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Technical Report

A sustainable analytical approach for detecting extra virgin olive oil adulteration using Nexera™ UC

Main feature: development of a green analytical method using supercritical fluid chromatography with UV detection

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Abstract:

In this study, a fast, simple and green methodology was optimized to detect intentionally adulterated extravirgin olive oil EVOO with cheaper seed oils at different levels by means of subcritical fluid chromatography (subFC) with UV detection, followed by statistical analysis. Separations were achieved with a CO_2-based green mobile phase, which provided high efficiency without reaching a prohibitive backpressure. Remarkably, small amounts of organic modifier (5 %) were sufficient for elution, thus minimizing both the solvent consumption and the waste generation. The developed method is a more sustainable alternative to conventional non-aqueous reversed phase methods (NARP-LC), for routine analysis aimed to guarantee EVOO quality.

Keywords: Adulterations, Bio-ethanol, Extra virgin olive oil, Green analytical chemistry, Supercritical fluid chromatography, Triacylglycerols

1. Introduction

Knowledge of the composition of triacylglycerols (TAGs) in vegetable oils is important for dietary and nutritional reasons, due to the influence of the different properties of each fatty acid on the human organism. Specifically, extravirgin olive oil (EVOO) is a popular food ingredient worldwide, and for this reason, detecting economically motivated adulteration is important to protect consumers' interest and health[1]. According to the latest report from the EU Food Fraud Network (European Union, 2021), olive oil (OO) tops the list of reported adulterated food products. The official methods to assess OO purity and detect the presence of extraneous vegetable oils include the analysis of fatty acids (FAs), triacylglycerols (TAGs) and sterols (Regulation (EU) No 2568/91 and its amendments) [2].

A variety of chromatographic techniques has been employed for TAGs analysis and separation. High-performance liquid chromatography (HPLC) methods, which use reversed-phase (RP) and silver-ion (Ag) columns for TAGs separation, have been employed either independently or in combination[3]. However, both these approaches have the drawback of relying on toxic organic solvents. The alternative separation technique employed in TAGs analysis is supercritical fluid chromatography (SFC) coupled with a UV detector at very low wavelengths, due to the weak UV absorption of the mobile phase. In this study, a fast, simple and green methodology was optimized to detect intentionally adulterated olive oil with cheaper seed oils at different levels by means of SFC with UV detection, followed by statistical analysis.

2. Experimental

2.1 Samples, chemicals and materials

Adulterated EVOO (*Olea europea* L.) samples were prepared by blending an EVOO sample with soybean oil (*Glycine max* L.) , corn (*Zea mays* L.) oil and peanut oil (*Arachis hypogaea* L.). These oils were chosen based on the likelihood of use as adulterants in EVOO. Each adulterant covered a wide range in concentration from 5 % to 50 % (*v/v*). The samples were vortexed for 1 min to ensure a homogenous mixture, without any further pretreatment. Compressed CO_2 (99.8 % grade) used as the main mobile phase was from Nippon Gases (Milan, Italy).). Bio-ethanol obtained from corn (gradient grade for liquid chromatography LiChrosolv®) was used as the modifier for SFC-PDA analyses.

2.2 SFC-PDA Nexera™ UC system

All experiments were performed on a Nexera™ UC system (Shimadzu Europa, Germany). The system was equipped with a CBM-20A communication bus module, a DGU-20A5R degassing unit, an LC-30ADSF CO_2 pump, one LC-20ADXR dual plunger parallel-flow pump, a Sil-30AC autosampler, a CTO-20AC column oven, a SPD-M20A photodiode array (PDA) detector, and a SFC-30A backpressure regulator (BPR). Chromatograms were recorded using Shimadzu LabSolution ver. 5.80 software. Four Ascentis Express C18 columns, provided by KGaA (Darmstadt, Germany), were connected in series for TAGs separation. The analytical conditions used are summarized in Table 1.

Table 1: Analytical conditions for the analysis of triacylglycerols in vegetable oils

Column	Ascentis Express C18 (4 × 15 cm × 4.6 mm, 2.7 µm)
Column oven	25 °C
Mobile phase	CO_2 (solvent A) and ethanol (solvent B)
Elution	Isocratic at 5 % B
Flow rate	2.0 mL min^{-1}
Injection volume	0.3 µL
UV detection wavelength	205 nm
BPR setting	200 bar

3. Results and discussion

Using four C18 columns packed with superficially porous particles, a highly efficient separation of TAGs with an isocratic elution mode was provided, allowed for complete sample TAG elution within 30 min with the conditions used in this method (Figure 1). Peak identification was carried out according to partition number (PN), the published composition of these common vegetable oils and the relative amount of major and minor peaks, and finally taking into account TAGs retention times. For compounds with identical PN, separation was achieved based on the number of unsaturations. The elution order of TAGs was primarily governed by the hydrophobicity of the solutes. As the PN increases, the non-polar character of TAGs becomes more dominant.

