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Determination of Bitterness of Extra Virgin Olive Oils by Amperometric Detection

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Abstract: A flow injection system with amperometric detection at potentials poised at +0.4 and +0.9 V was used to evaluate intensity of the bitter taste in monovarietal Extra Virgin Olive Oils (EVOO). Results from the proposed method were based on the extraction of the bitter constituents of the virgin olive oil samples in methanol-water, followed by the direct amperometric measurement. These potentials were selected according to the hydrodynamic voltammogram of oleuropein, one of the most prominent and bitter phenolic compound found in EVOO. The amperometric detection was applied on 32 monovariatal EVOO samples. Results were correlated with the phenolic profile measured by high performance liquid chromatography (HPLC). The amperometric signal at +0.9 V mainly correlated with the total phenols of the samples (R^2=0.81), whereas the signal at +0.4 V mainly correlated with oleuropein aglycone (3,4 DHPEA-EDA, R^2=0.79). Bitterness intensity of the samples was evaluated by a trained sensory panel of experts and the results compared to those obtained by the amperometric flow system. The best correlation with the bitter taste was achieved by the sensor at +0.4 V (R^2=0.72). A calibration model based on partial least squares was built with three variables, namely the sensors set at +0.4 and +0.9 V and the total phenol content of the EVOO extracts. The model showed a moderate capacity to predict the bitterness of the EVOO samples using leave one out method, (R^2=0.75) and in prediction of a test set of samples (R^2=0.7). Such approach is very promising for future studies.

Keywords: bitterness · amperometric detection · electrochemical sensors · extra virgin olive oil · amperometry · total phenols · food · flavonoids · electrode passivation

1 Introduction

Extra virgin olive oil (EVOO) is a typical product of the Mediterranean diet obtained directly from olives and solely by mechanical means, under conditions that do not lead to alter the oil composition [1,2]. EVOO is one of the few "fat foods" that are worldwide associated with health benefits. This aspect is essentially related to its peculiar chemical composition, among others, rich in phenolic compounds. Phenols are a wide class of redox species consisting of one or more aromatic rings bounded with hydroxyl (–OH) functionalities. One of the most important found in EVOO is oleuropein, a tyrosol ester of elenolic acid that can be further hydroxylated and glycosylated. Its content in olives can reach concentrations up to 140 mg g^{-1} on a dry matter basis [3]. The content of oleuropein in EVOO is quite variable. The molecule is certainly synthetized during the development of the olive fruit. So, its content is strictly dependent on pedoclimatic factors and agronomic practices [4]. Accumulation of oleuropein starts with the earlier growth phase of the fruit, where it can reach 14% of dry matter, but declines quickly during the subsequent maturation phase [5]. Accordingly, the ripening stage of the olive fruit plays a crucial role to tune the bitterness of the resulting oil.

Finally, the techniques of extraction, the kneading of the olive paste and the separation of the oily phase may also affect the resulting oleuropein content as a consequence of the activity of polyphenol oxidase (PPO), the enzyme responsible for the browning of green olives during processing [6].

The content of oleuropein on EVOO samples is of great importance for a number of reasons. In addition to its antioxidant [7], anti-cancer [8] and antimicrobial [9] properties, oleuropein is also responsible of the bitter and pungent taste of the resulting oils. A number of studies

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reported a strong correlation between the content of oleuropein-aglycone mono (3,4-DHPEA-EA) and di-aldehyde (3,4-DHPEA-EDA) with bitterness ($r^2 > 0.8$) [10]. Further proof was reported recently, with the discover of the receptor for p-HPEA-EDA in the oropharyngeal region of the oral cavity [11]. Since bitterness influences the consumer acceptance, there is a great interest in techniques able to measure such important sensorial variable.

Currently, sensory evaluation of bitterness in EVOO is performed mainly by Quantitative Descriptive Analysis (QDA) performed by a trained panel [12] as its direct connection with the consumer organoleptic judgment. However, sensory analysis is expensive, requires a trained panel of experts and lengthy time of training and data analysis. Both factors limit its use in routine work. Accordingly, there is a great interest to find new methods for the fast and simple evaluation of bitterness in EVOO.

Among the instrumental methods recently proposed, one of the simplest is the "Bitter index" based on optical UV spectroscopy. Briefly, the protocol consists on the extraction of an oil sample in hexane through a C18 cartridge, previously activated with methanol. The cartridge is washed with hexane to remove liposoluble substances and the retained compounds are eluted with a methanol-water mixture. The eluted sample is analzyed at 225 nm in a 1 cm cuvette. The result was positively correlated with the bitterness estimated by a panel of tasters [13–15]. Other examples of such systems use techniques, such as near and mid infrared [16,17], UV-VIS [18,19] and raman [20] spectroscopy. In addition, electrochemical sensors have been applied to the study of redox properties of olive oils [21].

