MEHMET KARAGÖZLÜ
DIRECT DETECTION OF COLD PRESSED OLIVE OIL ADULTERATION BY ELECTROCHEMICAL OXIDATION OF ALPHA-TOCOPHEROL WITH PGE
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A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF APPLIED SCIENCES OF NEAR EAST UNIVERSITY

By
MEHMET KARAGÖZLÜ

In Partial Fullfillment of the Reqirements for the Degree of Master of Science in Food Engineering

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Approval of the Graduate School of Applied Science

Prof. Dr. Nadire ÇAVUŞ

We certify this thesis is satisfactory for the award of the degree of Master of Science in Food Engineering

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ABSTRACT

In this study, a quick, easy, cost effective and environmentally friendly screening method for detection of cold pressed olive oil adulteration was proposed. Cold pressed olive oil was mixed with rapeseed oil, sunflower oil and maize (corn) oil in different proportions and analyzed by using Autolab Potentiostat with Ag-AgCl reference electrode, platinum counter electrode and pencil graphite electrode (PGE) system. Method was based on electrochemical detection of alpha-tocopherol (oxidation peak) on PGE. This study demonstrated that electrochemical analysis of cold pressed olive oil and mixture with other commercial oils have presented a potential for the detection of adulteration in cold pressed olive oil. Overall relative standard deviation of the method was 14% and cut off value of cold pressed olive oil was $30.98 \times 10^{-9} \pm 12.54$ A.

Keywords: Olive oil; adulteration; tocopherol; electrochemistry; differential pulse voltammetry; PGE

ÖZET

Bu çalışmada, soğuk sıkım zeytinyağının tağşiş tespiti için hızlı, kolay, uygun maliyetli ve çevre dostu bir tarama yöntemi ileri sürüldü. Soğuk sıkım zeytinyağı kolza yağı, ayçiçeği yağı ve mısır yağı ile farklı oranlarda karıştırılarak, Ag-AgCl referans elektrodu, platin karşıt elektrot ve kalem grafit elektrot (PGE) sistemli Autolab Potentiostat kullanılarak analiz edildi. Metot, PGE üzerindeki alfa-tokoferolün (oksidasyon piki) elektrokimyasal tespitine dayanıyor. Bu çalışma, soğuk sıkım zeytinyağı ile diğer ticari yağlarla karışımının elektrokimyasal analizinin, soğuk sıkım zeytinyağında tağşişin tespiti için bir potansiyel olduğunu göstermiştir. Bu metotta ortalama relatif standart sapma 14%, soğuk sıkım zeytinyağının cut off değeri ise $30.98 \times 10^{-9} \pm 12.54$ A olarak bulunmuştur.

***Anahtar Kelimeler*:** Zeytinyağı; tağşiş; tokoferol; elektrokimya; diferansiyel puls voltametrisi; PGE

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CHAPTER 1

INTRODUCTION

Olive is one of the world's healthiest foods due to its beneficial fatty acids, especially monounsaturated fats and other minor constituents, such as phenolic compounds, tocopherol (Vitamin E) and carotenoids (Uylaşer and Yıldız, 2014). It contributes low occurrence of cardiovascular diseases in the Mediterranean area and longevity. The nutritional and medical qualities of olive and olive products could be related to their high content of phenolic compounds, which are considered to be responsible for conferring its specific organoleptic value, antioxidants and free radical scavengers and hence it could be used as sources of potentially safe natural antioxidants for the food industry as well. The shelf life and nutritional quality of olive oil increases as the phenolic content increases (Visioli et al., 2002; Morello et al., 2005; Ben Othman et al., 2008; Uylaser and Yıldız, 2014; Frankel, 2011).

1.1 Chemical composition of olive oil

Chemical composition of olive oil is mainly triacylglycerols (~99%), free fatty acids, mono- and diacylglycerols and various lipids such as hydrocarbons, sterols, aliphatic alcohols, tocopherols and pigments. Also phenolic and volatile compounds which contributes to the unique character of olive oil presents.

1.1.1 Fatty acids

Fatty acids are the most important compounds in olive oil with $CH_3(CH_2)_nCOOH$ general formula. Fatty acids are carboxylic acids with generally cis-configuration, unbranched straight-chain with an even number of carbon atom. In nature, few fatty acids exist as trans-configuration, branched with an odd number of carbon atom.

Fatty acids are classified by their bonds on hydrocarbon chain as saturated or unsaturated. Fatty acids, which not contain double bond are saturated, contain only one double bond are monounsaturated and contain more than one double bond are polyunsaturated.

Olive oils have significant amount of monounsaturated fatty acids. Even gaining biological quality by unsaturated bonds, they are defenseless by oxygen and cause autoxidation. Rate of autoxidation is proportionally increase by number of double bonds and prevented by the amount and structure of antioxidants.

Palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (18:2) and linolenic (C18:3) acids are present in olive oil. In addition, some fatty acids are found in trace amounts such as myristic (C14:0), heptadecanoic and eicosonic acids (Buskou, 2006) as mentioned in Table 1.1.

Table 1.1: Fatty acid composition of olive oil as determined by gas chromatography (% m/m methylesters)

Fatty Acid		**Codex Alimentarius (2015)**	**IOC (2015)**
Myristic	C14:0	≤ 0.05	≤ 0.03
Palmitic	C16:0	7.50 – 20.00	7.50 – 20.00
Palmitoleic	C16:1	0.30 – 3.50	0.30 – 3.50
Heptadecanoic	C17:0	≤ 0.30	≤ 0.30
Heptadecenoic	C17:1	≤ 0.30	≤ 0.30
Stearic	C18:0	0.50 – 5.00	0.50 – 5.00
Oleic	C18:1	55.00 – 83.00	55.00 – 83.00
Linoleic	C18:2	3.50 – 21.00	2.50 – 21.00
Linolenic	C18:3		≤ 1.00
Arachidic	C20:0	≤ 0.60	≤ 0.60
Eicosenoic	C20:1	≤ 0.40	≤ 0.40
Behenic	C22:0	≤ 0.20	≤ 0.20
Lignoceric	C24:0	≤ 0.20	≤ 0.20

1.1.2 Hydrocarbons

Squalene and β-carotene are two hydrocarbons that present in considerable amounts in olive oil. Squalene is the last metabolite preceding sterol ring formation and is the major component of the unsaponifiable matter. In addition, it makes up more than 90% of the hydrocarbon fraction. Squalene presence is considered to be partly responsible for the beneficial health effects of olive oil and it acts as chemopreventive against certain cancers. β-carotene presence is related to the green pigments of olive oil (Boskou et al., 2006).

1.1.3 Sterols

Sterols consist of a large group of compounds with a broad range of biological activities and physical properties. Sterols are mentioned as steroid alcohols as well. Sterols are compounds that including side chain with 8-10 carbons and an alcohol group added to the steroid skeleton. They exist in oils as free, fatty acid ester and glucosides. Plant sterols which named phytosterols are the major component of the unsaponifiable fraction of lipids. Phytosterols are related to the quality of the olive oil and used for determination of olive oil adulteration and for checking its authenticity.

1.1.4 Phenolic compounds

Phenolic compounds in oils are defined as polyphenols. They are related to the stability of oil and increase the resistance of oxidative degradation of olive oil. Olive oil is composed of at least 30 phenolic compounds. Hydroxytyrosol and tyrosol are abundantly present in olive oil. Phenolic compounds are responsible for high quality olive oil due to their sensory and antioxidant properties. Also there is close relationship between taste and smell of olive oil and polyphenol content.

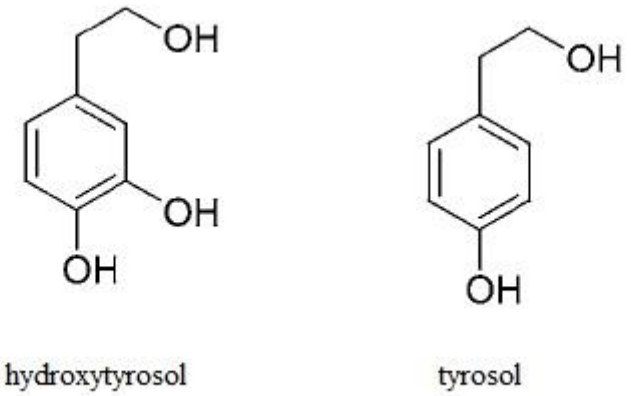


Figure 1.1: Hydroxytyrosol and tyrosol structure

1.1.5 Tocopherols

Tocopherols are natural phenolic antioxidants present in vegetable oils and are responsible for many of the healthful properties of these foods. Tocopherols are effective radical scavengers and defend body against free radical attack by protecting polyunsaturated fatty acids. Vitamin E has an important role at the intracellular level since its deficiency increases membrane fragility and encourages the damage of membranes by oxygen-reactive species, ozone or other free radicals (Diaz et al., 2004).

In nature, vitamin E exists as at least eight naturally occurring compounds, such as α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol. The α-tocopherol is the most biologically active compound and occurs naturally as one isomer (Dutta and Dutta, 2003). Olive oil is a significant source of vitamin E by maintaining between 10 and 150 mg of α-tocopherol each 100 g oil (Souci et al., 1994; USDA, 2016).