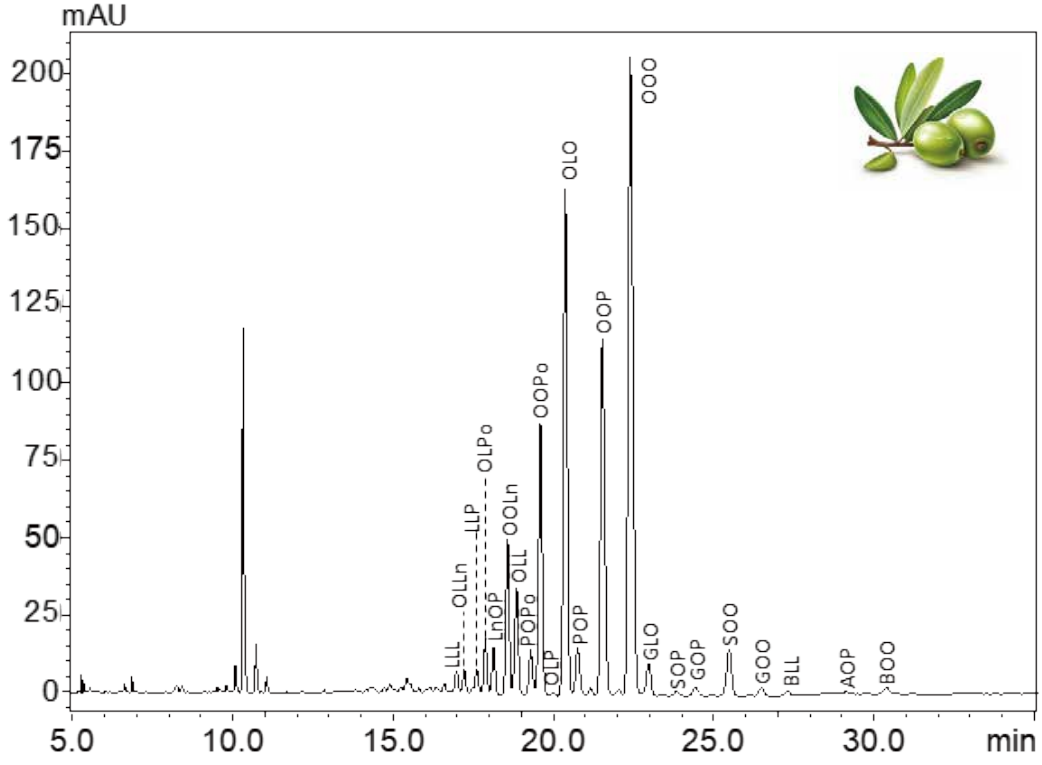


Figure 1: SFC-UV chromatograms of extra virgin olive oil, separated based on the partition number (PN). Fatty acid abbreviations: M= miristic acid (C14:0), P= palmitic acid (C16:0), Po= palmitoleic acid (C16:1), C17:0= margaric acid, C17:1= eptadecenoic acid, S= stearic acid (C18:0), O= oleic acid (C18:1), L= linoleic acid (C18:2), Ln= linolenic acid (C18:3), A= arachidic acid (C20:0), G= gadoleic acid (C20:1), B= behenic acid (C22:0).

Table 2: TAGs composition of EVOO blends, with one-way ANOVA experiments. Means followed by different letters (a-h) in the same ROW are significantly different according to one-way ANOVA performed.