Here, we present a direct electrochemical method able to evaluate the bitterness intensity of EVOO with minimal sample preparation and high throughput. This work is an adaptation of previous approaches based on the evaluation of the antioxidant power in wine [22], olive oil [23] and lipophilic extracts [24], but focused on the evaluation of bitterness on EVOO samples. The proposed method is simple, fast and provides a promising alternative to more advanced analytical techniques.

2 Experimental

2.1 Reagents

Oleuropein standard, gallic acid and ascorbic acid were purchased from Sigma-Aldrich. All solvents were LC grade (VWR). Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 μm membrane point-of-use cartridge (Millipak). Stock solutions of concentration 10 mmol L^{-1} were prepared by dissolving the exact mass of the gallic acid and ascorbic acid standards. The stock solutions were further diluted to obtain a calibration range.

2.2 Samples Extraction

Oils were produced in a small scale oil extraction system. Olives come from the olive germplasm collection of Santa Paolina experimental station of the National Research Council of Italy [25]. After production, monocultivar olive oils were stored in the freezer at −80° in dark glass bottles. Prior to analysis samples were thawed in a water bath at 25°. The melted samples were immediately placed in dark environment at 16° C to avoid oxidation. For extraction, a 2.0 g amount of olive oil was accurately weighed on analytical balance into a 15-mL tube. Then, 5 g of solvent (80 % methanol, 20 % water, final volume = 8.1 mL) was transferred to the weighed sample. The sealed sample tube was agitated for 60 seconds in IKA Genius 3 Vortex before further extraction in the ultrasonic bath (Bandelin Sonorex) for 15 minutes at room temperature. Afterwards, the sample was centrifuged at 5000 rev/min in Thermo Scientific SL 16R centrifuge for 25 minutes. The aliquot of the supernatant phase was transferred into a 10 mL plastic syringe, and filtered through a 0.45 mm filter before the injection into Flow injection analysis (FIA) system.

2.3 Flow Injection Analysis

Flow measurements were performed using Agilent 1260 Infinity Binary LC Autosampler. The following measurement settings were used: flow rate of 1.5 mL min^{-1}, injection volume of 5 μL. Electrochemical measurements were performed using thin-layer cell (ALS, Japan) equipped with a non-aqueous reference electrode (Ag/Ag+) and dual working electrode from glassy carbon of 3 mm diameter operating at a potential of +0.4 V; the layer thickness was 50 μm. For the experiment, only one working electrode was used. The data was recorded with Autolab analyzer (Metrohm, Netherlands). FIA experiments were performed at room temperature using a mixture of methanol and acetic buffer (pH 4, 0.2 M) in proportion 80 : 20 (v/v) as carrier solution. According to the literature, oleuropein oxidation proceeds with a two electrons and two protons transfer, showing a dependence of the peak potential toward the pH close to 60 mV per unit of pH change [25]. For the purpose of the current application, the working pH for oleuropein detection was chosen as 4.0. The olive oil extracts were diluted 1 : 10 with carrier solution and measured in duplicate. Quantification of antioxidants was based on the oleuropein standard.

2.4 High Performance Liquid Chromatography

Biophenols were measured according to COI/T.20/Doc No 29. HPLC equipment consisted of a Hewlett Packard 1200 diode-array detector system and a Hewlett Packard model 1100 autosampler (Agilent Technologies, Santa Clara, California, USA). Analytical conditions were: HPLC column, LiChrospher 100 endcapped RP-18, 5 μm, 250 × 4.6 mm ID; injection volume 20 μL; solvent, pH 2.5

H_2O/acetonitrile gradient as described in the method; wavelength, 280 nm. Phenolic compounds were identified according to International Olive Oil Council (IOOC) COI/T.20/Doc. No. 29 method and quantitative results were expressed in mg kg^{-1} equivalent of tyrosol.

2.5 Sensory Analysis

The sensory analysis of the samples was performed by the expert panel of the chamber of commerce of Grosseto, recognized by Italian Ministry for Agriculture under the conditions described in the IOOC regulations (COI/T.20/DOC.15/Rev. 8 – 2015, COI/T.20/Doc. No. 15/Rev. 1, 1996 and COI/T.20/Doc. No. 22, 2005) [26] as described in the previous study [27].

2.6 Statistical Analysis

XLSTAT (Addinsoft) Version 2015.4.01.22162 and Minitab 16 (Minitab Ltd., United Kingdom) were used for statistical analysis of data.

