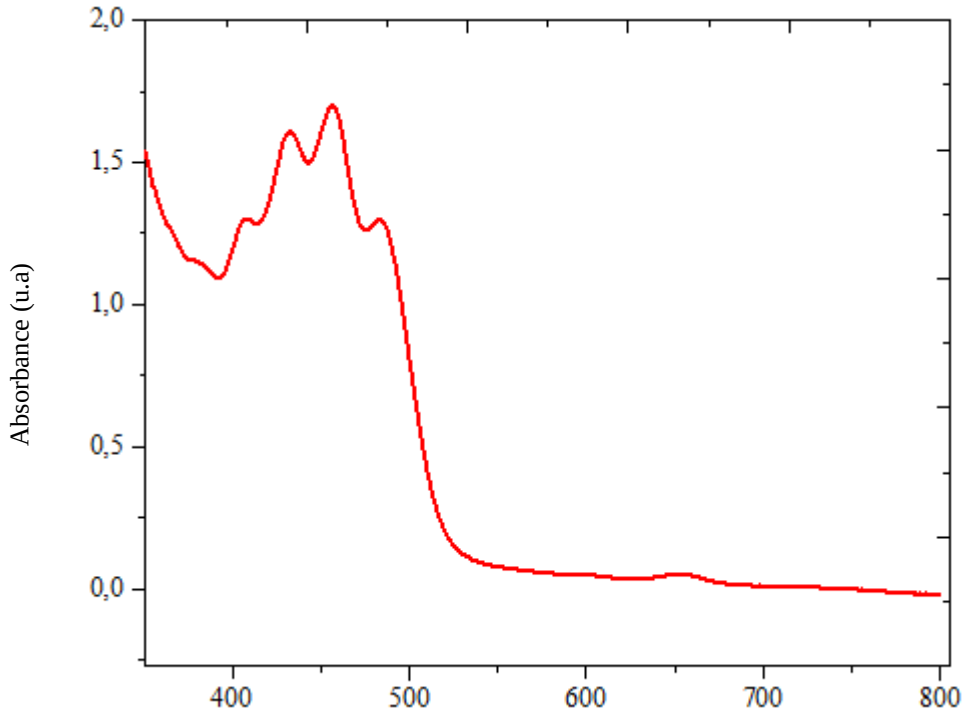
[a]: (v/v);P: standard olive oil; SO: refined soybean oil; CO: refined canola oil; COO: refined corn oil and SUO: refined sunflower oil.
Source: Authors themselves (2020).

3. Results and Discussion

3.1 P-scan analysis

To check the quality of the standard to be used, a scan was carried out from 300 to 800 nm to determine the highest peak intensity characteristic of extra virgin olive oils (Figure 1).

Figure 1. Standard olive oil scanning spectroscopy.



Wave-length (nm)
Source: Authors themselves (2020).

The absorption maxima occur ram in the range of 400 the 500 nm (Figure 1), region scanning spectrum in which there is the absorption of chlorophylls and carotenoids. The maximum wavelength chosen was 456 nm, since it is the region defined for analysis of absorption at the points of the calibration curve and samples acquired from the market. In this sense, it is known that vegetable oils did not absorb in this region, this is because they undergo a refining process, eliminating the chlorophylls and carotenoids present (Jorge, 2009)

3.2 Calibration curves for vegetable oils

With the increasing addition of vegetable oil (VO) to the pattern (P), absorbance readings of the samples were taken at 456 nm (Table 3) to verify the occurrence of the same.

Table 3. Percentage of vegetable oil (VO) in pattern (P) and absorbances of different commercial oils.

% of oil	Abs Soy (nm)	Abs Canola (nm)	Abs Corn (nm)	Abs Sunflower oil (nm)
0	$1{,}784^{a,\,a} \pm 0{,}022$	$1{,}815^{a,\,a} \pm 0{,}022$	$1{,}801^{a,\,a} \pm 0{,}022$	$1{,}835^{a,\,a} \pm 0{,}022$
1	$1{,}742^{a,\,b} \pm 0{,}020$	$1{,}700^{a,\,b} \pm 0{,}020$	$1{,}701^{a,\,b} \pm 0{,}020$	$1{,}716^{a,\,b} \pm 0{,}020$
5	$1{,}662^{a,\,c} \pm 0{,}026$	$1{,}612^{a,\,c} \pm 0{,}026$	$1{,}603^{a,\,c} \pm 0{,}026$	$1{,}618^{a,\,c} \pm 0{,}026$
10	$1{,}606^{a,\,c} \pm 0{,}041$	$1{,}506^{a,\,c} \pm 0{,}041$	$1{,}548^{a,\,c} \pm 0{,}041$	$1{,}542^{a,\,c} \pm 0{,}041$
20	$1{,}419^{a,\,d} \pm 0{,}032$	$1{,}427^{a,\,d} \pm 0{,}032$	$1{,}434^{a,\,d} \pm 0{,}032$	$1{,}364^{a,\,d} \pm 0{,}032$
50	$0{,}965^{a,\,e} \pm 0{,}048$	$0{,}883^{a,\,e} \pm 0{,}048$	$0{,}878^{a,\,e} \pm 0{,}048$	$0{,}853^{a,\,e} \pm 0{,}048$
70	$0{,}512^{a,\,f} \pm 0{,}031$	$0{,}475^{a,\,f} \pm 0{,}031$	$0{,}536^{a,\,f} \pm 0{,}031$	$0{,}473^{a,\,f} \pm 0{,}031$
90	$0{,}188^{a,\,g} \pm 0{,}031$	$0{,}182^{a,\,g} \pm 0{,}031$	$0{,}209^{a,\,g} \pm 0{,}031$	$0{,}130^{a,\,g} \pm 0{,}031$
100	$0{,}042^{a,\,h} \pm 0{,}027$	$0{,}037^{a,\,h} \pm 0{,}027$	$0{,}066^{a,\,h} \pm 0{,}027$	$0{,}000^{a,\,h} \pm 0{,}027$