TAGs	EVOO	EVOO5	EVOO10	EVOO15	EVOO20	EVOO30	EVOO40	EVOO50
LLL	0.701^{h}	5.489^{g}	6.555^{f}	8.594^{e}	11.934^{d}	13.550^{c}	16.338^{b}	17.882^{a}
OLLn	0.712^{g}	2.339^{f}	2.474^{f}	3.053^{e}	4.116^{d}	4.677^{c}	5.652^{b}	6.210^{a}
OLPo	1.863^{h}	3.766^{g}	4.212^{f}	5.098^{e}	6.401^{d}	7.086^{c}	8.391^{b}	9.263^{a}
LnOP	1.558 ^{ab}	1.828^{a}	1.576^{ab}	1.555^{ab}	1.473^{b}	1.479^{b}	1.477^{b}	1.570^{ab}
OOLn	5.869^{h}	8.296^{g}	9.154^{f}	10.263^{e}	12.091^{d}	13.000^{c}	14.679^{b}	15.678^{a}
OLL	4.011^{a}	3.758^{ab}	3.799^{ab}	3.790^{ab}	3.659^{b}	3.609^{b}	3.533^{b}	3.534^{b}
POPo	1.799^{b}	2.129^{a}	1.798^{b}	1.822^{b}	1.832^{b}	1.832^{b}	1.836^{b}	1.877^{b}
OLP	10.650^{a}	9.609^{b}	9.581^{b}	9.302^{b}	8.717^{c}	8.459^{cd}	7.945^{de}	7.653^{e}
OOPo	0.107^{a}	0.305^{a}	0.121^{a}	0.106^{a}	0.111^{a}	0.104^{a}	0.102^{a}	0.103^{a}
OLO	20.720^{a}	17.926^{b}	17.851^{b}	16.831^{c}	15.213^{d}	14.408^{e}	12.917^{f}	11.992^{g}
POP	2.051^{d}	2.647^{c}	2.709^{c}	2.844^{c}	3.262^{b}	3.341^{b}	3.777^{a}	3.958^{a}
OOP	15.034^{a}	12.366^{b}	11.895^{c}	10.782^{d}	9.088^{e}	8.199^{f}	6.545^{g}	5.563^{h}
OOO	30.397^{a}	24.671^{b}	24.086^{c}	21.908^{d}	18.302^{e}	16.513^{f}	13.094^{g}	11.005^{h}
SOP	0.315^{a}	0.381^{a}	0.281^{a}	0.270^{a}	0.257^{a}	0.251^{a}	0.232^{a}	0.241^{a}
SOO	2.455^{a}	2.241^{ab}	1.991^{abc}	1.839^{bcd}	1.547^{cde}	1.438^{def}	1.213^{ef}	1.036^{f}

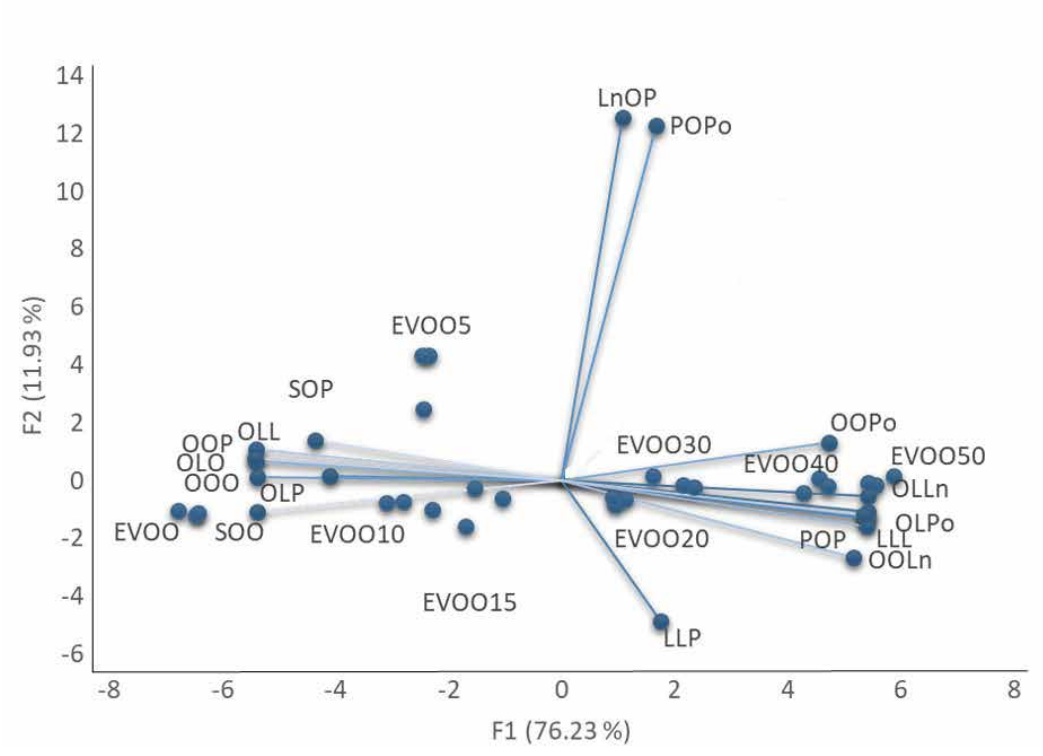


Figure 2: Biplot from the first and second principal components of PCA of EVOO/soybean oil blends. Variables: twenty-two different TAGs quantified in each oil sample.