3 Results and Discussion

Flow injection with amperometric detection is a technique able to detect and quantify redox species with very high throughput. The fast response, high sensitivity, high selectivity toward redox species and stability are inherent characteristics of the technique that are desired for the development of routine quality control assays. In this work, we used a single glassy carbon electrode positioned in an electrochemical flow cell to develop a simple and fast estimation of bitterness in EVOO. Before the analysis of EVOO samples, the performance of the amperometric sensors was tested with the analysis of oleuropein standard solutions. Figure 1 shows the hydrodynamic voltammogram (HDV) of 18.5 μM oleuropein solution. The plot (**A**) shows the current peak height as a function of the applied potential under laminar flow conditions. The result is a sigmoidal curve from which it is possible to identify two onset potentials. The first onset is located in the plateau region at about +0.9 V (Figure 1-B). In this zone, the peak height is independent of the potential and the detection is governed only by the diffusion, which is the ideal condition for analytical purposes. However, at such high potential, the selectivity of the electrode towards oleuropein is poor, as most of the redox species present in the EVOO samples can be detected as well. Also, in this range, the background current is rising exponentially, decreasing the overall performance of the sensor (Figure 1-C).

Thus, we selected a second onset from the HDV. This is located in the earlier part of the hydrodynamic voltammogram, at potentials around +0.4 V. In this zone, the peak height is rising with increasing potential, as the potential is controlling the kinetics of the heterogeneous electron transfer. In Figure 2 the peak currents of two oleuropein solutions (18.5 μM and 185 μM) is shown. De-

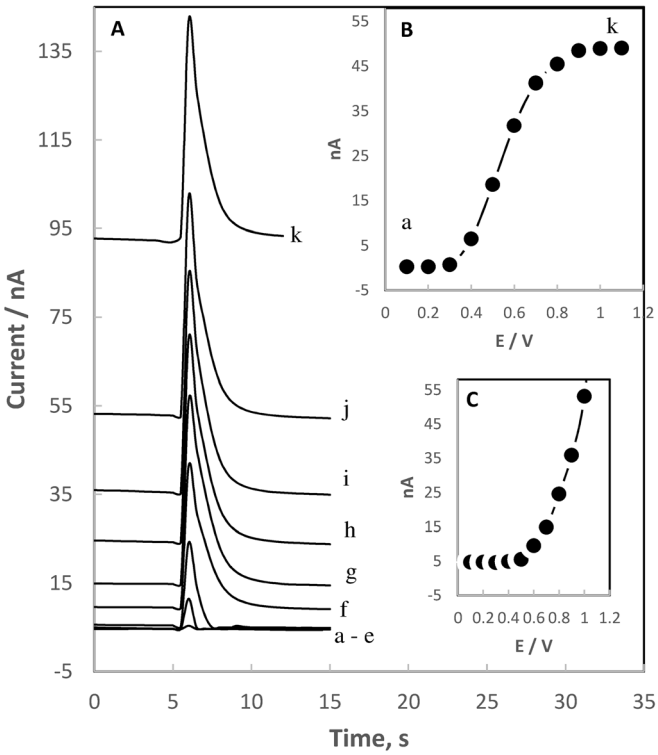

Fig. 1. Hydrodynamic voltammogram of oleuropein solution (18.5 μM), flow rate of 1.5 mL min^{-1}. **A** shows the peak current as a function of the applied potential. Inset **B** shows the peak height (mean value, n = 3) as a function of applied potential. Inset **C** is describing background current as a function of the applied potential.

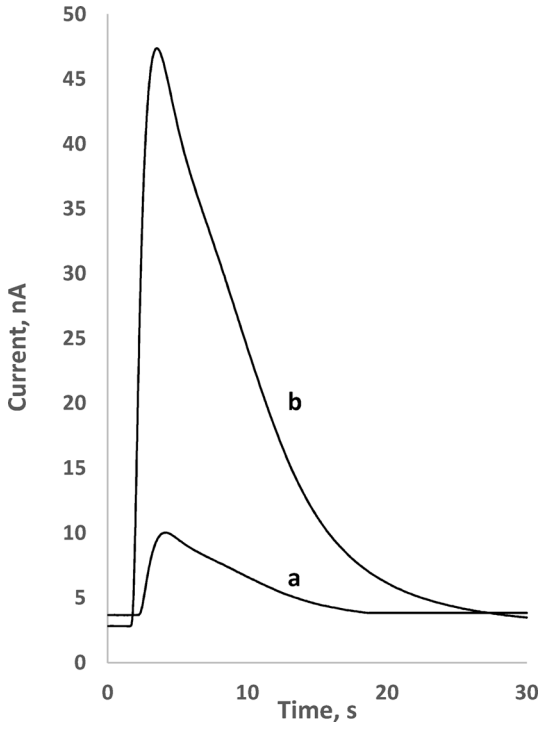

Fig. 2. The peak current of 18.5 μM (**a**) and 185 μM (**b**) oleuropein solution with applied potential of +0.4 V.