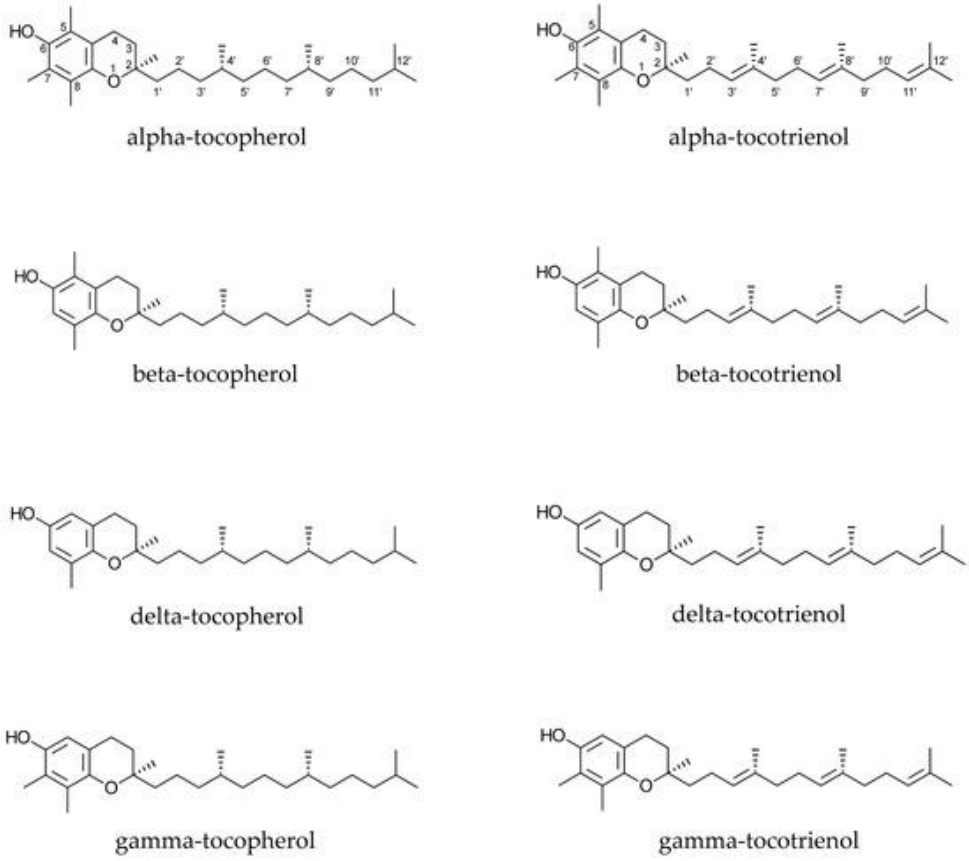


Figure 1.2: Structure of tocopherols and tocotrienols

1.1.6 Volatile and aroma compounds

Volatile compounds are formed during and after the separation of the oil that gives a unique and delicious taste of olive oil. While the nutritional value of olive oil is related to the presence of high amounts of oleic acid and some minor components, its aroma is directly related to the volatile compounds. Volatile compounds occur by oxidation of fatty acids.

Around two hundred and eighty compounds identified as volatile fraction of olive oil such as hydrocarbons, alcohols, aldehydes, ketons, acids, esters, ethers, furan derivatives, thiophene derivatives, pyranones, thiols and phyrazines. Although, only 67 of them are present at levels higher than their odor threshold contribute to the aroma (Boskou et al., 2006).

1.1.7 Fatty alcohols, diterpene alcohols and waxes

Fatty alcohols exist in the free and esterified form and consist of linear saturated alcohols with more than 16 carbon atoms such as docosanol, tetracosanol, hexacosanol and octacosanol. Tricosanol, pentacosanol and heptacosanol found in trace amounts which are fatty alcohols with odd carbon atoms.

Waxes are esterified form of fatty alcohols and fatty acids. Esters of oleic or palmitic acid with 36, 38, 40, 42, 44, 46 carbon atoms are the main waxes detected. Waxes are important minor compounds of olive oil due to distinguish olive oil types.

Alcohol fraction of olive oil includes diterpene alcohols such as phytol and geranylgeraniol which are two acyclic diterpenoids. Their levels are used in calculation of alcoholic index and useful parameter for detecting solvent extracted olive oil in virgin olive oil (Boskou et al., 2006).

1.1.8 Pigments

Chlorophylls and carotenoids are the main pigments that give olive oil color which is shade of green and yellow. Olive cultivar, maturation index, production zone, extraction system and storage condition may influence the color of olive oil (Boskou et al., 2006).