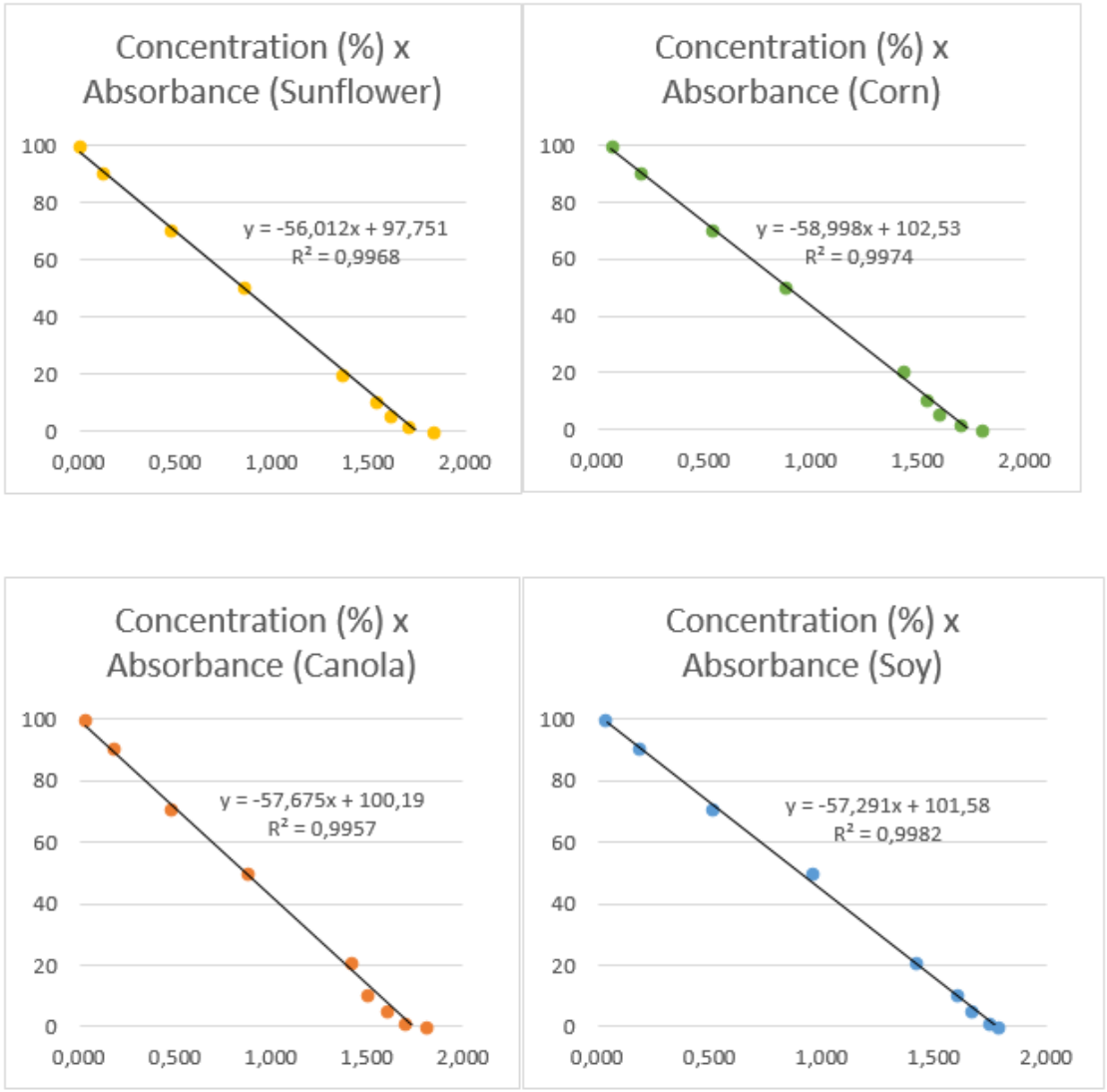
Same letters in the same column, are statistically equal to the 95% significance level (Tukey)
Equal letters on the same line, are statistically equal to the 95% significance level (Tukey)
The standard deviation (± σ) is shown for each result.
Source: Authors themselves (2020).