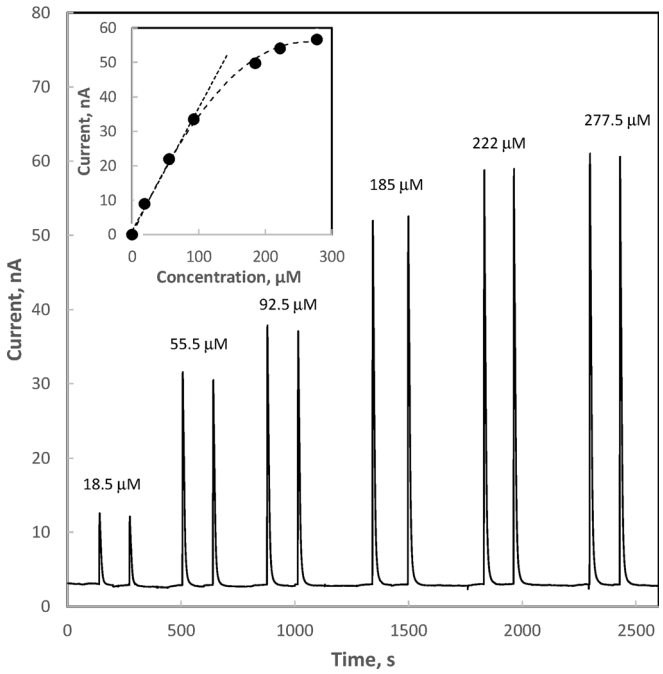

Fig. 3. Calibration for oleuropein standard solution obtained by a flow injection system equipped with at a glassy carbon working electrode poised at the potential of +0.4 V vs. Ag/Ag^+. Conditions: flow rate: 1.5 mL min^{-1}, methanol, 80%; as supporting electrolyte acetic buffer pH 4 0.2 M, 20%. Inset shows the peak height (mean value, n=3) as a function of oleuropein concentration.

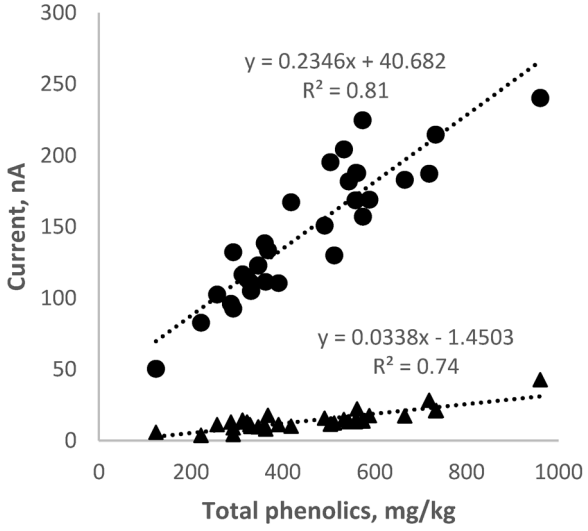

Fig. 4. Correlation plot between the amperometric signal obtained with glassy carbon electrodes set at +0.4 V (triangles) and +0.9 V (circles) versus the total phenol content of 32 monovarietal oil samples (HPLC data).

spite the low signal, such choice offers maximum selectivity, as the potential is low enough to get rid of most of the signals from other phenolic compounds present in EVOO samples.

At these two detection potentials (+0.4 and +0.9 V), the resulting current signal was proportional to the concentration of oleuropein. Figure 3 shows the signals obtained from successive injections of oleuropein (from 18.5 to 277.5 µM) when the electrode was set at the potential of +0.4 V. The resulting peak current increased linearly with the concentration of oleuropein (R^2=0.996), with a slope of 0.0004. When the detection potential was poised at +0.9 V, the peak current also increased linearly (R^2=0.996) with a higher slope (0.0023). The limit of detection were 1.58 µM and 1.71 µM, respectively, for +0.4 and +0.9 V (based on 3*St.Dev./Slope of calibration curve). Furthermore, the current signal obtained for n=10 subsequent injections of oleuropein standard (185 µM), resulted in a relative standard deviation of 0.6% and 0.19%, respectively for +0.4 and +0.9 V.

3.1 Amperometric Detection of Extra Virgin Olive Oils

The above described method was applied to the analysis of 32 monovarietal EVOO samples. The extracts in methanol/water (80:20) of the samples were analyzed with the amperometric detector set at +0.4 and +0.9 V (Table 1). The signals of the two amperometric sensors were scarcely correlated (R^2=0.56). This was desired as it indicates that the information contained in each of the two signals explains different properties of the samples.

Figure 4 shows the two resulting current signals in connection with the total phenol content. The best correlation was achieved with the amperometric detector poised at +0.9 V, showing a correlation of R^2=0.82. This result is important for two reasons. First, it indicates that most of the redox species detected by the amperometric sensor are phenolic compounds. Second, it confirms that the detection potential is high enough to account for most of the phenols contained in the EVOO samples.

The EVOO samples were next analyzed by HPLC. Table 2 reports the phenolic profile of the samples. The correlation between the amperometric signal measured at +0.4 and +0.9 V with the content of the single phenolic compounds showed that the aglycone form of the decarboxy methyl oleuropein was well correlated with the amperometric electrode poised at +0.4 V (R^2=0.79). This result shows that such amperometric signal is largely derived from the content of oleuropein derivatives.

