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Mut (Mersin) Bölgesi Natürel Sızma Zeytinyağlarının Senkron Floresans Spektroskopisi ve Kemometri ile Orijinalliğinin Belirlenmesi: LDA ve HCA Model Yaklaşımları

Authenticity Verification of Extra Virgin Olive Oils Harvested in Mut (Mersin) Region by Synchronous Fluorescence Spectroscopy and Chemometrics: Approaches on LDA and HCA Models

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Özet: Bu çalışmada, Mut (Mersin) bölgesinden iki farklı hasat döneminde (2019 ve 2020 Ekim) elde edilen ekstra saf sızma zeytinyağı (VOO) ve ticari temin edilen diğer yemeklik yağların senkron floresans (SyF) spektroskopi verileri, doğrusal diskriminant analizi (LDA) ve hiyerarşik küme analizi (HCA) ile analiz edilerek orijinalliklerinin belirlenmesi sağlanmıştır. Spektroskopi analizlerinde emisyon/uyarma monokromatörleri senkron kullanılmış ve veriler 200-800 nm emisyon dalga boyu bölgesinde elde edilmiştir. VOO ve diğer yenilebilir yağ numunelerinin muhtemel tağşiş durumunu doğrulamak için yüksek doğrulukla LDA modeli elde edilmiş: test edilen numunelerin %98.28'i kendi bölgesinde yerleşim göstermiş ve HCA dendogramları yüksek hassasiyet ve özgüllük ile numunelerde başarılı ayrımlar sağlamıştır. Elde edilen bulgular, SyF spektroskopisinin kemometri ile birleştiğinde sınıflandırmadaki uygulanabilirliğini açıkça göstermekte ve VOO numunelerinin ucuz yenilebilir yağlarla olası tağşişini tespit edebilmek için umut verici bir yöntem

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sunmaktadır. Üstelik çalışmada önerilen basit ve hızlı bu yöntem, analiz sırasında herhangi bir kimyasal kullanımına ve zahmetli işlemlere ihtiyaç duymadan uygulanabilmektedir.

Anahtar Kelimeler: Senkron floresans spektroskopi; Ekstra Saf Sızma Zeytinyağı; Kemometri; Tağşiş

Abstract. In this work, synchronous fluorescence (SyF) spectroscopy data of extra virgin olive oils (VOOs) harvested from two different harvest terms (2019 and 2020 October) in Mut (Mersin) region and other edible oils were analyzed by linear discriminant analysis (LDA) and hierarchical cluster analysis (HCA) to verify their authenticity. The emission and excitation monochromators were synchronously employed, and the data were obtained in the 200–800 nm emission region. A robust LDA model was gained to authenticate the extra VOOs and other edible oils: 98.28% of the samples were correctly predicted, and the HCA dendrograms showed successful discriminations with high sensitivity and specificity. The findings show the capability of SyF spectroscopy coupled with chemometrics and offer a promising methodology for confirming the possible adulteration of extra VOOs with inexpensive edible oils. Furthermore, this simple and rapid method doesn't need any chemical usage and laborious procedures.

Key words: Synchronous fluorescence spectroscopy; Extra virgin olive oil; Chemometrics; Authenticity

1. Introduction

Extra virgin olive oil (VOO), manufactured from the fruit of an olive tree through a mechanical cold pressing without chemicals, has high nutritional properties and a unique aroma with high production costs [1–3]. Its nutritional value primarily features the high levels of phenolic substance and monounsaturated fatty acid (MUFA) content, which are positively active on antioxidant activity and prevent a number of diseases such as obesity, diabetes, and different types of cancer, metabolic and cardiovascular syndromes [1, 2, 4]. Owing to its health benefits, it has become progressively more popular and recently made it attractive to adulteration with cheaper vegetable oils. Many different factors make it complicated to authenticate olive oils by traditional methods like high performance liquid

chromatography (HPLC) and gas chromatography (GC); however, the majority of these methods require high maintenance and gaining costs and engage time-consuming and laborious applications [5–7]. Spectroscopic techniques like infrared (IR), mass (MS), Raman, nuclear magnetic resonance (NMR), and fluorescence (FL) spectroscopy have been recently employed for the authentication analysis of extra VOOs. To offer successful discrimination between different vegetable oils and extra VOO samples and provide better estimation for adulteration, these methods based on the simultaneous detection of diverse chemical compounds of samples in combination with multivariate analyses [1, 2, 7–10]. Until now, no research has been reported in the literature involving the usage of SyF and chemometrics to authenticate the extra VOO samples from Mut (Mersin) region and other edible oils from different brands. Given these, to verify their authenticity, the SyF spectroscopy data of extra VOOs harvested from two different harvest terms in Mut (Mersin) region and some commercial edible oils were analyzed by LDA and HCA. The emission and excitation monochromators were synchronously used, and the data were obtained in the emission wavelength region of 200–800 nm.

