FINAL SCIENTIFIC REPORT

for the entire implementation period of the project **Novel biosensors and smart tools for ultrasensitive detection of olive oils adulteration, PN-III-P4-ID-PCE-2020-0923**

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- **Objectives foreseen/achieved**

The main objective of this project was the development of new electrochemical and optical biosensors and smart tools, integrated in a portable device, for the detection of adulteration of olive oils.

The project had the following specific objectives:

O1: development of new sensitive materials and suitable nanotechnologies for the development of biosensors;

O2: development and characterization of new biosensors useful for the detection of adulteration of olive oils;

O3: calibration and validation of biosensors for the ultrasensitive detection of compounds related to the adulteration of olive oils;

O4: integration of biosensors in a multiparametric portable device;

O5: development of a multivariate algorithm based on data fusion and intelligent tool models for the rapid recognition of adulteration of olive oils;

O6: demonstration and validation of multiparametric systems for detecting adulteration of olive oils;

O7: integration and training of students in the multidisciplinary team.

The results of the research activities carried out during the implementation period of the project, as well as their dissemination in ISI journals, indicate that the general objective and the specific objectives of the project have been fully met.

The general objective was achieved through the development and implementation of new electrochemical and optical biosensors specially designed for the ultrasensitive detection of olive oil-specific compounds, as well as markers present in adulterants. The data that originated from different biosensors was processed by developing and using advanced statistical models, and the information resulting from these models enabled the rapid and accurate detection of adulteration of olive oils at concentration levels between 1% and 5%.

The following table presents the planned deliverables/indicators, the deliverables/achieved results, as well as the degree of their achievement, which demonstrates the full achievement of the planned deliverables/indicators and thus, the 100% fulfillment of the foreseen specific objectives.

#	Deliverables/planned indicators	#	Deliverables/achieved indicators	Achievement degree
1	Chromatographic analysis of markers		Yes	100%
2	Development of new sensitive materials		Yes	100%
3	Synthesis of conducting polymers and molecularly imprinted polymers		Yes	100%
4	Development of a multi-sensor platform		Yes	100%
5	Characterization of biosensors in the presence of markers		Yes	100%
6	Development of biosensors arrays for the analysis of markers in olive oil		Yes	100%
7	Developing intelligent models and applying them to data analysis		Yes	100%

8	Validation of the multi-parametric system in the analysis of real samples		Yes	100%
9	Participating in conferences, publication in ISI journals, patent application	6 6 1	Yes, 26 Yes, 12 Yes, 1	100%
10	Acquiring activities		Yes	100%
11	Elaboration of scientific reports Report for stage 1 Report for stage 2 Executive summary for stage 1 and 2 Final report	 1 1 1 1	 Yes Yes Yes Yes	100%

- **Presentation of the obtained results, of the achieved results indicators; of the non-achievements recorded against the next estimate through the funding request (if applicable), with their justification;**

The project implementation process was carried out in three stages in accordance with the project implementation contract. Following, the main results obtained within the research activities and the result indicators achieved during the entire project implementation period are presented.

Task 1. Detection of target molecules and development of new sensitive materials

Task 1.1. Chromatographic analysis of markers

The oil samples investigated in the project were commercial oils, namely: olive oils (extra virgin olive oils from different producers, obtained by different technologies, from different olive varieties, from different countries as well as olive pomace oils), but also vegetable seed oils (corn, sunflower, linseed, hemp, rape etc.), which were analyzed by liquid chromatography, mass spectrometry and using different biosensors. Extra virgin olive oils (obtained from local producers in the Bari region, Italy), provided by Prof. Dr. Maria Lisa Clodoveo from the Carlos Moro University of Bari, as well as deodorized and refined olive oils, were also analyzed. The project also analyzed samples of adulterated oils, prepared in the laboratory from extra virgin olive oils and controlled additions from other vegetable oils. In total, more than 250 oil samples were analyzed, the data obtained being used for the construction and validation of the multiparametric system.

The present research aimed at a first stage to develop a targeted method for the quantification of compounds specific to vegetable oils and, in particular, olive oil, namely tyrosol, hydroxytyrosol, trigonelline, oleuropein, verbacoside, oleic acid and oleanolic acid by UHPLC -MS/MS (FullMS-vDIA), which was subsequently used to quantify the respective compounds in vegetable oil samples in order to discriminate them using principal component analysis (PCA) and cluster analysis (HCA) and classification using discriminant analysis (PLS-DA), artificial neural networks (ANN) and machine learning (ML). Also, the determination of minoritary phenolic compounds in oils was carried out by UHPLC-MS/MS, using the analysis method developed in step 1. HRMS data processing was carried out with the help of Compound Discoverer v. 2.0 software (Thermo Scientific, USA) using a working template for metabolomics, making it possible to identify other classes of compounds by reporting to databases including Chemspider, MzCloud, etc.

The determination of specific phenolic compounds (tyrosol, hydroxytyrosol, oleuropein), terpenic compounds (maslinic and oleanolic acids) and trigonelline in vegetable oils by UHPLC-MS/MS involves three important steps, namely: (i) sampling and extraction of oil samples (solid phase extraction - SPE); (ii) data acquisition in FullMS-vDIA mode, which was performed using the Q Exactive Focus high-resolution mass spectrometer (Thermo Scientific, USA) coupled to a UHPLC Dionex high-performance

liquid chromatograph for analyte separation; (iii) the processing and identification of data which was carried out with the help of the Xcalibur software. Also, by processing the spectral data with the software Compound Discoverer v. 2.0 (Thermo Scientific, USA), using a working template for metabolomics, it was also possible to identify other classes of compounds by reporting to databases, including Chemspider, MzCloud, MassBank, etc.

Isolation of the compounds of interest from the investigated vegetable oils was achieved by SPE extraction using SPE cartridges and a vacuum elution system (Vaccum Manifold, Varian). Three types of cartridges were used to optimize the extraction, namely: Strata NH2 cartridges (55 µm, 70 Å), 500 mg/6 mL, Strata Silica cartridges (55 µm, 70 Å), 500 mg/6 mL, and cartridges HyperSep silica 500 mg/6 mL, recovery studies performed showing better performance (75-98% recovery rate) for Strata Silica (55 µm, 70 Å), 500 mg/6 mL cartridges that were further used for the extraction of the majoritary phenolic compounds from vegetable oils. The SPE extraction protocol consists of: conditioning the SPE cartridges with 6 mL methanol and 6 mL hexane, followed by the addition of the sample solution (2.5 g oil in 6 mL hexane) and its penetration into the cartridge; washing the cartridges with 3x3 mL hexane and eluting the compounds from the cartridge with 10 mL methanol. The resulting sample solution is concentrated to dryness in a stream of nitrogen, after which the extract is resuspended with 1 mL methanol:ultrapure water = 80:20 solution, filtered through a 0.45 µm membrane and subjected to analysis.

For UHPLC-MS/MS detection, the following categories of operational parameters were optimized and subsequently set: HESI ionization parameters, chromatographic separation parameters (mobile phase composition, gradient, flow rate), MS operating parameters (Full acquisition scan followed by fragmentation). The separation of the analytes was carried out on an analytical Kinetex C18 column (100 × 2.1 mm, 1.7 µm), at 30 °C, under the action of a gradient of mobile phases: HPLC water with 0.1% formic acid and HPLC methanol with 0.1% formic acid. The mass spectrometer was run in H-ESI (negative) ionization mode at an applied voltage of 3.0 kV. The nitrogen flow was 48 L/min, and the auxiliary 11 L/min at a temperature of 413°C, capillary temperature of 320°C. Data were acquired in Full MS – vDIA mode with simultaneous recording of precursor and resulting MS/MS fragments. Mass spectral data were recorded in the scan range of m/z 100–1500, with full scan resolution at 70,000, and MS/MS acquisition resolution at 35,000. The step normalized collision energy (NCE) was set to 35 eV. Data processing was carried out using Xcalibur software, and data quantification was carried out using the external standard method, in a linearity range of 25-1000 ng/mL. The coefficient of linearity ranged from 0.990 to 0.995, while the detection limit of the methods was calculated based on a signal to noise ratio of 3:1. To evaluate the performance of the analysis method, the investigation of the matrix effect was followed by adding known concentrations of the standard at a concentration level of 100 ng/mL, followed by the analysis of the resulting samples and the estimation of the recovery percentage, the results obtained being between 75-98% . The identification of the compounds was carried out based on the comparison of the retention times with those of the reference substances and by the identification of the molecular ion and fragments resulting from fragmentation in the negative mode (Table 1).

Table 1. Identification of phenolic compounds in vegetable oils by UHPLC-MS/MS

Co					s
Trigc					
Hydr					
Tyro					.0230

Verbascoside	8.85	$C_{29}H_{36}O_{15}$	624.2054	623.1976	623.198
Oleur					
Olear					
Maslinic acid	18.08	$C_{30}H_{48}O_4$	472.3552	471.3474	471.3478; 472.3513

The content of specific majoritary phenolic compounds in the analyzed oils varies according to the oiling time, as follows: (i) in the original olive oils, the major quantified compounds are trigonelline with values between 0.834-22.514 mg/kg and hydroxytyrosol, with values between 0.008-24.582 mg/kg, while tyrosol, verbacoside and oleuropein showed lower values, respectively n.d.-2.553 mg/kg, 0.314-0.758 mg/kg and n.d.-0.243 mg/kg; (ii) higher amounts of trigonelline (5.774-34.062 mg/kg) and tyrosol (n.d.-4.363 mg/kg) were identified in EVOO compared to original olive oils, while the content of hydroxytyrosol, verbacoside and oleuropein is smaller; (iii) higher amounts of trigonelline (2.074-26.985 mg/kg) and tyrosol (n.d.-10.386 mg/kg) were identified in commercial olive oils compared to original olive oils, while the content of verbacoside and oleuropein it is alike; (iv) in the analyzed vegetable oils, among the majority compounds are trigonelline (n.d.-65.129 mg/kg), with higher concentrations in grape seed oil and rapeseed oil and tyrosol (n.d.-12.758 mg/kg), with higher concentrations high in walnut, flax and hemp oils.