Sensory analysis of the EVOO samples was next performed by a panel of experts in agreement with International Olive Oil Council (IOOC) COI/T.20/Doc. No. 29 method for sensory analysis of olive oils [27]. The results were correlated with those obtained by the two amperometric detectors. Figure 5 shows the correlation between the amperometric signals vs. the bitterness scores obtained by the panel of experts. The amperometric sensors poised at +0.4 V showed the highest correlation with the bitterness intensity (R^2=0.72), whereas the sensors at +0.9 V showed only a scarce correlation (R^2=0.58). Again, this information confirms that most of the bitter intensity in the EVOO samples derives from oleuropein compounds. Not surprisingly, the sensors poised at

Table 1. Total phenolic content of oils (HPLC data), current signal detected with the electrode set at +0.4 and +0.9 V and the bitterness intensity as measured by a panel of experts.

Cultivar	Taste attribute	"400 mV" nA	"900 mV" nA	Total Phenols mg/kg	Bitterness Index a.u.
MELAIOLO	MILD	11.2	110.6	390.669	4.6
PENDOLINO	MILD	17.4	168.9	588.441	4.9
GINESTRINO	BITTER	20.8	214.6	732.663	5.1
FILARE	MILD	15.6	150.8	491.17	4.8
MADONNA IMPRUNETA	MILD	12.2	129.9	511.663	4.5
COLOMBANA	MILD	17.3	183	665.809	4.95
COLOMBINO	BITTER	42.5	240.3	960.975	5.35
MADREMIGNOLA	MILD	14.7	187.9	559.426	4.85
LAZZERO GUADALUPE	MILD	13.1	181.7	543.752	4.5
TISIGNANA	SWEET	3.6	82.7	221.986	3.85
ROSINO	BITTER	20.9	433.3	734.524	5.15
PESCIATINO	MILD	13.6	157.1	574.332	4.65
MIGNOLO	SWEET	4.4	92.5	292.314	4.35
ARANCINO	MILD	13.2	168.5	558.513	4.6
MORCHIAIO	MILD	14.8	204.4	532.667	4.75
ROSSELLINO CERRETANO	SWEET	7.9	111.5	362.28	4.5
ARETINO	SWEET	9.5	122.8	346.338	4.5
LAZZERO PRATIGIANO	MILD	14.4	224.8	573.466	4.8
ROSSELLO	SWEET	8.9	132.0	292.667	4.55
LASTRINO	MILD	11.3	195.2	503.881	4.8
MORAIOLO	MILD	10.5	138.4	360.138	4.3
GREMIGNA TONDA	MILD	10.1	167.2	417.779	4.35
PUNTERUOLO	BITTER	28.2	187.3	718.944	5
QUERCETANO	MILD	13.0	95.8	287.596	4.7
AMERICANO	MILD	17.6	133.5	367.523	4.65
DA CUCCARE	BITTER	22.2	187.7	562.066	4.85
CUCCA	SWEET	5.8	50.2	123.781	3.55
OLIVO DEL MULINO	MILD	14.4	116.5	312.605	4.5
FRANTOIO	MILD	9.7	104.9	331.347	4.5
MIGNOLO CERRETANO	MILD	11.1	102.5	257.197	4.7
OLIVO DI CASAVECCHIA	MILD	11.1	111.2	328.917	4.8
PUNTINO	MILD	12.9	113.4	322.313	4.7

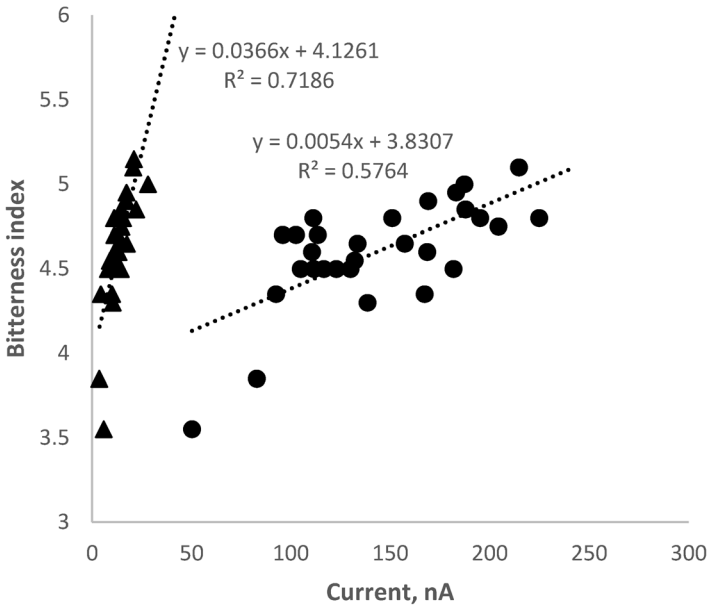

Fig. 5. Correlation plot between the amperometric signal obtained with glassy carbon electrodes set at 0.4 (triangles) and 0.9 V (circles) versus bitter index evaluated by a sensory panel.