In Table 3 it can be seen that as the percentage of oil rises, it decreases its absorbance. This is due to the VO having gone through the refining process, eliminating the carotenoids present, and thus, its absorption being zero at 456 nm (Milanez et al., 2017).

There was no significant difference in the type of VO used immediately, regardless of VO used, can we say only that there was an adulteration of olive oil with refined vegetable oil, in other words, absorbances with 0% oil or 100% oil do not differ. In addition, regardless of the oil used, there is no statistical difference between them. The percentages of 5 and 10% of vegetable oil addition do not differ statistically. The others are different.

From these absorbances, it was possible to plot calibration curves for each vegetable oil (Figure 2), so that, from reading in the UV-Vis in 456 nm of olive oil samples, it was possible to know if there was adulteration with vegetable oils and how much was inserted.

Figure 2. Calibration curves for each vegetable oil.



Source: Authors themselves (2020).

3.3 Application of the technique to commercial samples

Six oils were purchased in local shops to check the methodology applied, the last the control sample(PS) an oil compound, which was informed to the consumer containing 88% oil made of soybeans and 12% of olive oil. Table 4 shows the concentration (%) of oil present in each oil for each curve.

Table 4. Calculation of the oil concentration (%) using the calibration curves.

Olive oil	Soy curve (%)	Canola curve (%)	Corn curve (%)	Sunflower curve (%)
1	32,93[a, a] ± 1,046	30,98[a, a] ± 1,046	31,73[a, a] ± 1,046	30,54[a, a] ± 1,046
2	31,73[a, a] ± 1,039	29,77[a, a] ± 1,039	30,49[a, a] ± 1,039	29,36[a, a] ± 1,039
3	22,74[a, b] ± 0,993	20,71[a, b] ± 0,993	21,23[a, b] ± 0,993	20,57[a, b] ± 0,993
4	0[a, c]	0[a, c]	0[a, c]	0[a, c]
5	70,46[a, d] ± 1,523	68,81[a, d] ± 1,523	70,44[a, d] ± 1,523	67,28[a, d] ± 1,523
6	0[a, e]	0[a, e]	0[a, e]	0[a, e]
7	96,03[a, f] ± 2,245	94,60[a, f] ± 2,245	96,81[a, f] ± 2,245	92,32[a, f] ± 2,245
PS	95,40[a, f] ± 1,962	93,96[a, f] ± 1,962	96,16[a, f] ± 1,962	91,70[a, f] ± 1,962

Same letters in the same column, are statistically equal to the 95% significance level (p<0,05).
Equal letters on the same line, are statistically equal to the 95% significance level (p<0,05).
The standard deviation (± σ) is shown for each result.
Source: Authors themselves (2020).

Arranged by the calibration curves for each oil and the absorbances of the oils, they were calculated as percentages of vegetable oil added as the oils. At 4 and 6 dissipation equals zero, which shows the absence of vegetable oil in extra virgin olive oil. Samples 1, 2, and 3 dissipated from 20% to just over 33% of vegetable oil addition in extra virgin olive oil, that is, showing in advance the probable oils that may have been adducted. The 5 and 7 were the extra virgin olive oils with the highest addition of vegetable oil, with the increase in the percentage of vegetable oil, not olive oil standing out as compound olive oil since extra virgin olive oil does not have any addition of vegetable oil. The sample called PS was used as a control, which should have 12% olive oil, however, it is possible to observe that there was less addition of olive oil than reported on the label.

In Table 4, it is also possible to identify that, regardless of the application curve, the result do not appear to differ ($p < 0.05$). Furthermore, olive oils 1 and 2 are statistically equal, differing from the others. Olive oil 7 and PS are statistically equal ($p < 0.05$), however, olive oil 7 does not have the words compound olive oil, being labeled as extra virgin olive oil, that is, it can be considered that the consumer is not receiving the correct labeling information.

4. Final Considerations

Given the above, it is clear that the 456 nm region in UV-Vis can be used for prior analysis in the investigation and inspection of olive oils available on the market in an effective way to check adulteration by refined vegetable oils, and that the UV technique -Vis proves to be a fast, inexpensive technique, easy to access compared to those already used at the moment, using chromatographic techniques and expanding the opportunity to analyze the largest number of samples for verifying adulteration of oils in loco, making the opinion of professionals will research, for example, the Ministry of Agriculture, Livestock and Supply (MAPA) in Brazil.

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