The quantitative data resulting from the investigation of the minoritary phenolic compounds in the analyzed oils indicated that the main phenolic acids are hydroxybenzoic (HyB), dihydroxybenzoic (DHyB), p-coumaric (Ap-Coum), ferulic (AF), ellagic (Ael), cinnamic (Acin), but also flavonoids such as apigenin (Apg) and pinocembrin (PinoC), their content varying depending on the type of vegetable oil. Higher concentrations of dihydroxybenzoic acid correspond to olive oils (n.d.-0.118 mg/kg), while hydroxybenzoic acid was quantified in higher amounts in vegetable oils (n.d.-0.381 mg/kg), with higher values for olive oil walnuts and pumpkin seed oil. p-Coumaric acid was quantified in higher amounts in commercial olive oils (n.d.-0.806 mg/kg) and virgin olive oils (0.008-0.708 mg/kg), while higher amounts of ferulic acid (n.d.- 1.007 mg/kg) and ellagic acid (0.015-0.731 mg/kg) correspond to the other types of vegetable oils investigated. Cinnamic acid is predominant in olive oils, with values ranging from 0.023-4.832 mg/kg in original olive oils and n.d.-5.076 in commercial olive oils. Also, the content of apigenin and pinocembrin is higher in olive oils and extra virgin olive oils compared to the other types of vegetable oils.

Also, specific markers were identified in extra virgin olive oil such as: elenolic acid, including hydroxylated derivatives and aldehyde forms, oleacin and the hydroxylated derivative, derived from oleuropein, oleocanthal, ligstroside, elenaic acid.

Determination of sterols and triterpene diols in vegetable oils by RP-UHPLC-DAD involves the following steps: (i) sampling of oil samples; (ii) saponification of the sample with an ethanolic solution of KOH in order to isolate the unsaponifiable fraction; (iii) L-L extraction of the unsaponifiable fraction; (iv) drying and evaporation to dryness of the extraction solvent; (v) retaking the extract and filtering through the 0.45 µm filtering membrane; (vi) RP-UHPLC-DAD analysis.

The isolation of the unsaponifiable fraction from the oils represented by sterols and triterpene compounds was achieved by saponification, followed by L-L extraction. The extraction protocol is as follows: 1 g of the sample is subjected to saponification with 50 mL of 2M ethanolic KOH solution, at a temperature of 70 °C, under constant stirring. After cooling to room temperature, the mixture was transferred to a separatory funnel to extract the unsaponifiable fraction with 2 x 25 mL of hexane

(washing with shaking for 3 min each time). The hexane (containing the sterols) was dried with anhydrous sodium sulfate and evaporated to dryness using the Multivapor MP6 concentrator at 50°C. The residue was dissolved in 2 mL of methanol, then filtered through a 0.45 µm membrane and subjected to chromatographic analysis. The schematic representation of the extraction stage of sterols and triterpenic compounds from olive oils and seed oils is presented in Figure 1.

Figure 1. Schematic representation of the extraction of sterols and triterpene diols from vegetable oils before analysis by RP-UHPLC-DAD

Separation of compounds was achieved by reverse phase chromatography using a Prevail C18, 5 µm (15 × 0.4 cm i.d.) column and isocratic elution of the mobile phase methanol:acetonitrile 30:70 (v/v) at a flow rate of 1.0 mL/min. Detection was performed at a wavelength of 205 nm, and the injection volume was 25 µL.

Data processing was carried out using the Chromeleon software (Thermo Fisher Scientific), and the quantification of the results was carried out by the external standard method, in a concentration range between 1-100 mg/L. The coefficient of linearity ranged from 0.990 to 0.995, while the detection limit of the methods, which was calculated based on a signal to noise ratio of 3:1. To evaluate the performances of the analysis method, the investigation of the matrix effect by enriching the samples was aimed of oil with known concentrations of the standard at a concentration level of 10 mg/L, followed by the analysis of the resulting samples and the estimation of the recovery percentage, the results obtained being between 80-96%. The identification of the compounds was carried out based on the comparison of the retention times with those of the reference substances.

Table 2 shows the quantitative results regarding the content of sterols and triterpene diols in the analyzed oil samples.

Table 2. The content of sterols and triterpene diols in the analyzed oils

Oil	mg/100 g	Erythrodiol	Brassicasterol + Avenasterol	Cholesterol	Campestrol + Stigmasterol	Squalene	Sitosterol
P	Mi						0
	Ma						2
	Av						9
V	Mi						
	Ma						6
	Av						8
I	Mi						3
	Ma						2
	Av						5
E	Mi						1
	Ma						2
	Average	2.11	35.10	n.d.	n.d.	389.01	89.19

P-olive pomace oils, V – seed oils, I – extra virgin olive oils of Italian origin and E – commercial extra virgin olive oils, n.d. undetected.

The quantitative results obtained indicate that the compositional profile of sterols and triterpene diols determined in the analyzed oils varies according to the type of oil, as follows: (i) erythrodiol is present in larger quantities in olive pomace oils, with an average value of 38 .88 mg/100 g, compared to 2.30 mg/100 g in extra virgin olive oils from Italy, 4.48 mg/100 g in commercial extra virgin olive oils and 5.71 mg/100 g in oils from seeds; (ii) cholesterol was detected only in oils from olive pomace, with values in the range of 8.50-25.44 mg/100g; (iii) squalene is the majority compound quantified in oils, with higher values in extra virgin olive oils (155.46-469.80 mg/100g) and lower values in seed oils (n.d.-162.36 mg/100g).

Olive oil samples were also analyzed by IR spectroscopy, UV spectrophotometry and Vis spectrophotometry. To determine the compounds with antioxidant properties present in the oil samples, the Folin-Ciocâlteu method was used to determine the total content of polyphenolic compounds. Other ways of assessing the antioxidant activity were free radical scavenging methods. DPPH, ABTS and galvinoxyl free radical scavenging methods were used.

These results were used in the validation stages of the biosensors, in order to be able to appreciate the concordance between the responses of the biosensors and other types of physico-chemical analyses, but also the differences between the pure samples and those adulterated with other vegetable oils.

Task 1.2. Development of new sensitive materials

A series of new biosensors have been developed for the analysis of biomarkers in olive oil samples. For the construction of biosensors, screen-printed carbon-based electrodes (SPCE), indium tin oxide (ITO), gold (Au) and platinum (Pt) were used which were modified with different nanomaterials, peptides and enzymes. The nanomaterials used were carbon nanotubes, carbon nanofibers, mesoporous carbon, and graphene as such or functionalized with different types of hydrophilic groups: -OH, >C=O, -COOH, -$COONH_2$, etc. For example, manganese phthalocyanine, cobalt phthalocyanine, Prussian Blue or peptides were used as electron exchange mediators. A wide range of enzymes from the oxidase class were also used, for example tyrosinase, laccase, peroxidase, cholesterol oxidase.

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These screen-printed electrodes were used as a support for the immobilization of some nanomaterials to increase the sensitivity and selectivity of electrochemical sensors. Also, these sensors have been used for enzyme modification in order to increase sensitivity and selectivity. The nanomaterials used were carbon nanotubes functionalized with carboxyl or amide groups in order to favor the interactions between the enzyme and the immobilization matrix, without the need for the crosslinking step. Carbon nanofibers and graphene functionalized with hydroxyl groups were also used.

For the colorimetric biosensors, silica gel layers with and without enzyme (laccase or tyrosinase, a mixture of tetramethoxysilane and methyl-trimethoxysilane) were prepared and deposited on ITO screen-printed electrodes.

For example, in one study, the first step for constructing enzyme biosensors was to modify carbon screen-printed electrodes (SPE/C) with single-layer carbon nanotubes. This procedure was carried out

by dispersing on the active surface of the supporting electrode a volume of 10 μL of a suspension previously prepared from SWCNT. To prepare the SWCNT suspension, 10 mg of single layer carbon nanotube powder was dispersed in a solvent mixture consisting of dimethylformamide and ultrapure water in a 1:1 ratio. For optimal dispersion, the suspension was ultrasonicated for 30 minutes.

The optimal suspension volume (10 μL) was added to the surface of the SPE/C electrodes using an Eppendorf micropipette in two successive steps. After each step, the electrodes were dried in a desiccator at a temperature of 25 °C for 2 hours.

The second stage consisted in the construction of two types of enzymatic biosensors by modifying SPE/SWCNT with laccase and tyrosinase, respectively. The enzyme solutions were added onto the SWCNT/SPE surface by the casting technique, followed by cross-linking using glutaraldehyde vapors. After adding 5 μL of enzyme solution, the electrodes were kept in the desiccator for one hour for drying. Another 5 μL was added in the same way. Exposure to glutaraldehyde vapor was for 1 minute for each electrode. The cross-linking ensures a favorable connection of the SWCNT with the enzyme. However, a longer exposure time could decrease the enzyme activity due to changes in the three-dimensional structure of the heteroprotein. After immobilization, the biosensors were stored at 4 °C until use, a maximum of 72 hours. Figure 2 shows the stages of the preparation process of laccase and tyrosinase-based biosensors and the schemes of the reactions that take place between glutaraldehyde and the enzyme.