Table 2. Composition of phenolic extracts from extra-virgin olive oils at production ($\mu g\,g^{-1}$) measured by HPLC.

Cultivar	Hydroxytyrosol (or 3,4-DHPEA)	Tyrosol (or p-HPEA)	Total Vanillic and caffeic acids	Vanillin	Para-coumaric acid	I Hydroxytyrosyl acetate	Ferulic acid	Orto-coumaric acid	Decarboxymethyl oleuropein aglycone, oxidised dialdehydic form	Decarboxymethyl oleuropein aglycone, dialdehydic form (or 3,4 DHPEA-EDA)	Oleuropein	Oleuropein aglycone, dialdehydic form
MELAIOLO	1.23	1.38	2.06	3.05	0.10	1.28	1.54	1.33	16.12	144.85	5.47	10.91
PENDOLINO	1.89	1.77	1.14	2.08	0.11	0.62	0.77	1.76	68.53	235.76	22.89	30.17
GINESTRINO	3.96	1.22	1.31	2.24	0.16	1.48	1.27	0.87	145.89	56.92	64.65	58.13
FILARE	1.13	1.10	0.49	1.95	0.08	2.52	0.92	0.93	61.84	112.64	23.65	28.16
MADONNA IMPRUNETA	1.80	1.74	0.59	3.08	0.19	0.39	0.40	1.84	31.33	232.97	12.28	16.16
COLOMBANA	2.24	2.46	1.04	2.56	0.19	0.37	0.56	0.62	63.72	243.09	24.69	28.40
COLOMBINO	2.73	3.52	0.37	1.23	0.22	1.87	0.54	1.87	173.07	96.39	49.33	55.09
MADREMIGNOLA	1.51	2.26	0.49	1.41	0.06	0.47	0.39	1.23	59.15	110.38	24.77	25.21
LAZZERO GUADALUPE	1.25	1.99	0.66	1.45	0.11	0.72	0.31	1.82	54.18	126.36	25.81	23.37
TISIGNANA	0.64	0.89	1.19	1.59	0.19	0.99	0.81	1.48	6.08	42.50	1.89	4.00
ROSINO	2.05	1.06	0.97	1.52	0.18	1.41	0.46	1.18	107.12	281.27	58.56	41.35
PESCIATINO	2.23	1.72	2.72	2.43	0.32	1.02	1.83	1.39	95.59	42.36	39.02	38.72
MIGNOLO	1.01	1.79	0.99	2.69	0.09	0.39	4.16	0.39	10.11	86.76	4.09	5.05
ARANCINO	3.00	1.93	2.21	2.21	0.34	0.93	3.18	0.76	84.43	49.24	51.53	34.25
MORCHIAIO	2.46	0.74	1.97	2.00	0.44	1.07	2.56	0.72	82.00	180.01	35.88	22.02
ROSSELLINO CERRETANO	0.89	1.20	1.25	2.80	0.25	0.75	0.78	1.45	5.57	186.15	2.25	1.99
ARETINO	1.91	1.62	2.90	1.16	0.25	0.29	5.31	0.75	42.03	53.50	17.56	24.48
LAZZERO PRATIGIANO	1.49	0.85	2.06	3.01	0.27	0.80	5.67	1.50	41.13	283.70	23.29	17.23
ROSSELLO	1.25	0.72	2.23	2.34	0.19	3.01	0.54	0.60	31.44	77.90	15.56	15.97
LASTRINO	1.03	0.91	1.68	2.03	0.32	0.92	2.49	0.95	19.70	228.70	13.59	9.45
MORAIOLO	1.80	1.13	0.69	1.39	0.27	1.21	0.30	0.90	33.73	126.91	16.37	12.10
GREMIGNA TONDA	0.68	0.67	1.00	2.41	0.38	0.50	1.49	1.54	11.55	273.54	3.36	3.12
PUNTERUOLO	3.22	2.39	0.86	1.32	0.38	0.65	0.99	1.84	129.37	82.22	52.85	51.31
QUERCETANO	1.31	0.80	0.63	1.36	0.24	0.30	1.68	0.71	20.88	122.02	11.53	8.27
AMERICANO	1.90	1.25	0.71	1.03	0.39	0.61	0.18	1.25	65.81	50.56	31.34	22.13
DA CUCCARE	2.04	1.30	3.17	1.84	0.43	2.17	0.47	2.19	86.53	79.05	33.82	40.94
CUCCA	0.57	1.37	1.33	1.89	0.35	1.10	0.40	0.59	4.37	15.20	2.01	2.07
OLIVO DEL MULINO	1.82	1.13	0.55	1.11	0.27	1.25	0.17	0.84	61.38	31.70	18.81	25.51
FRANTOIO	0.66	1.43	0.93	2.12	0.18	0.36	0.46	1.02	12.65	60.67	6.33	8.12
MIGNOLO CERRETANO	0.98	0.76	0.27	0.82	0.16	0.12	0.11	1.10	28.12	79.62	15.59	11.60
OLIVO DI CASAVECCHIA	1.77	3.21	1.22	1.23	0.22	1.10	0.76	0.19	25.44	38.16	12.67	11.58
PUNTINO	1.07	0.85	0.26	0.81	0.29	0.31	0.53	1.42	28.69	144.92	10.40	12.59