2. Materials and Methods

2.1. Samples and reagents

The extra VOOs harvested in Mut region (Mersin City, Turkiye) were poured directly from the producers during two harvest terms (n=30; 2019–VOO–1 to 30 and n=30; 2020–VOO–1 to 30) (Table 1) [5]. The refined edible oils; cottonseed oil (CSO) (n=5; CSO–1 to 5), virgin olive oil (VOO) (n=5; VOO–1 to 5), hazelnut oil (HNO) (n=6; HNO–1 to 6), soybean oil (SBO) (n=5; SBO–1 to 5), sunflower oil (SFO) (n=5; SFO–1 to 5), pomace oil (PO)(n=3; PO–1 to 3) and Riviera olive oil (ROO) (n=3; ROO–1 to 3) were obtained from local supermarkets in Karaman City, Turkiye.

sample code	producer	type of oil tree	harvest term
2020-1	A*** T***	Ayvalık	2020/ Mut
2020-2	N*** S***	Ayvalık	2020/ Mut
2020-3	Y*** E***	Gemlik	2020/ Mut
2020–4	Y*** E***	Ayvalık	2020/ Mut
2020–5	M*** A*** E***	Gemlik	2020/ Mut
2020-6	M*** U***	Ayvalık	2020/ Mut
2020–7	İ*** D***	Ayvalık	2020/ Mut
2020-8	A*** S***	Ayvalık	2020/ Mut
2020–9	A*** T***	Ayvalık	2020/ Mut
2020-10	A*** Y***	Ayvalık	2020/ Mut

Table 1. Information table for the VOO samples which were harvested in Mut (Mersin) region

2020-11	M*** A***	Ayvalık	2020/ Mut
2020-12	A*** K***	Ayvalık	2020/ Mut
2020-13	A*** D***	Ayvalık	2020/ Mut
2020-14	M*** M***	Ayvalık	2020/ Mut
2020-15	M*** D***	Ayvalık	2020/ Mut
2020-16	N*** E***	Erdek	2020/ Mut
2020-17	M*** S***	Domat	2020/ Mut
2020-18	İ*** Ö***	Erdek	2020/ Mut
2020-19	K*** O***	Ayvalık	2020/ Mut
2020-20	M*** S***	Domat	2020/ Mut
2020-21	O*** U***	Ayvalık	2020/ Mut
2020-22	N*** E***	Domat	2020/ Mut
2020-23	A*** D***	Avvalık	2020/ Mut
2020-24	İ*** S***	Delice (Harap)	2020/ Mut
2020-25	A*** U***	Delice (Harap)	2020/ Mut
2020-26	M*** Y*** C***	Avvalık	2020/ Mut
2020-27	F*** K***	Avvalık	2020/ Mut
2020–28	V*** G***	Avvalık	2020/ Mut
2020–29	İ*** S***	Avvalık	2020/ Mut
2020-30	N*** D***	Avvalık	2020/ Mut
2019–1	N*** S***	Avvalık/Gemlik	2019/ Mut
2019–2	Y*** G***	Gemlik	2019/ Mut
2019–3	A*** E***	Avvalık	2019/ Mut
2019–4	H*** Y***	Avvalık/Gemlik	2019/ Mut
2019-5	D*** A*** K***	Avvalık	2019/ Mut
2019-6	D*** A*** K***	Avvalık	2019/ Mut
2019–7	M*** A***	Avvalık	2019/ Mut
2019-8	M*** K***	Avvalık	2019/ Mut
2019–9	M*** A*** A***	Gemlik	2019/ Mut
2019-10	N*** Ö***	Avvalık	2019/ Mut
2019–11	K*** K***	Gemlik	2019/ Mut
2019–12	H*** Y***	Avvalık	2019/ Mut
2019–13	A*** B***	Avvalık/Gemlik	2019/ Mut
2019–14	H*** İ*** K***	Gemlik	2019/ Mut
2019–15	M*** A*** C***	Avvalık	2019/ Mut
2019–16	Y*** S***	Avvalık	2019/ Mut
2019–17	H*** D***	Avvalık/Gemlik	2019/ Mut
2019–18	M*** Ö***	Gemlik	2019/ Mut
2019–19	C*** K***	Avvalık	2019/ Mut
2019-20	M*** G***	Avvalık/Gemlik	2019/ Mut
2019-21	İ*** []***	Avvalık	2019/ Mut
2019-22	Y*** Y***	Gemlik	2019/ Mut
2019–23	A*** C***	Avvalık	2019/ Mut
2019–24	M*** C***	Gemlik	2019/ Mut
2019–25	M*** K***	Avvalık	2019/ Mut
2019–26	H*** K***	Avvalık/Gemlik	2019/ Mut
2019–27	İ*** D***	Avvalık/Gemlik	2019/ Mut
2019–28	 M*** S***	Avvalık/Gemlik	2019/ Mut
2019–29	M*** Ü***	Avvalık/Gemlik	2019/ Mut
2019-30	E*** S***	Avvalık/Gemlik	2019/ Mut