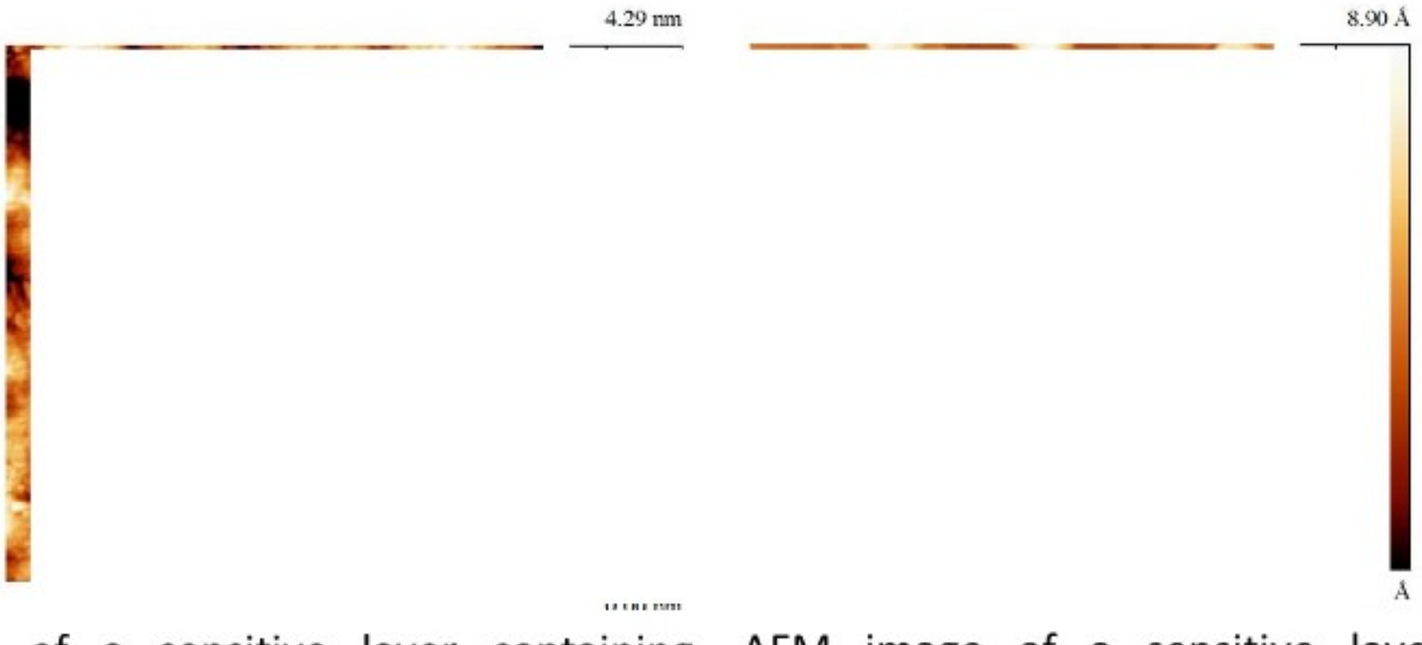
Figure 2. Preparation process of biosensors based on tyrosinase and laccase, respectively immobilized on screen-printed carbon electrodes modified with carbon nanotubes

The characterization of the deposited sensitive materials (of the active surface of the biosensors) was performed by scanning electron microscopy and atomic force microscopy. Electrochemical methods (cyclic voltammetry (CV), differential pulse voltammetry (DPV), electrochemical impedance spectroscopy (EIS) and spectrometric methods (FTIR) were also used. Some of the obtained results are presented below. The following figure shows the SEM images of some biosensors made, GPH-MnPc-Tyr/SPE (Figure 3) and OMC-Lac (Figure 4).

Figure 3. SEM image of the GPH-MnPc-Tyr/SPE biosensor surface

Figure 4. SEM image of the OMC-Lac biosensor surface

Figure 5 shows AFM images of the sensitive element of some biosensors made within the project.



AFM image of a sensitive layer containing tyrosinase and manganese phthalocyanine

AFM image of a sensitive layer - laccase deposited on mesoporous carbon

Figure 5. AFM images of the sensing elements

For optical biosensors, silica gel matrices with and without enzyme (laccase or tyrosinase) were prepared using a mixture of silanes, tetramethoxysilane and methyl-trimethoxysilane, forming a gel in the presence of H_2O and HCl followed by deposition on ITO screen-printed electrodes.

The procedure consisted of preparing a solution consisting of 1400 µL of tetramethoxysilane and 600 µL of methyl-trimethoxysilane, 500 µL of water, and 50 µL of 0.01 M HCl solution and mixing it slowly for one hour. The second solution prepared was the phosphate buffer solution of pH 5 (for laccase) or 7 (for tyrosinase) and 0.1 M concentration containing 5 mg/mL enzyme. Then equal volumes of the two solutions are quickly mixed and then 20 µL of the mixture is deposited on the ITO electrode and after gel formation (5 minutes) the electrode is placed in phosphate buffer solution of pH 6.5 and concentration 0.1 M and store at 4 °C until use. UV-Vis spectra were recorded for the phenolic compounds and the biosensor before and after the biocatalytic interaction using a quartz cuvette containing 3 mL of 0.1 M phosphate buffer solution of pH 5 (for laccase) or 7 (for tyrosinase).

Next, the results obtained from the catechol analysis for the laccase-based biosensor are presented. Following the enzyme-substrate interaction, a decrease in absorbance at 275 nm and the appearance of an absorption band at 400 nm can be observed, which can be used to monitor the catechol oxidation process biocatalyzed by laccase. Figures 6 and 7 show the spectrum of catechol before and after the interaction with laccase from the sensitive layer of the biosensor and the calibration curve of the optical biosensor based on laccase versus catechol obtained by monitoring the absorbance at 400nm.

Figure 6. Absorption spectrum of catechol (red line) and reaction product (blue line) obtained in the laccase-catalyzed reaction.

Figure 7. Laccase vs. catechol biosensor calibration curve obtained by monitoring absorbance at 400nm

Table 3 shows the main optical biosensors based on silica gel made in this project that differ in the enzymes used, the target compounds and the limits of detection (LOD).

Table 3. Optical biosensors developed in the project

Enzyme	Analyte	LOD/μM
Ty		
La		
Ty		
La		

It can be seen that optical biosensors generally have higher detection limits compared to electrochemical sensors, so they are less sensitive to the detection of markers in olive oil.

Task 1.3. Synthesis of conducting polymers and molecularly imprinted polymers

Synthesis of conducting polymers and molecularly imprinted polymers

In order to make new electrochemical sensors, electro conducting organic polymers were deposited using monomers derived from thiophene (3,4-ethylenedioxythiophene (EDOT), hydroxymethyl-EDOT) and pyrrole in the presence of different dopant anions (potassium hexacyanoferrate (II), perchlorate of lithium, sodium sulfate, Prussian Blue, etc.), some of them with a dual role, both doping agents and electron exchange mediators, an important process during electrochemical detection. Thus, innovative electrochemical sensors based on conductive polymers doped with different anions were obtained that can successfully detect markers in olive oil. In Table 4, the manufacturing method of the sensors is presented centrally.

Table 4. Experimental conditions for obtaining conductive polymers

Monomer	c (M)	Doping agents	Electropolymerization	Electrolyte solutions used for
E				
F				
F				
		$Fe_4[Fe(CN)_6]_3$	0.8 V; 75 s	

Polymer layers electrosynthesized by chronoamperometry in the presence of different doping agents were characterized by SEM and electrochemical. Figure 8 shows SEM images of conductive polymer layers obtained by electropolymerization.

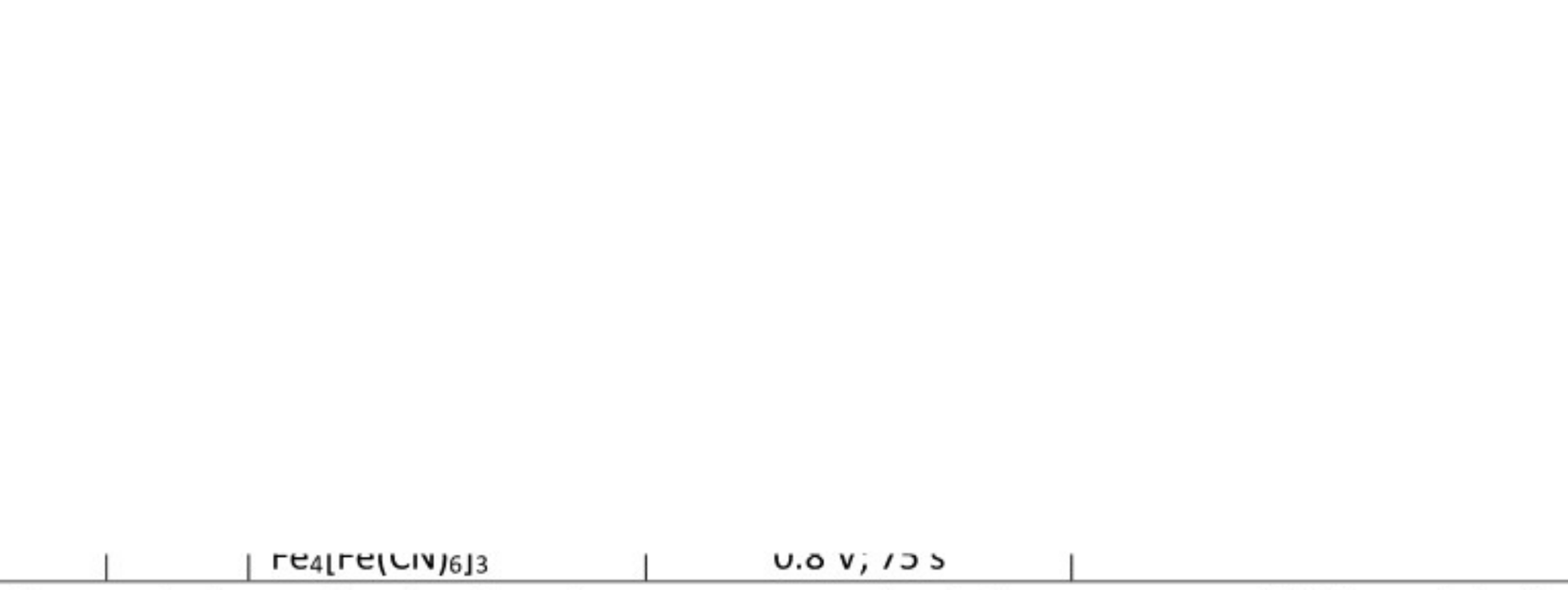


Figure 8. SEM images of sulfate ion-doped PEDOT (left) and sulfate ion-doped PPy (right)

The electrochemical responses of sensors based on conducting polymers varied depending on the nature of the polymer and the nature of the dopant. Thus, Figure 9 shows the responses of sensors based on PPY/FCN and PEDOT/FCN immersed in 0.1M KCl solution. Redox peaks related to the conducting polymer and ferrocyanide doping ion (FCN) and peaks due to it are observed.