ANOVA treatment demonstrates that the approach can adequately separate EVOO from other common oil adulterants. Table 2 shows the TAGs composition of EVOO and EVOO blends (5-50 % of soybean oil in EVOO). Means followed by different letters in the same column are significantly different according one-way ANOVA ($p < 0.05$) performed. By choosing only TAGs that are best correlated with the adulterant and contribute the most to the variance between the adulterant and EVOO, such as LLL, significant discrimination between EVOO blends can be achieved.

The 2D-PCA-plot obtained from the two principal components, representing the sixteen TAGs quantified for EVOO and the distinct clustering for EVOO blends with soybean oil as potential oil adulterant is shown in Figure 2. Using TAG profiles combined with PCA can differentiate EVOO from soybean oil at adulteration levels above 5 %. The distinct position of the EVOO cluster on the far left of the plot along the F1 axis indicates the potential of this approach for detecting the adulteration of EVOO with the common oil adulterant used in this study.

Utilizing the software-based AGREE tool, the greenness features of the novel SubFC-PDA method were investigated. The AGREE analysis offers an assessment approach that yields easily interpretable and informative results. The assessment criteria are drawn from the 12 principles of green analytical chemistry and are converted into a unified 0-1 scale, and the final score is then automatically generated according to these principles. The performance of the procedure in each principle is indicated with a color scale (red-yellow-green), while the weight of each principle is reflected by the width of its segment.

The AGREE evaluation yielded a final score of 0.83, demonstrating the higher sustainability of the new SFC-PDA method, compared to conventional NARP-HPLC, for which a score of 0.48 was calculated.

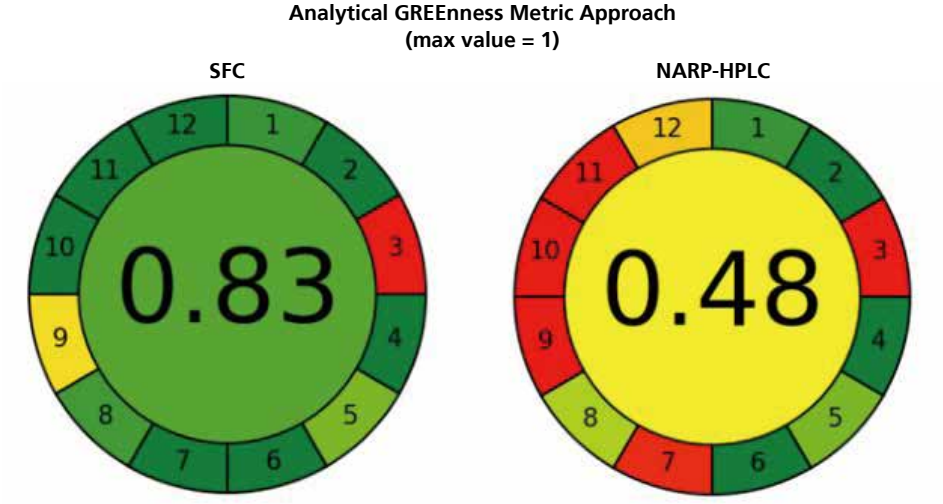


Figure 3: AGREE graphic assessment of: (left) the SFC-PDA method and (right) conventional NARP-HPLC method for the analysis of TAGs components in vegetable oils.

Conclusions

The Nexera™ UC system provided excellent results for assessing TAGs composition in ten different vegetable oils examined in this study employing an SFC-PDA analytical method using isocratic mode and UV detection at 205 nm. This approach does not require any sample preparation and enables analyses to be completed with less waste and less cost compared to traditional methods, making it feasible for routine analyses, while providing reliable quantitative values.

Quantification of adulterated EVOO was performed by establishing curves based on peak areas of LLL marker versus seed oils concentrations. Statistical analysis using PCA and one-way ANOVA also visually distinguished adulterated EVOO from pure EVOO, and the result was in accordance with the present quantitative method. This fast and environmental method exhibits a huge potential for quality control and authenticity evaluation of EVOO.

Reference

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