Spectroscopy grade chemicals and reagents were procured from Sigma–Aldrich Inc. (St. Louis, Missouri, US) and VWR–BDH Inc. (West Chester, Pennsylvania, US) chemical companies. After the filtration through 0.45 μ m membrane filter, all

samples were stored in the dark bottles at +4°C until they were analyzed by SyF spectroscopy.

2.2. Synchronous fluorescence (SyF) spectroscopy measurements

The SyF measurements were recorded on a Cary Eclipse spectrophotometer (Agilent Tech., US). The samples were analyzed using a microplate reader cuvette without pretreatment or chemical usage. The excitation and emission monochromators were synchronously used with different wavelength intervals ($\Delta\lambda$; $\Delta\lambda$ =10 to 80 nm), and the fluorescence data were gathered in the emission region of 200–800 nm. The slit widths, acquisition intervals, and integration time were used as 5 nm, 1 nm, and 0.3 s, respectively [5, 11]. The data were exported as an Excel file (Office–365, Microsoft Corporation, Redmond, WA).

2.3. Multivariate data analysis and software packages

The applied supervised and unsupervised multivariate data analysis models were LDA and HCA, respectively. The exported SyF data were analyzed by Unscrambler[®]X10.4 (CAMO Software, Oslo, Norway) software package. Matrix files were built from the spectroscopy data of full wavelength region $[D_{rows (correspond to emission wavelengths) \times columns}$ (correspond to samples with their repeats)], and then they were imported to the Unscrambler[®]X10.4 software for the chemometrics analyses.

3. Results

The SyF spectroscopy measurements were carried out to authenticate extra VOOs from two different harvest terms in Mut (Mersin) region and other edible oils from different brands. The SyF data of full wavelength region (200–800 nm) obtained with $\Delta\lambda$ =10 nm was used to construct a supervised LDA model (Figure 1). The linear LDA model was generated with eight predictors (2019–VOOs, 2020–VOOs, CSOs, VOOs, HNOs, SBOs, SFOs, POs, and ROOs). The data of selected nine different emission wavelengths (200 nm, 300 nm, 400 nm, 500 nm, 600 nm, 650 nm, 700 nm, 750 nm and 800 nm) were removed from the data of full region (200–800 nm).



Figure 1. LDA discrimination plot for the 2019–VOOs, 2020–VOOs and other refined edible oils $(\Delta\lambda=10 \text{ nm})$ recorded from the SyF analysis in the region of 200–800 nm using (a) linear and (b) quadratic methods.

As seen figure 1a, 92.24% of the samples could be classified with high accuracy on the basis of their origin by the linear LDA model whereas quadratic LDA model could discriminate 98.28% of the samples. The extra VOO samples harvested from 2019 and 2020 terms were located in a very close area but differed depending on the years. The same samples were located in a very different region from other edible oil types (*CSOs, VOOs, HNOs, SBOs, SFOs, POs, and ROOs*). The PO samples are closest to the extra VOO samples harvested from 2019 and 2020 terms, and the SFO, SBO, and HNO samples are located utmost from them. In the LDA models, 27 refined edible oils from different commercial brands and 60 extra VOO samples from Mut (Mersin) region were presented as "training set" to construct the model; the commercial VOO samples (*VOO–1 to VOO–5*) were "testing set". To validate this model, the data of the "testing set" [12, 13] were used, and the results of prediction are presented as a confusion matrix in the 109

Table 2, verifying the high prediction ability of LDA approach. Thus, most of the samples (92.24%) could be classified in their origin. As obviously seen in the classification table of LDA, the commercially available VOO samples defined as the "testing set" were in the 2019–VOOs or 2020–VOOs classes according to the prediction test of the LDA model. In conclusion, the discrimination results of LDA show that the SyF spectroscopy based on the intensity of fluorescent compounds is well responsive to differentiations for studied samples.