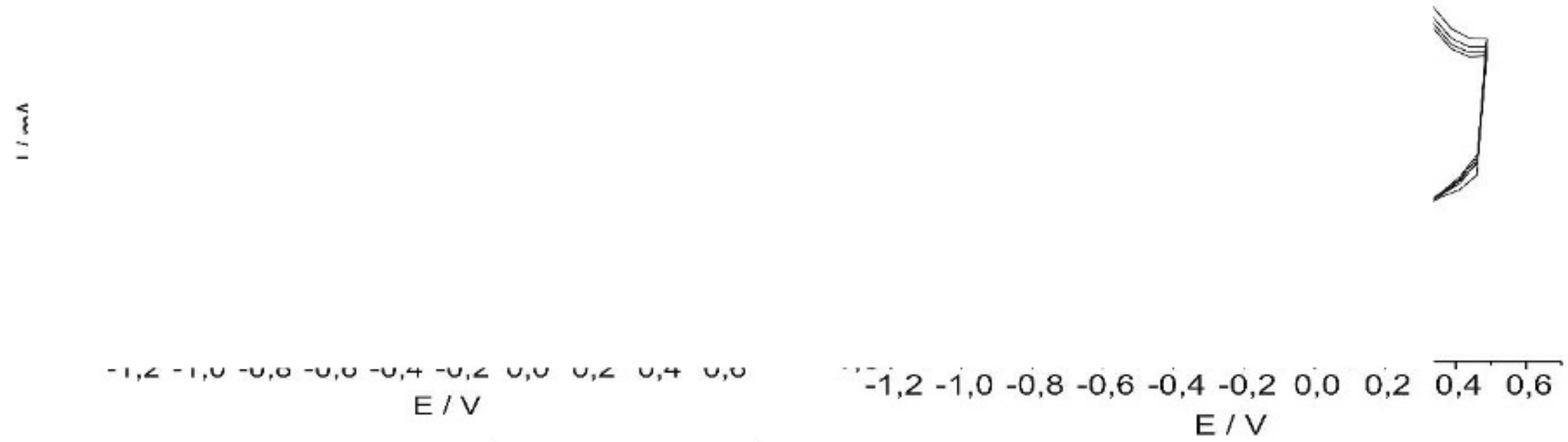


Figure 9. Sensor signals of PPY/FCN and PEDOT/FCN sensors immersed in 0.1 M KCl solution during voltammetric signal stabilization process.

The obtained sensors can be used as such to obtain the chemical fingerprint of olive oils or they can be transformed into biosensors by immobilizing some enzymes, with a biocatalytic role in the determination of markers in olive oil. Biosensors with good sensitivity and selectivity for olive oil biomarkers were thus obtained.

Figure 10 shows the cyclic voltammograms of the PPY/FCN/Tyr biosensor when immersed in tyrosol solution of variable concentration and the corresponding calibration curve.

Figure 10. Cyclic voltammograms of PPY/FCN/Tyr immersed in tyrosol solution of variable concentration; b) Calibration curve

The main biosensors based on conducting polymers, the enzymes used, the target compounds and the limits of detection (LOD) are presented in Table 5.

Table 5. Biosensors based on conductive polymers

Sensor	Enzyme	Analyte	LOD/nM
PEDOT/FCN	Tyrosinase	Hydroxytyrosol	0.045
PE			
PH			
PH			
PF			
PF			
PF T/T B	Laccase from *Agaricus bisporus*	Oleuropein	0.059

For the synthesis of molecularly imprinted polymers, acrylic acid and methacrylic acid were used as monomers, and oleuropein, tyrosol, hydroxytyrosol, verbascoside and trigonelline as target molecules. The procedure is similar for the two monomers and the crosslinking agent and initiator were ethylene glycol dimethyl acrylate and benzoyl peroxide for all cases. Next, the process carried out to obtain sensors of this type is presented.

0.02 g of acrylic acid (monomer) and 0.02 g of trigonelline (template molecule) were dissolved in 20 mL of ethanol and the mixture was ultrasonicated for 30 min. In another beaker, 400 µL of ethylene glycol dimethyl acrylate (crosslinker) and 0.0050 g of benzoyl peroxide (initiator) were dissolved in 5 mL of ethanol and the solution was left to stand for 30 min. The two solutions are mixed and then shaken vigorously for 1 hour at 25 °C. Afterwards, the mixture is heated to 50 °C for 90 min to complete the polymerization process. The molecular polymer-trigonelline adduct obtained in solid form is repeatedly washed with ethanol until the trigonelline template molecule is completely removed from the adduct. The molecularly imprinted molecular polymer is dried and stored cold in the absence of light.

The molecularly imprinted polymer thus obtained is dispersed in ethanol by ultrasonication and then deposited on the surface of screen-printed carbon electrodes, thus obtaining selective molecularly imprinted polymer-based sensors for the detection of markers in olive oil.

Figure 11 shows the responses of the C/polyacrylate/Hydroxytyrosol sensor to increasing amounts of hydroxytyrosol recorded by DPV and calibration curve. The sensor has adequate selectivity and sensitivity for the determination of hydroxytyrosol in complex samples.

Figure 11. Responses of the sensor in hydroxytyrosol solution in the range 50-300 nM and the calibration curve

Table 6 shows the main sensors based on molecularly imprinted polymers developed in this project and their detection limits.

Table 6. Sensors based on molecularly imprinted polymers

Sensor	LOD/nM	Sensor	LOD/nM
C/p			
C/p			
C/p			

Through this method, molecularly imprinted polymer-based sensors with biomarkers from olive oil were obtained, which can be successfully used for the detection of additions of other oils in olive oil. The best results were obtained with trigonelline.

The results obtained in Task 1 are consistent with the project objectives, the specific activities have been achieved 100% and the general and specific objectives have been fully achieved.

Task 2. Development and characterization of biosensors

Task 2.1. Development of a multi-sensor platform

In the project, a series of activities were carried out to create a modular multisensor system, which would include several measurement possibilities using different detection techniques: amperometry, voltammetry, potentiometry and conductometry. Also, the way of integration and simultaneous measurement with colorimetric biosensors using UV or Vis spectrometry was studied. The possibility of performing the measurements simultaneously or successively, what the design of the electrochemical cell should be and what amount of sample is minimally necessary to perform the measurements were evaluated.

Task 2.2. Characterization of biosensors in the presence of markers

The biosensors prepared and characterized morphologically, spectrometrically and electrochemically in the previous steps were used for the detection of some biomarkers in olive oil.

Next, the studies carried out for the electrochemical detection of hydroxytyrosol with 3 new biosensors based on lutetium phthalocyanine and tyrosinase, peroxidase or laccase are presented.

The role of the electron exchange mediator in the electrochemical detection of hydroxytyrosol was highlighted by recording cyclic voltammograms in a 10^{-4} M hydroxytyrosol and 10^{-1} M KCl solution, shown in Figure 12.

Figure 12. Cyclic voltammograms of biosensors in 10^{-4} M hydroxytyrosol and 10^{-1} M KCl solution

It is obvious that the sensitivity of the biosensor is greatly increased due to the mediator effect of $LuPc_2$, which facilitates the electron transfer between the redox reaction of hydroxytyrosol and the sensitive surface of the biosensor. The mechanism of the quasi-reversible redox reaction of hydroxytyrosol is shown in the following scheme and involves the exchange of 2 electrons and 2 protons.

For the most sensitive determination of hydroxytyrosol, the experimental conditions were optimized, the pH of the solution to be analyzed, the buffer solution and the ionic strength, temperature, etc.

To determine the sensitivity of the biosensor, the calibration curve was made by recording the cyclic voltammograms in hydroxytyrosol solution of different concentrations, in the range of 2-200 µM. The cyclic voltammograms obtained are presented in Figure 13.

Figure 13. Cyclic voltammograms of the tyrosinase-based biosensor in hydroxytyrosol solutions of different concentrations

From the graphical representation of the anodic and cathodic peak currents as a function of the analyte concentration, good linearities were obtained, with correlation coefficients greater than 0.95 (Figures 14 a and b).

Figure 14. a) Calibration line corresponding to the anodic peak for hydroxytyrosol; b) Calibration curve corresponding to the cathodic peak for hydroxytyrosol

Good detection limit values were obtained for both peaks, 1.36 μM for the cathodic process and 2.38 μM for the anodic peak.