Table 2 (Continued)

Cultivar	Decarboxymethyl ligstroside aglycone, oxidised dialdehydic form	Decarboxymethyl ligstroside aglycone, dialdehydic form	Ligstroside aglycone, dialdehydic form	Oleuropein aglycone, oxidised aldehydic and hydroxylic form	Oleuropein aglycone, aldehydic and hydroxylic form	Ligstroside aglycone, oxidised aldehydic and hydroxylic form	Ligstroside aglycone, aldehydic and hydroxylic form	Pinoresinol, 1 acetoxy-pinoresinol	Cinnamic acid	Luteolin	Apigenin	Methyl luteolin
MELAIOLO	42.41	29.46	10.70	20.25	50.17	18.19	4.09	5.60	5.65	4.49	5.04	5.31
PENDOLINO	11.73	43.98	2.55	30.86	62.35	18.16	2.82	11.93	8.57	9.35	11.26	7.41
GINESTRINO	23.14	7.45	19.34	55.62	146.62	21.87	4.47	71.60	15.62	10.47	9.44	8.94
FILARE	11.15	31.09	6.05	36.46	83.08	16.81	2.99	30.51	14.12	5.30	8.17	10.04
MADONNA IMPRUNETA	13.20	73.39	4.28	20.25	47.08	15.14	5.00	3.87	5.96	6.71	7.63	6.37
COLOMBANA	23.02	70.82	4.65	39.09	73.17	19.53	3.52	16.97	13.00	10.32	13.55	8.23
COLOMBINO	43.03	50.69	9.54	62.35	140.63	43.95	11.95	87.71	72.45	10.07	8.22	34.17
MADREMIGNOLA	27.68	58.94	6.03	40.78	42.98	18.16	2.92	79.73	23.33	8.67	14.38	8.52
LAZZERO GUADALUPE	25.89	61.34	6.60	31.74	41.79	16.64	3.68	63.90	21.58	8.62	14.75	9.19
TISIGNANA	18.71	15.70	3.06	8.55	7.04	6.12	2.20	88.60	2.14	2.92	1.01	3.67
ROSINO	22.71	33.53	10.50	33.49	66.78	15.60	3.94	9.07	14.67	8.34	13.34	5.43
PESCIATINO	28.22	9.22	10.48	40.00	112.30	20.57	9.08	29.40	20.40	11.12	42.46	11.70
MIGNOLO	12.59	27.56	6.03	7.83	21.27	9.35	4.78	68.99	7.98	1.75	4.54	2.11
ARANCINO	25.26	10.06	11.70	44.55	96.51	15.87	7.05	29.02	16.71	12.75	45.72	9.30
MORCHIAIO	18.78	22.15	9.74	30.13	64.22	10.84	3.45	8.21	9.69	7.34	13.33	2.93
ROSSELLINO CERRETANO	14.58	52.12	4.16	14.91	20.62	7.72	2.44	19.51	3.83	4.60	10.73	1.73
ARETINO	17.79	12.94	7.54	29.18	36.88	10.98	2.21	42.71	7.05	5.03	17.64	4.62
LAZZERO PRATIGIANO	16.86	57.02	6.28	19.49	44.02	11.10	1.27	14.30	6.06	4.58	8.72	2.77
ROSSELLO	21.94	8.35	6.80	12.63	34.70	8.34	8.91	22.39	3.40	2.01	7.25	4.19
LASTRINO	70.37	51.42	4.28	16.22	23.88	12.44	3.27	12.26	3.33	9.13	10.85	4.67
MORAIOLO	9.06	18.42	4.23	27.79	51.82	8.85	2.29	21.83	4.33	3.36	7.41	3.97
GREMIGNA TONDA	30.34	27.92	3.26	9.58	15.45	9.19	0.75	8.35	2.72	2.52	5.36	2.09
PUNTERUOLO	41.02	27.55	16.95	52.72	104.47	25.54	5.29	32.99	44.10	10.51	12.79	17.62
QUERCETANO	10.96	23.07	2.00	12.71	39.38	5.04	3.57	7.89	4.74	1.55	2.10	4.87
AMERICANO	22.52	12.15	6.76	26.11	68.94	11.47	2.69	10.97	12.52	4.27	6.00	5.97
DA CUCCARE	35.92	27.98	11.93	28.85	86.39	16.79	4.68	54.91	18.68	4.83	8.56	8.58
CUCCA	12.78	14.63	3.73	10.30	12.53	7.53	4.24	4.38	3.74	4.06	5.56	9.05
OLIVO DEL MULINO	20.98	12.99	4.95	15.86	39.33	10.13	7.78	23.84	15.30	3.72	8.11	5.08
FRANTOIO	16.60	39.66	5.05	17.77	34.79	14.78	10.24	69.35	8.18	4.89	10.08	5.05
MIGNOLO CERRETANO	9.62	22.60	4.16	12.07	34.33	7.58	3.31	6.47	7.18	2.22	2.57	5.83
OLIVO DI CASAVECCHIA	13.39	19.72	3.54	23.06	54.39	11.40	5.83	61.41	11.44	4.16	11.36	11.68
PUNTINO	7.41	33.23	3.48	11.70	36.14	6.80	2.68	4.51	4.63	2.32	2.12	5.14