Table 2. Confusion matrix tables for the samples ($\Delta\lambda$ =10 nm) recorded from the region of 200–800 nm using (a) linear and (b) quadratic methods, and (c) classification table using linear method.

(a) comasion matrix table asing mean method	(a)	confusion	matrix	table	using	linear	method
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Confusion matrix	Actual	2019-VOO	PO	ROO	2020-VOO	HNO	SFO	SBO	CSO
Predicted	P	1	2	3	4	5	6	7	8
2019-VOO	1	116	0	0	8	0	0	0	0
PO	2	0	12	0	0	0	0	0	0
ROO	3	0	0	12	0	0	0	0	0
2020-VOO	4	4	0	0	112	0	0	0	0
HNO	5	0	0	0	0	18	4	1	0
SFO	6	0	0	0	0	5	16	4	0
SBO	7	0	0	0	0	1	0	15	0
CSO	8	0	0	0	0	0	0	0	20

(b) confusion matrix table using quadratic method

Confusion matrix	Actual	2019-VOO	PO	ROO	2020-VOO	HNO	SFO	SBO	CS0
Predicted	(<mark>P</mark>	1	2	3	4	5	6	7	8
2019-VOO	1	117	0	0	3	0	0	0	0
PO	2	0	12	0	0	0	0	0	0
ROO	3	0	0	12	0	0	0	0	0
2020-VOO	4	3	0	0	117	0	0	0	0
HNO	5	0	0	0	0	24	0	0	0
SFO	6	0	0	0	0	0	20	0	0
SBO	7	0	0	0	0	0	0	20	0
CSO	8	0	0	0	0	0	0	0	20

(c) classification table using linear method

Classified_10		2019-VOO	PO	ROO	2020-VOO	HNO	SFO	SBO	CS0	Class
	Â	1	2	3	4	5	6	7	8	9
V00-1_1	1	-15.8888	-99.2860	-155.7194	-16.0289	-556.8710	-593.7990	-508.1974	-136.1547	2019-VOO
V00-2_1	2	-27.0201	-73.7884	-100.9103	-27.3458	-453.1124	-484.8618	-407.2424	-86.0074	2019-VOO
V00-3_1	3	-13.7847	-98.5897	-152.0289	-17.6166	-553.6832	-589.5584	-506.2117	-136.4883	2019-VOO
V00-4_1	4	-13.6395	-110.9092	-197.8197	-10.7827	-639.9856	-683.9057	-594.0891	-182.7426	2020-VOO
V00-5_1	5	-17.4550	-82.5378	-124.3154	-17.6879	-500.9923	-535.3310	-453.7205	-107.0816	2019-VOO
V00-1_2	6	-24.9342	-106.3166	-162.7291	-20.9720	-559.1636	-593.6741	-510.4100	-136.2575	2020-VOO
V00-2_2	7	-34.5623	-80.5952	-108.4411	-33.9556	-463.5874	-496.2990	-414.0982	-87.7842	2020-VOO
VOO-3_2	8	-10.3962	-94.6839	-152.7195	-16.7950	-565.6174	-604.7118	-518.5331	-138.2840	2019-VOO
V00-4_2	9	-12.3858	-110.2329	-196.4586	-11.1124	-641.2426	-684.6413	-594.0394	-181.5555	2020-VOO
VOO-5_2	10	-25.4611	-89.1687	-131.2979	-23.1766	-507.0883	-540.6993	-457.9669	-107.7337	2020-VOO
V00-1_3	11	-22.2503	-114.4078	-168.2454	-25.2702	-572.3585	-608.4081	-524.4050	-151.2408	2019-VOO
VOO-2_3	12	-24.0188	-72.4917	-99.8882	-23.7314	-450.7796	-482.6427	-402.6150	-85.9139	2020-VOO
VOO-3_3	13	-11.1156	-92.0522	-154.3176	-17.6031	-571.0632	-610.4416	-522.2432	-139.5484	2019-VOO
VOO-4_3	14	-15.4923	-110.8553	-197.2793	-11.2657	-637.8456	-680.9884	-589.5623	-178.9628	2020-VOO
VOO-5_3	15	-19.2010	-89.5161	-130.1332	-20.5672	-507.6354	-541.5918	-459.5764	-114.6438	2019-VOO
V00-1_4	16	-21.1129	-106.6358	-161.5536	-18.8446	-561.3453	-597.8521	-513.1335	-138.4731	2020-VOO
V00-2_4	17	-26.7281	-73.4155	-102.1466	-24.9776	-455.1188	-488.8776	-405.8879	-85.3815	2020-VOO
VOO-3_4	18	-11.4297	-99.9146	-155.4653	-16.5220	-567.2271	-605.1464	-518.7245	-140.6809	2019-VOO
V00-4_4	19	-21.1524	-115.4346	-202.0804	-16.4397	-638.1722	-679.3530	-591.4450	-184.9135	2020-VOO
VOO-5_4	20	-20.8267	-86.9319	-128.7564	-18.8174	-505.1383	-540.2711	-456.4170	-108.8336	2020-VOO