It was determined that the kinetics of the cathodic process, which corresponds to the reduction of the orthoquinone derivative of hydroxytyrosol formed in the enzymatic reaction, is of the Michaelis-Menten type. The obtained results, K_M (66.77μM) and I_{max} (24.22μA) demonstrate that the enzyme immobilized in the biosensor retains its activity, has a good affinity towards the substrate and therefore the biosensor has high sensitivity.

The results obtained for the three biosensors in this study are presented in Table 7.

Table 7. Biosensor sensitivity data and enzyme kinetics parameters

Enzyme	Oxidation peak		Reduction peak		Michaelis-Menten parameters	
	E (V)	LOD(μM)	E (V)	LOD(μM)	K_M(μM)	I_{max}(μA)
Ty						
Pe						
l						

Below are presented, as an example, the studies carried out for the electrochemical detection of oleuropein with two biosensors based on tyrosinase and laccase, respectively. In the first step, the electrochemical behavior of oleuropein was studied using the two newly developed biosensors,

SPE/SWCNT/Lac and SPE/SWCNT/Tyr, by means of CV and square wave voltammetry (SWV). A 10^{-4} M solution of oleuropein was prepared for each biosensor, with pH 5.0 (for Lac) and pH 7.0 (for Tyr). In both situations, well-defined peaks corresponding to the oxidation and reduction of oleuropein are observed, the differences being due to the different biocatalytic effect of the two enzymes.

To create the calibration curve, the square wave voltammograms of the two biosensors were recorded in oleuropein solutions in the concentration range 0.01 μM-28.62 μM. Table 8 shows the linear regression equations used to calculate the limits of detection and quantification.

Table 8. Linear dependence equation, R^2, LOD and LOQ for the two biosensors

Biosensor	Sensibility	R^2	LOD (M)	LOQ (M)
SP				
SP				

SPE/SWCNT/Tyr is notable for lower detection and quantification limits, thus confirming the very good electrocatalytic properties of this biosensor.

Table 9 shows the main biosensors made in the project, the target compounds and the detection limits.

Table 9. Biosensors based on nanomaterials and enzymes

Sensitive material	Enzyme	Analyte	LOD/nM
M			
C n			
M fu			
M fu			
C			
Si fu			
G			
C			
C n			
C			
O			
G			
G pl			
C			
C			
C		acid	

Most of the biosensors made in the project proved to be suitable for the construction of the biosensor network for the determination of adulteration of olive oils. Eight biosensors, with the best characteristics and different selectivities, were chosen to build the network of biosensors to determine the adulteration of olive oils: PEDOT/FCN/Tyrosinase, PPY/FCN/Tyrosinase, C/polyacrylate/Trigonelline, Carbon nanofibers/nanoparticles of gold/Cholesterol dehydrogenase, Single-wall carbon nanotubes, functionalized with carboxyl groups /Lacase, Carbon /Cobalt phthalocyanine/Lacase, Graphene oxide /Lacase, C/polymethacrylate/Oleuropein.
The conclusion obtained based on the results obtained was that for a higher reliability of the system, the electrochemical detection technique (amperometry and voltammetry) should be used, the measurements should be carried out successively and the electrochemical cell should have the smallest possible volume.

Task 2.3. Development of biosensor networks for the analysis of markers in olive oil
For the analysis of pure or adulterated olive oils using electrochemical biosensors, several procedures have been carried out for the extraction of the polar fraction: extraction with methanol-water, extraction in HCl solution, emulsions with Triton x-100 and eutectic solvents. In all cases, good electrochemical signals were obtained with electrochemical sensors or biosensors. However, the methanol-water extraction method proved to be the simplest to perform experimentally, the extract can be analyzed with both biosensors and HPLC, and therefore this method was used for the pre-treatment of oil samples in order to analysis with the multiparametric system.

For the design of the biosensor network that includes the biosensors with the best analytical performance and cross-selectivity for the simultaneous or sequential analysis of olive oil samples, several constructive variants were considered. After a thorough analysis, it was decided to use a system that includes 8 different biosensors, each equipped with a counter electrode and a reference electrode. By means of a multiplexer each three-electrode system is sequentially activated and the electrochemical signals with each biosensor are recorded.
The 8 biosensors can be part of the same alumina plate or each one can be individual, and the connection to the potentiostat is made through a cable for control and data acquisition. Figure 15 shows the design of the multibiosensor system as well as the individual electrochemical cell (capacity 100 μL) for each biosensor.

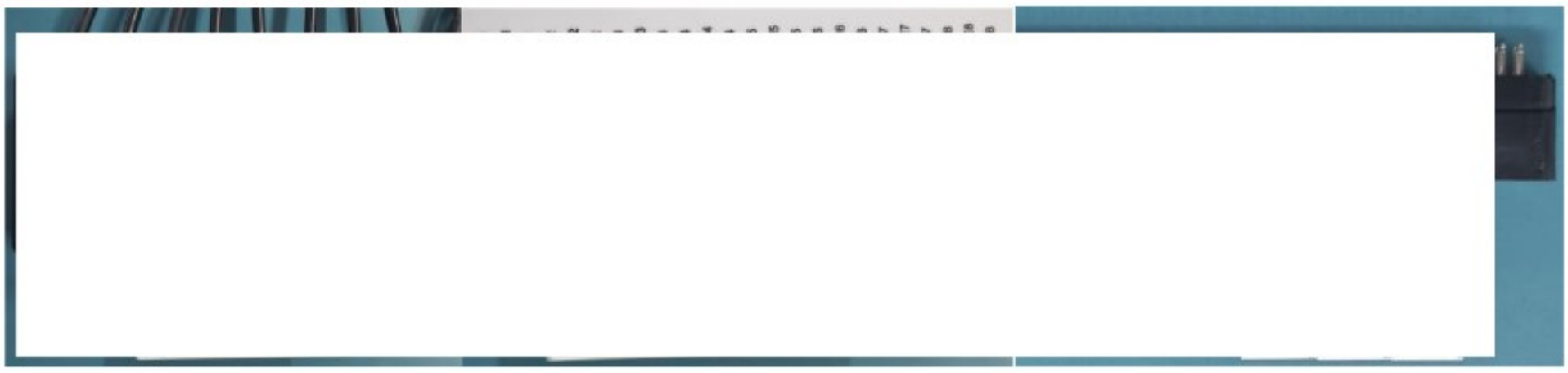


Figure 15. The array of 8 biosensors on the same alumina plate, the electrochemical cell for each biosensor and the individual biosensor system

After the biosensor signals are recorded they are processed to determine the concentration of the markers and/or the purity of the oil samples. The portability of the system is ensured by the use of a portable potentiostat/galvanostat and electrochemical biosensors, liquid/liquid extraction of the polar fraction in methanol/water oil and a maximum analysis time of 5 minutes for a sample.

The specific activities provided for in Task A2 have been completed in full, and the degree of achievement of the objectives is 100%.

Task 3. Developing intelligent models and applying them to data analysis

In this activity, intelligent models were developed by applying multivariate data analysis methods from different types of measurements made for samples of olive oils (extra virgin or from olive pomace), other vegetable oils such as sunflower oil, corn oil, canola oil, soybean oil, peanut oil, pumpkin oil, grape seed oil, coconut oil, sesame oil, palm oil and mixtures thereof. The oil samples were analyzed individually and as mixtures of extra virgin olive oil and other oils in different proportions to train the systems and determine the detection limits. The models were made using several methods, among which it can mention principal components analysis (PCA), heatmap analysis (HMA), discriminant analysis solved by partial least squares (PLS-DA), soft and independent class analogy modeling (SIMCA) and partial least squares regressions (PLS1 and PLS2). In addition, classification models based on artificial neural networks (ANN) and machine learning (ML) were developed.

For example, PCA and HMA methods have been used to differentiate extra virgin olive oils from other vegetable oils based on targeted and untargeted high resolution mass spectrometry (HRMS) data for the determination of phenolic, triterpene and other biologically active compounds. Figure 16 shows the distribution of vegetable oils in the PCA scores PC1-PC2 plot.

Figure 16. PCA results (scores and loadings) of different vegetable oils based on: (A) targeted HRMS analysis of biomarkers phenolic compounds and triterpenic acids and (B) non-targeted HRMS screening analysis. (EVOO*-extra virgin olive oil of Italian origin; EVOO-commercial extra virgin olive oil; VOO-commercial virgin olive oil; SF-sunflower oil; GS-grape seed oil; P-pumpkin oil; L-linseed oil; Se - sesame oil; He - hemp oil; Rp - rapeseed oil; W - walnut oil; P-palm oil; R-rice oil; A-almond oil; CN- coconut oil and So - soybean oil).

PCA indicated a clear discrimination between olive oils (EVOO*, EVOO and VOO) and other vegetable oils, but no clear discrimination was observed between the different seed and nut oils, probably due to the very large variety of vegetable oils analyzed .

For HMA the studied oil samples were grouped into two main groups, cluster C1 corresponding to olive oils (EVOO*, EVOO and VOO), and cluster C2 corresponding to oils obtained from seeds and nuts. In addition, the quantified variables were grouped into two groups: G1, which groups variables representative of olive oils, and group G2, which includes phenolic compounds representative of seeds and nuts. The obtained results are shown in Figure 17 A and B.