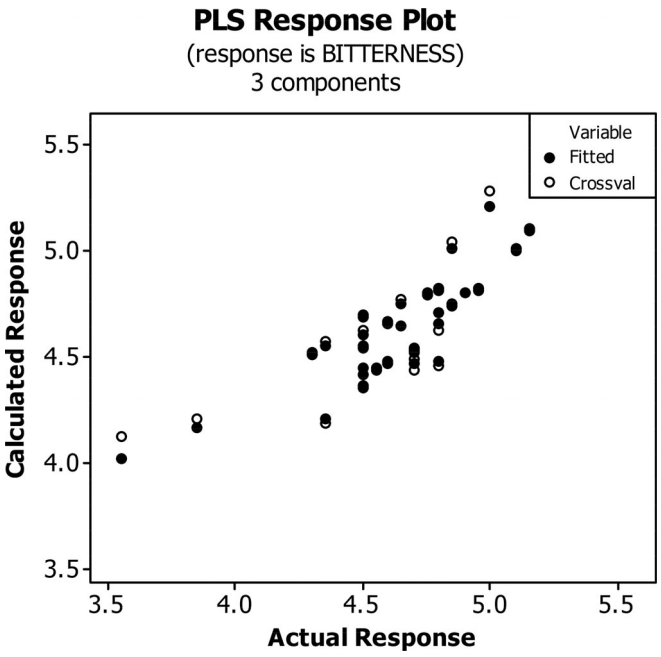

Fig. 6. Partial least square model for prediction of bitterness of EVOO.

+0.4 V achieved the best correlation with the bitterness intensity as we showed previously that this sensor mainly correlated with the content of oleuropein compounds. Instead, the sensor poised at +0.9 V has lower capacity to predict bitterness. The amperometric signal of this sensor is a result of the oxidation of most of the phenolic compounds present in the samples and, thus, is less specific toward oleuropein compounds. Ultimately, these results confirm that not all the phenolic compounds present in the EVOO samples contribute to the bitter taste.

Finally, the partial least squares (PLS) regression model (Figure 6) was applied to tentatively improve the correlation between the bitterness of the EVOO samples and three variables, respectively, two variables from the amperometric sensors: E1 (+0.4 V), E2 (+0.9 V) and one variable from the total phenol values (TP). PLS regression model is considered optimal when explanatory variables are correlated, such as the case for total phenol and the sensor poised at +0.9 V. The resulting multivariate model, validated by the leave one out method was:

$$\text{BITTERNESS} = 3{,}37 + 8{,}04 \cdot [400\,\text{mV}] + 0{,}73 \cdot [900\,\text{mV}] - 0{,}00006 \cdot [\text{TP}].$$

The method shows acceptable correlation with the bitter taste intensity in fitting ($R^2 = 0.75$) but failed to improve the prediction with the leave one out validation procedure ($Q^2 = 0.70$). Taking into account that the reference method used here (sensory analysis) can be notoriously affected by a large uncertainty, amperometric sensors are showing promising approach for prediction of bitterness intensity of EVOO.

Although the verification of the model with EVOO was limited only to one single season of production, the results achieved are consistent with the most recent literature [28,29]. The signal coming from the amperometric sensor poised at +0.4 V provided a promising approach for the correlation with bitterness. Future studies should improve the amperometric sensor by modifying its surface with inorganic or biological catalysts to enhance its selectivity toward oleuropein compounds. Through this approach we expect to further improve the correlation with the compounds responsible for bitter taste.

4 Conclusions

Present study shows how a simple flow injection analysis system equipped with a single glassy carbon electrode set at +0.4 V vs. Ag/AgCl can be used to provide a good estimation of bitterness in EVOO sample extracts, whereas the amperometric sensor poised at higher potential (+0.9 V) was used to estimate the total phenol content in EVOO samples. The correlation observed is satisfactory also keeping into account the inherent uncertainty of the data from the reference methods based on a sensorial panel of experts.

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Determination of Bitterness of Extra Virgin Olive Oils by Amperometric Detection