The HCA models were constructed for the authentication of extra VOOs from two different harvest terms (2019 and 2020 October) in Mut (Mersin) region and other edible oils from different brands (Figures 2–10). The SyF data of the full wavelength region (200–800 nm) obtained with $\Delta\lambda$ =10 nm were used to construct unsupervised HCA models. As seen from the dendrograms, the samples coded VOO–4, VOO–5, VOO–6, VOO–7, VOO–8, VOO–9 and VOO–28 from the 2019 harvest term formed a different cluster from the other samples (Figure 2); while the samples coded VOO–16, VOO–18, VOO–24 and VOO–25 from the 2020 harvest term formed a different cluster from the other samples (Figure 3). In commercial edible oils, the samples coded VOO–4, HNO–5, SFO–2, SFO–4, SBO–2, and CSO–1 formed a different cluster from other samples in their origins (Figures 4–10). Other commercial edible oil samples were successfully clustered by constructing similar clusters. Therefore, it could be revealed that a combination of SyF spectroscopy and HCA offered a fast, effortless, and inexpensive approach for the authentication of 2019–VOOs, 2020–VOOs, and other edible oils.



Figure 2. HCA dendrogram graph for the 2019–VOOs samples ($\Delta\lambda$ =10 nm) recorded from the SyF analysis in the region of 200–800 nm.



Figure 3. HCA dendrogram graph for the 2020–VOOs samples ($\Delta\lambda$ =10 nm) recorded from the SyF analysis in the region of 200–800 nm.



Figure 4. HCA dendrogram graph for the commercial VOO samples ($\Delta\lambda$ =10 nm) recorded from the SyF analysis in the region of 200–800 nm.



Figure 5. HCA dendrogram graph for the commercial HNO samples ($\Delta\lambda$ =10 nm) recorded from the SyF analysis in the region of 200–800 nm.



Figure 6. HCA dendrogram graph for the commercial SFO samples ($\Delta\lambda$ =10 nm) recorded from the SyF analysis in the region of 200–800 nm.



Figure 7. HCA dendrogram graph for the commercial SBO samples ($\Delta\lambda$ =10 nm) recorded from the SyF analysis in the region of 200–800 nm.



Figure 8. HCA dendrogram graph for the 2020–VOOs samples ($\Delta\lambda$ =10 nm) recorded from the SyF analysis in the region of 200–800 nm.



Figure 9. HCA dendrogram graph for the commercial PO samples ($\Delta\lambda$ =10 nm) recorded from the SyF analysis in the region of 200–800 nm.



Figure 10. HCA dendrogram graph for the commercial ROO samples ($\Delta\lambda$ =10 nm) recorded from the SyF analysis in the region of 200–800 nm.

4. Discussion

The SyF spectroscopy method combined with multivariate LDA and HCA was developed to authenticate the extra VOOs harvested in Mut (Mersin) region and commercially available edible oils. The normalized emission spectra recorded from the 200-800 nm wavelength region were used to construct the qualitative models of supervised LDA and unsupervised HCA. A 98.28% of the samples could be discriminated with high accuracy on the basis of their origin by LDA model; whereas, the samples could be clustered by the HCA model. Thus, the findings demonstrate that authentication of the extra VOOs harvested in Mut (Mersin) and commercially available edible oil samples could be successfully realized by multivariate data analyses combined with SyF spectroscopy.

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