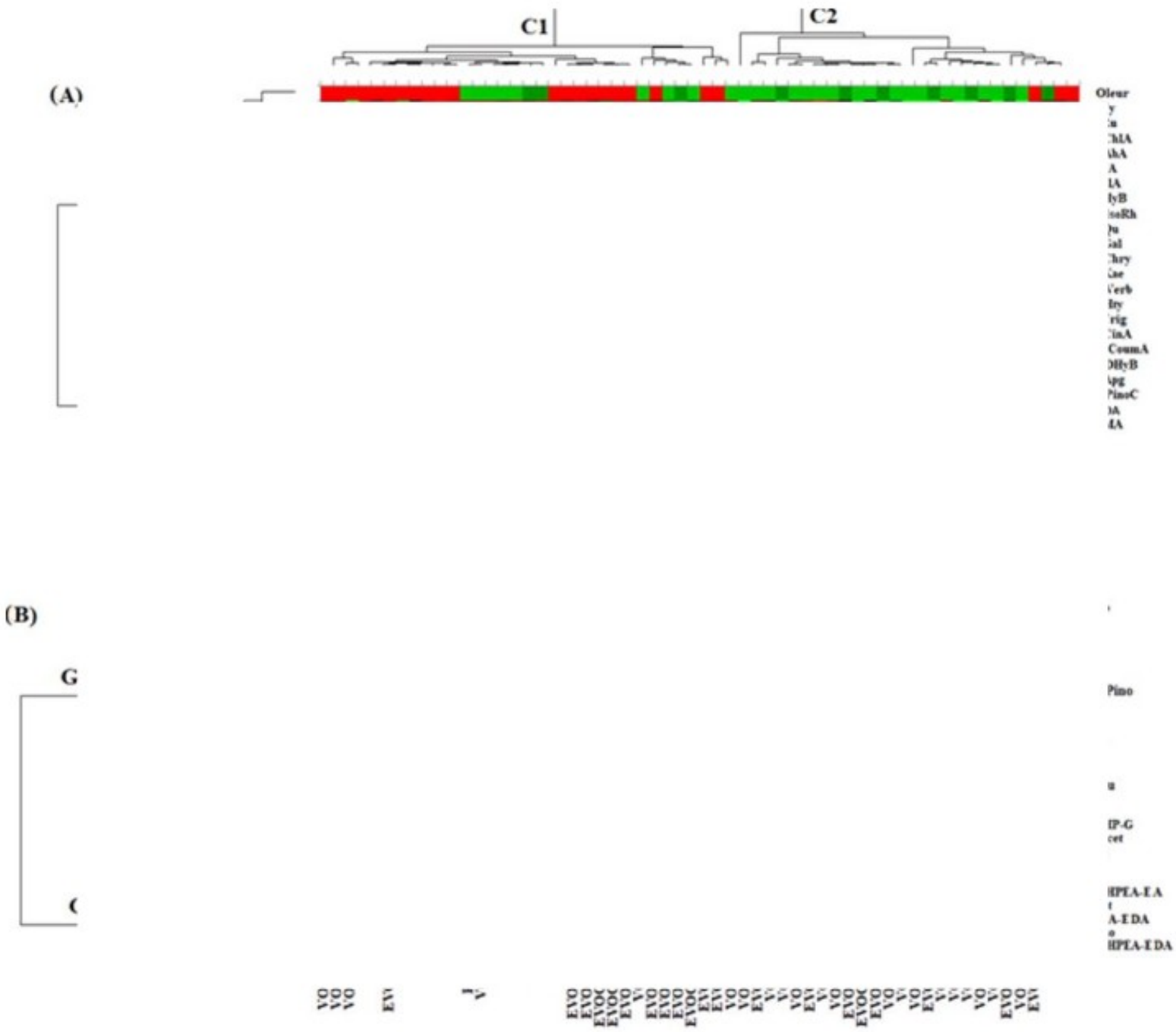


Figure 17. Heatmap of discriminating features according to the different types of vegetable oils analyzed (red and green cells correspond to low and high levels of compounds in the samples, respectively. Columns are oil samples and rows are determined compounds, colored according to their weight in the different types of oil). (A) Targeted HRMS analysis of phenolic compounds and triterpenic acids and (B) untargeted HRMS screening analysis. Color scale: red (higher values) to green (lower values).

To establish the correlations between the signals obtained with the biosensors and the results obtained by HPLC, multivariate regression models were made for the specific phenolic compounds in extra virgin olive oils. Figure 18 shows the graph of the correlation between the oleuropein content practically determined by HPLC and that predicted by the multibiosensor system from the PLS2 regression model.

Figure 18. Correlation between oleuropein content practically determined by HPLC and that predicted by the multibiosensor system

It is observed that the values predicted by the biosensor system are close in value to those obtained experimentally, with correlation coefficients greater than 0.96 in both calibration and validation.

In A3, the specific activities were carried out in full (100%) and the data analysis methods were selected and optimized and discrimination, classification and regression models were created.

Task 4. Validation of the multi-parametric system in the analysis of real samples

The system consisting of 8 biosensors with the best performance was validated by analyzing samples of commercial extra virgin olive oils and samples of extra virgin olive oils provided by the University of Bari as well as samples adulterated at laboratory level with refined olive oil, with deodorized olive oil, with olive pomace oils, with sunflower, corn, soybean, rapeseed, walnut, grape seed and flax oils. The added oil concentrations in the extra virgin olive oil samples were 1%, 2% and 5%. The preparation of the samples was carried out by weighing the oils and their homogenisation was carried out by using vortex type shakers.

Pure and adulterated samples were analyzed by HPLC for the identification of phenolic and steroid biomarkers, by IR spectrometry, UV-Vis spectrophotometry and with the multibiosensor system.

The obtained data were used to create classification models and PLS2 regression models to evaluate the correlations between the data obtained with biosensors and those obtained by spectrometry and HPLC. For the classification of adulterated oil samples at the laboratory level, classification methods such as PLSDA, SIMCA, neural networks - multilevel perceptron and machine learning - were used. The use of other classification methods such as support vector based classifiers, decision trees, nearest neighbor and Bayesian networks has also been studied. As the main result of the classification methods, the confusion matrix, also called the misclassification table, which is a representation of statistical classification accuracy, was obtained and analyzed.

The results obtained in the case of extra virgin olive oil samples adulterated with other oils with adulterant oil concentrations of 1%, 2% and 5% are presented below (Table 10).

Table 10. The oil samples used for the validation of the multibiosensor system

Extra virgin olive oil	Adulterant oil
B F II T D C F	;rape seed, linseed, ıut oil
	costa d'Oro, Il Biologico	

All samples were analyzed in triplicate with the biosensor system, and below are presented some of the results obtained when discriminating or classifying pure or adulterated oil samples using the voltammetric signals of the biosensors.

In the first stage, the data obtained with the sensors were analyzed for the analysis of oil extracts from pure oils (6 samples of extra virgin olive oils, 6 olive pomace oils, 3 refined olive oils, 3 partially deodorized olive oils , 2 corn oils and 6 sunflower oils) (Figure 19).

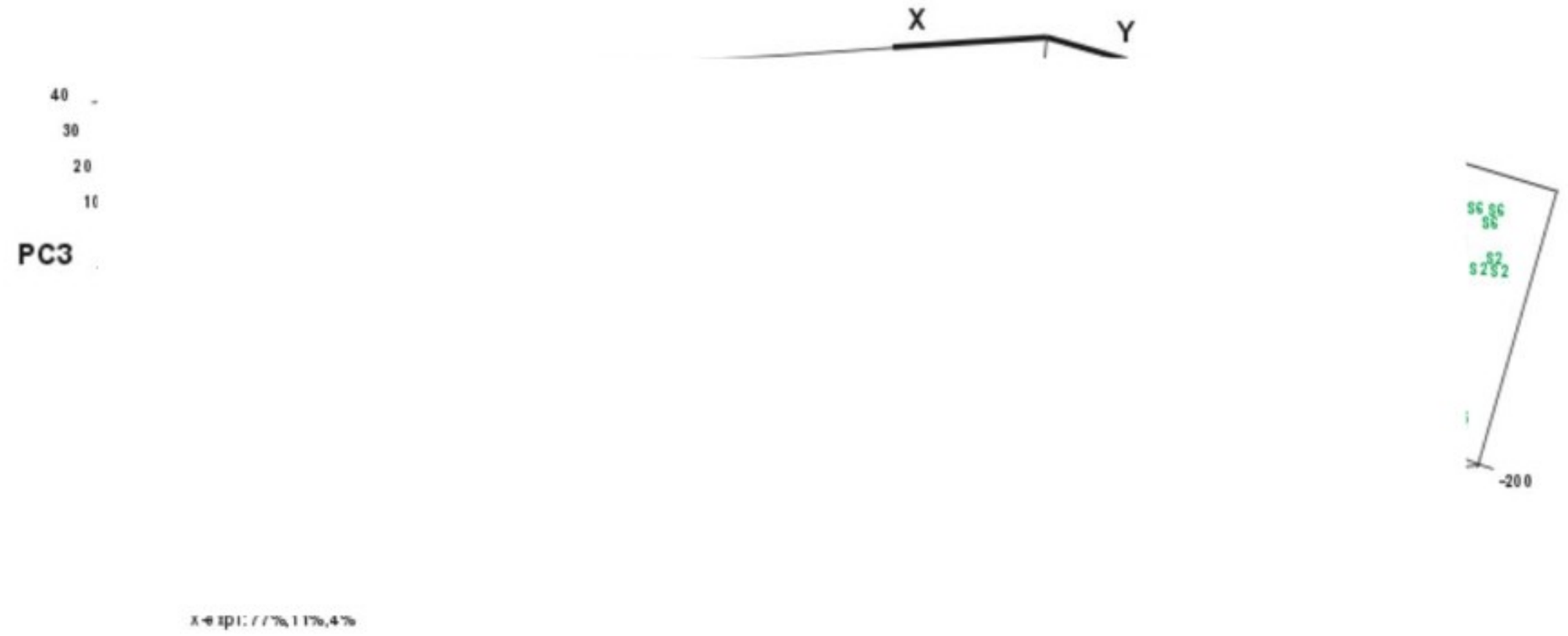


Figure 19. Scores graph of PCA regarding the discrimination of oil samples from vegetable oils

The PCA scores plot illustrates that the biosensor system is able to differentiate the oils according to the vegetable source (olive, sunflower, corn) and within the same production source they are separated by categories (extra virgin, pomace, refined, deodorized). Discriminant analysis, PLS-DA, of samples of extra virgin olive oils adulterated with corn or sunflower oils, on the one hand, and refined or deodorized pomace olive oils, on the other hand, led to the following results, presented in Table 11.

Table 11. Quantitative data of PLS-DA for the classification of extra virgin olive oil samples and those adulterated with other vegetable oils in percentage of 5%.

The type of oil	Calibration				Validation			
	Slope	Offset	R_c	RMSEC	Slope	Offset	R_p	RMSEP
Ex								78
Ex pc								31
Ex oli								33
Ex de								51
Ex oi								41

R_c—correlation coefficient at calibration; R_p— correlation coefficient at validation; RMSEC— root mean square error of calibration; RMSEP— Root Mean Squared Error in Validation.

It can be appreciated that adulterated oil samples are differentiated from pure oils at adulterant concentration levels below 5% with good precision with correlation coefficients greater than 0.9 and small errors, both in calibration and validation.

To apply the SIMCA classification method, PCA models were made for the pure oils and the adulterated samples were used as test samples. According to the results presented in the form of Coomans plots,

the adulterated samples are formed in the form of clusters separated from those of the pure oils. As seen in Figure 20, extra virgin oil samples adulterated with olive pomace oils are statistically different from pure ones at 1% concentration levels.

Figure 20. Coomans plot of SIMCA regarding the classification of extra virgin oil samples adulterated with 1% olive pomace oils.

Table 12 presents some of the results obtained from the classification of oil samples analyzed with the SIMCA method.

Table 12. Sample classification table by the SIMCA method

Model	Sample analyzed	Correctly classified samples / %	Incorrectly classified samples / %
Extra virgin oils (EV) / olive pomace oils	EV	100	0
Ext oils			
Ext oliv			
Ext (S)			
	S/EV 5%	100	0

It can be concluded that SIMCA models provide a correct classification of oil samples according to the degree of adulteration if the adulterant concentration is in the range of 2-5% for low quality olive oils and 2% if the adulterant oil is corn or sunflower.

It is therefore necessary to develop more complex models to accurately classify adulterated samples at 1% concentration levels. The application of classification methods neural networks - multilevel perceptron and learning machine - machine learning led to the following results, presented in the form

of classification tables. To validate the models, the total crossover method or the test group method was used, using three groups, two being the pure oils and the third the adulterated oils at the same concentration level (1%, 2%, 5%).

The machine learning algorithm classification is presented in tables 13, 14, 15 for the samples of extra virgin olive oils, olive pomace oils and extra virgin olive oils containing 1% olive pomace oils.

Table 13. Summary of the machine learning classification model

Cor	148	98.6667 %
Inc		
Kap		
Me		
Roo		

Table 14. Detailed accuracy by the class of the samples

Sample	TP	FP Rate	Precision	Recall	F	MCC	ROC	PRC
E								
A								
O.. from olive pomace	1.000	0.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 15. Confusion matrix

Samples			Samples classified as
E			
			il
			%
			Oil from olive pomace

It can be seen that the oil samples are correctly classified except for 2 extra virgin olive oil samples, which proves the very good accuracy of the multibiosensor system.

The same data set was used to perform the classification using neural networks - multilevel perceptron and the results obtained are presented in Table 16.

Table 16. Summary of the classification model neural networks - multilevel perceptron

C		%
In		
sa		
K		
M		
R		

Classification models performed with classifiers based on support vectors, decision trees, nearest neighbor and Bayesian networks mostly led to 100% correct classification of all oil samples, both pure and adulterated (Table 17).

Table 17. Data summary of classification models

	Support vectors	Random forest	Nearest	Bayesian networks
Cc				
In				
sa				
Ka				
M				
Root mean squared error	0.2722	0.0551	0.0692	0.0943

The correlations between the electrochemical signals of the biosensors and the results of other analyzes (HPLC, FTIR, UV-Vis) determined from PLS2 multivariate regression models by were very good,

the best results being obtained for phenolic biomarkers, with correlation coefficients greater than 0.95 for both calibration and validation.

From these results it can be concluded that the electrochemical data obtained with biosensors can be successfully used to determine the adulteration of olive oils with lower quality olive oils and vegetable oils at concentrations of 1% adulterant oil in extra virgin olive oil.

Within Task 4, the activities provided for in the project were completed in full (100%). The multisensor system was validated on pure and adulterated oil samples.

Task 5. Participation in conferences, publication in ISI journals, submission of patent application

Articles published in Clarivate Analytics journals:

1. Munteanu, I.G.; Apetrei, C. Analytical Methods Used in Determining Antioxidant Activity: A Review. International Journal of Molecular Sciences 2021, 22, 3380. IF 5,923. https://doi.org/10.3390/ijms22073380

2. Bounegru AV, Apetrei C. Laccase and Tyrosinase Biosensors Used in the Determination of Hydroxycinnamic Acids. International Journal of Molecular Sciences 2021; 22(9):4811. IF 5,923. https://doi.org/10.3390/ijms22094811

3. Bounegru, A.V.; Apetrei, C. Evaluation of Olive Oil Quality with Electrochemical Sensors and Biosensors: A Review. International Journal of Molecular Sciences, 22, 12708. IF 5,923. https://doi.org/10.3390/ijms222312708

4. Bounegru, A.V.; Apetrei, C. Studies on the Detection of Oleuropein from Extra Virgin Olive Oils Using Enzymatic Biosensors. International Journal of Molecular Sciences 2022, 23, 12569. IF 6,208. https://doi.org/10.3390/ijms232012569

5. Munteanu, I.G.; Grădinaru, V.R.; Apetrei, C. Sensitive Detection of Rosmarinic Acid Using Peptide-Modified Graphene Oxide Screen-Printed Carbon Electrode. Nanomaterials 2022, 12, 3292. IF 5,719. https://doi.org/10.3390/nano12193292

6. Dăscălescu, D.; Apetrei, C. Development of a Novel Electrochemical Biosensor Based on Organized Mesoporous Carbon and Laccase for the Detection of Serotonin in Food Supplements. Chemosensors 2022, 10, 365. IF 4,229. https://doi.org/10.3390/chemosensors10090365

7. Bounegru, A.V.; Apetrei, C. Sensitive Detection of Hydroxytyrosol in Extra Virgin Olive Oils with a Novel Biosensor Based on Single-Walled Carbon Nanotubes and Tyrosinase. International Journal of Molecular Sciences 2022, 23, 9132. IF 6,208. https://doi.org/10.3390/ijms23169132

8. Munteanu, I.G.; Apetrei, C. Assessment of the Antioxidant Activity of Catechin in Nutraceuticals: Comparison between a Newly Developed Electrochemical Method and Spectrophotometric Methods. International Journal of Molecular Sciences 2022, 23, 8110. IF 6,208. https://doi.org/10.3390/ijms23158110

9. Bounegru, A.V.; Apetrei, C. Simultaneous Determination of Caffeic Acid and Ferulic Acid Using a Carbon Nanofiber-Based Screen-Printed Sensor. *Sensors* 2022, 22, 4689. IF 3,847. https://doi.org/10.3390/s22134689

10. Trifan, A.G.; Apetrei, I.M. Development of Novel Electrochemical Biosensors Based on Horseradish Peroxidase for the Detection of Caffeic Acid. Appl. Sci. 2023, 13, 2526. https://doi.org/10.3390/app13042526

11. Munteanu, I.G.; Apetrei, C. Classification and Antioxidant Activity Evaluation of Edible Oils by Using Nanomaterial-Based Electrochemical Sensors. *International Journal of Molecular Sciences* 2023, 24, 3010. IF 5,6. https://doi.org/10.3390/ijms24033010

12. Geana, E.-I.; Ciucure, C.T.; Apetrei, I.M.; Clodoveo, M.L.; Apetrei, C. Discrimination of Olive Oil and Extra-Virgin Olive Oil from Other Vegetable Oils by Targeted and Untargeted HRMS Profiling of Phenolic and Triterpenic Compounds Combined with Chemometrics. *International Journal of Molecular Sciences* 2023, 24, 5292. IF 5,6. https://doi.org/10.3390/ijms24065292

Participation in international conferences

1. Constantin Apetrei, Alexandra Virginia Bounegru, Irina Georgiana Munteanu, Irina Mirela Apetrei. Electrochemical sensors and biosensors based on polypyrrole for detection of phenolic compounds in olive oils. Scientific Conference of Doctoral Schools CDS-UDJG 2021, The Ninth Edition, Galați, 10-11 June 2021. Oral presentation, abstract published in Abstract Book, p. 117

2. C. Apetrei, I.M. Apetrei. Detection of Olive Oil Adulteration Using Electrochemical Sensors and Biosensors. XXVIth International Symposium on Bioelectrochemistry and Bioenergetics, Online, 9-13 May 2021, Cluj-Napoca, Romania. Oral presentation, abstract published in Abstract Book, p. 97.

3. Alexandra Virginia Meresescu (Bounegru), Constantin Apetrei. Development of novel biosensor for the detection of p-coumaric acid in phenolic extracts from virgin olive oils. Biosensors 2021, The 31st Anniversary World Congress on Biosensors, 26-29 iulie 2021, poster, P1.008

4. C. Apetrei, A. V. Bounegru, I.G. Munteanu, I.M. Apetrei. Development of a sensitive method for the voltammetric detection of phenolic compounds in extra virgin olive oils. CSAC2021: 1st International Electronic Conference on Chemical Sensors and Analytical Chemistry, session Electrochemical Devices and Sensors, 1–15 July 2021, poster, sciforum-046141

5. A. V. Bounegru, I. G. Munteanu, C. Apetrei. Development of an electroanalytical method for detecting adulteration of extra virgin olive oils. SCDS-UDJG 2022 The Tenth Edition, Galaţi, 9-10 iunie 2022. Invited oral presentation, abstract published in Book of abstracts, p. 39.

6. Alexandra Virginia Bounegru, Constantin Apetrei. Sensors and Biosensors for Evaluating Olive Oil Quality. SCDS-UDJG 2022 The Tenth Edition, Galaţi, 9-10 June 2022. Oral presentation, abstract published in Book of abstracts, p. 161.

7. Andreea Loredana Comănescu, Constantin Apetrei. Discrimination of vegetable oils by using spectrometric data and chemometrics methods. SCDS-UDJG 2022 The Tenth Edition, Galaţi, 9-10 June 2022. Poster, abstract published in Book of abstracts, p. 241.

8. Andrei Geman, C. Apetrei. Determination of the addition of maize oil to extra virgin olive oil by FTIR method coupled with multivariate data analysis methods. SCDS-UDJG 2022 The Tenth Edition, Galaţi, 9-10 iunie 2022. Poster, abstract published in Book of abstracts, p. 242.

9. C. Apetrei. Spectrometric and electroanalytical methods for the determination of virgin olive oils adulteration. International Summer School - FOOD SAFETY AND HEALTHY LIVING -FSHL–2022, 5-8 September 2022, Brașov – Romania, Invited oral presentation, abstract published in Book of abstracts, p. 142.

10. Constantin APETREI, Elisabeta-Irina GEANĂ, Irina Mirela APETREI. Electroanalytical method coupled with chemometry for detection of virgin olive oil adulteration. The 6th International Conference New Trends on Sensing - Monitoring – Telediagnosis for Life Sciences NT-SMT-LS 2022 Brașov, September 8-10, 2022. Oral presentation, abstract published in Book of abstracts, p. 43.

11. Alexandra Virginia BOUNEGRU, Constantin APETREI. Electrochemical determination of hydroxytyrosol in extravirgin olive oils using screen-printed electrodes modified with single wall carbon nanotubes and tyrosinase The 6th International Conference New Trends on Sensing - Monitoring – Telediagnosis for Life Sciences NT-SMT-LS 2022 Brașov, September 8-10, 2022. Poster, abstract published in Book of abstracts, p. 62.

12. Irina-Georgiana Bulgaru (Munteanu), Constantin Apetrei. Comparative study on the antioxidant activity of extra virgin olive oil samples using a newly developed electrochemical method and DPPH spectrophotometric assay. The 6th International Conference New Trends on Sensing - Monitoring – Telediagnosis for Life Sciences NT-SMT-LS 2022 Brașov, September 8-10, 2022. Poster, abstract published in Book of abstracts, p. 81.

13. Constantin Apetrei, Andreea Loredana Comănescu, Andrei Daniel Geman, Irina Georgiana Munteanu, Alexandra Virginia Bounegru, Irina Mirela Apetrei, Elisabeta Irina Geană. Electrochemical (bio)sensor array coupled with multivariate data analysis for the assessment of virgin olive oil quality. "Priorities of Chemistry for a Sustainable Development", PRIOCHEM XVIII, București, 26-28 October 2022. Invited oral presentation - keynote, abstract published in Book of abstracts, p. 11.

14. Elisabeta-Irina Geana, Corina Teodora Ciucure, C. Apetrei. Authentication and detection of adulteration of extra virgin olive oil based on the composition of phenolic compounds. "Priorities of Chemistry for a Sustainable Development", PRIOCHEM XVIII, București, 26-28 Octombrie 2022. Oral presentation, abstract published in Book of abstracts Nr. 18/2022 ISSN 2601 - 419X, p. 63.

15. A. D. Geman, A. L. Comănescu, C. Apetrei. Classification of extra virgin olive oils using chemometry analysis applied to spectrometric data. The 18th International Conference of Constructive Design and Technological Optimization in Machine Building Field OPROTEH 2023, Bacău, 11-13 May 2023, Oral presentation, abstract published in Conference Proceedings-ABSTRACTS, p. 78.

16. A. L. Comănescu, A. D. Geman, C. Apetrei. Chemometric analysis of FTIR spectroscopic data used for the detection of olive oils adulteration with vegetable oils. The 18th International Conference of Constructive Design and Technological Optimization in Machine Building Field OPROTEH 2023, Bacău, 11-13 May, 2023, Oral presentation, abstract published in Conference Proceedings-ABSTRACTS, p. 78.

17. Irina Mirela Apetrei, Alexandra Virginia Bounegru, Irina Georgiana Munteanu, Constantin Apetrei. Detection of olive oils adulteration with electrochemical sensors and biosensors based on nanomaterials and enzymes. The 18th International Conference of Constructive Design and Technological Optimization in Machine Building Field OPROTEH 2023, Bacău, 11-13 May, 2023, Oral presentation, abstract published in Conference Proceedings-ABSTRACTS, p. 79.

18. C. Apetrei, I.G. Munteanu, A.V. Bounegru. Determination of antioxidant properties by spectrometric and electrochemical methods. Correlations among results. International Summer School FOOD SAFETY AND HEALTHY LIVING FSHL – 2023. Oral presentation, Book of Abstracts, p. 64.

19. Irina Georgiana Munteanu, Constantin Apetrei. Novel peptide-based sensors designed to detect antioxidant phenolic compounds. Eurosensors XXXIV, Lecce, Italy, 10-13 Septembrie 2023. Poster. abstract published in Proceedings 2023.

20. Elisabeta-Irina Geană, Corina Teodora Ciucure, Constantin Apetrei. Authenticity of olive oil: classification of botanic and geographic origins and detection of adulteration based on phenolic compounds composition coupled with chemometric analysis. SCDS-UDJG 2023, The Eleventh Edition, GALAŢI, 8-9 June 2023, Oral presentation, abstract published in Book of abstracts, p. 105.

21. Irina-Georgiana Munteanu, Constantin Apetrei. Electrochemical peptide-based Sensor for direct detection and quantification of verbascoside in extra virgin olive oil. SCDS-UDJG 2023, The Eleventh Edition, GALAŢI, 8-9 iunie 2023, Oral presentation, abstract published in Book of abstracts, p. 106.

22. Andreea-Loredana Comănescu, Constantin Apetrei. Determination of extra virgin olive oil adulteration by using multivariante methods. SCDS-UDJG 2023, The Eleventh Edition, GALAŢI, 8-9 June 2023, Poster, abstract published in Book of abstracts, p. 297.

23. A.D. Geman, C. Apetrei. Study of olive oil quality with spectrometric methods. SCDS-UDJG 2023, The Eleventh Edition, GALAŢI, 8-9 June 2023, Poster, abstract published in Book of abstracts, p. 297.

24. C. Apetrei, I. G. Munteanu, A. V. Bounegru, I. M. Apetrei. Biosensors array for the detection of virgin oils adulteration with seed oils. 24th International Conference "New Cryogenic and Isotope Technologies for Energy and Environment" - EnergEn 2023, Băile Govora, Romania, 18–20 October, 2023, Oral presentation keynote, abstract published in Book of abstracts ISSN 2601-9965, p. 235-236.

25. Irina-Georgiana MUNTEANU, Constantin APETREI. An ecofriendly approach for the electrochemical determination of olive oils polyphenols using deep eutectic solvents. "PRIORITIES OF CHEMISTRY FOR A SUSTAINABLE DEVELOPMENT" PRIOCHEM -XIXth Edition, Bucharest, 11-13 October, 2023, Poster, abstract published in Book of abstracts Nr. 19/2023 ISSN 2601 - 419X, p. 27.

Participation in national conferences

26. A. V. Bounegru, C. Apetrei. Noi biosenzori enzimatici pentru determinarea electrochimică a oleuropeinei din uleiuri de măsline extravirgine. A XXXVI-a Conferinţă Naţională de Chimie, Călimăneşti–Căciulata. October 4-7, 2022, Oral presentation, Book of abstracts, p. 28.

Patent application

1. Constantin Apetrei. Molecularly imprinted polymeric layers with trigonelline embedded in carbon nanomaterials for the realization of electrochemical sensors and their manufacturing process, OSIM, national patent application, A/00669, 9.11.2023.

Task 6. Project management

Acquiring activities within the project were carried out in very good conditions, with some delays in deliveries in 2021 due to the covid-19 pandemic. The project website, www.busdoa.ugal.ro , has been updated and includes the plan, achievements and reports for each stage of project implementation as well as a brief presentation of the results obtained (a text for public understanding) – the summary of the project implementation and the most important results.

• The estimated impact of the results obtained, emphasizing the most significant result obtained

The most important impact of this project is scientific, namely the publication of 12 scientific articles in ISI journals with a cumulative impact factor of 64, participation in international scientific conferences with 25 papers and submission of 1 patent application - which can be considered ***the most significant result of the project.*** **The socio-economic impact** of the project is to support the career development of four young researchers, two students and two PhD students, who worked in a multidisciplinary scientific field alongside experienced researchers, the results obtained being disseminated to the scientific community both through the publication of scientific articles in ISI journals with a high impact factor as well as by participating in international conferences. Also, the project led to the increase of the scientific experience of the team members and the results obtained increased the international visibility of the team. Carrying out a research internship at the University of Ilmenau, Germany, expands international collaborations by allowing the project team to participate with new research proposals at an international level. **The economic impact** will increase through the transfer of the patent to industry for the application of the molecularly imprinted sensors developed in the project in the control of adulteration of olive oils.

Project manager,
Apetrei Constantin