1.2 Effect of environmental factors on chemical composition of olive oil

Environmental, climatic, genetic and agronomic factors influence the quality and analytical characteristics of olive oil. During development and fruit ripening, olive cultivars have different resistance to thermal or stress conditions. Oil composition may be affected by altitude, temperature of environment, rain and/or irrigation. Phenotypic stability of olive oil may be increase or decrease by temperature of environment or harvesting year. Olives demonstrate early pigmentation which caused rapid degradation of chlorophyll by high temperatures. However, higher content of unsaturated fatty acids observed at low temperature environment. Also, content of volatile compounds reduce by temperature. Higher water availability cause to reduce the oil content of phenolic compounds by rain or irrigation. Fatty acid content can be effected by altitude. Oils from plants grown at higher altitudes have more stability against oxidation (Di Vaio et al., 2012).

1.3 Nutritional value of olive oil

Olive oil constitutes an important part of the Mediterranean diet as the main fat source. Consuming olive product reduces the occurrence of cardiovascular disease in the Mediterranean countries due to presence of low saturated fatty acid content and high monounsaturated fatty acid content. In addition, regularly consumption of olive oil helps to lower the blood pressure significantly (Psaltopoulou et al., 2004). HDL cholesterol plays a protective and anti-atherogenic role by promoting the elimination of LDL cholesterol. HDL cholesterol considerably decreases by rich high-polyunsaturated fat diets which include seed oils. Even, olive oil reduces serum cholesterol to the same rate as polyunsaturated fats without reducing HDL cholesterol. Phenolic compounds of olive are important in human diet and health for acting as antioxidants and free radical scavengers.

Lipid peroxidation is a degradative, free radical mediated process which is responsible for unpleasant odors and flavors formation in oils and foods. In addition, functional abnormalities and pathological changes occur due to oxidation of polyunsaturated fatty acids of the biomembranes. Tocopherol isomers are chain-breaking antioxidants and they are known as free radical scavengers. α-tocopherol is the most biologically active form of vitamin E and efficiently transfers a hydrogen atom to a lipid free radical (peroxyl, alkoxyl and carbon-centered radicals) giving the corresponding non-radical product of the lipid and an α-tocopheroxyl radical. To form a non-radical product, α-tocopheroxyl radicals react with a second free radical or with another α-tocopheroxyl radical. Each molecule of α-tocopherol consumes two lipid free radicals and eliminates the free-radical chain reaction (Yamauchi, 1997).

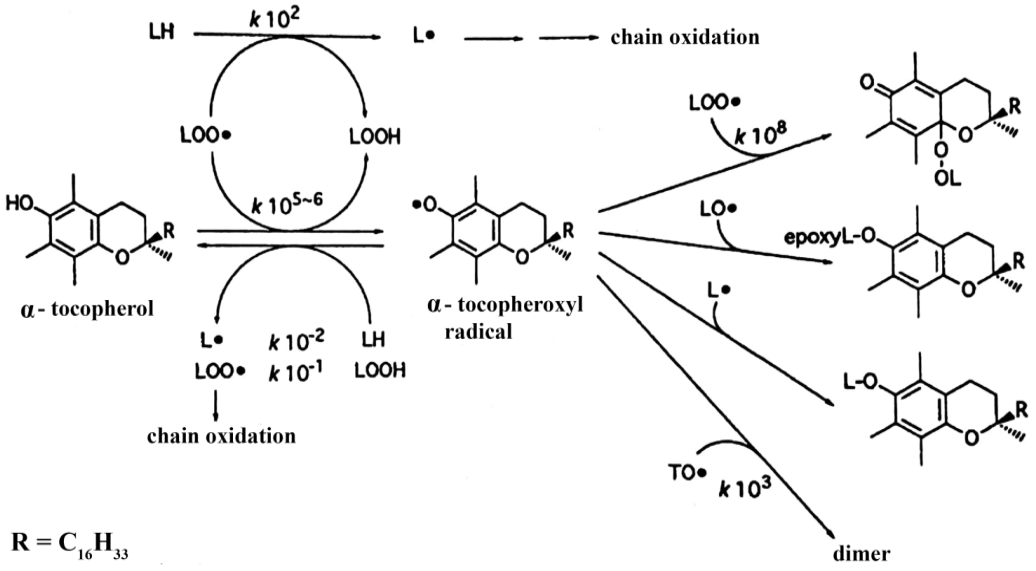


Figure 1.3: α-tocopherol reaction during autoxidation of unsaturated lipids (Yamauchi, 1997)

1.4 Olive oil processing and classification

Olive oil is the oil obtained only from the fruit of olive tree (*Oleaeuropaea L.*).

Through the centuries, fresh, high quality virgin olive oils have become one of the most widely accepted and used oils in culinary applications (Visioli and Galli, 1998; Boskou, 2009) especially for the people of the Mediterranean countries. However its use has nowadays expanded to other parts of the world due to its unique flavor, high contents of healthy monounsaturated fatty acids, and the presence of biologically important minor constituents (Uylaser and Yıldız, 2014). A chemical-free process using only pressure, cold pressing produces a higher quality of olive oil which is naturally lower in acidity.

The oil is obtained through grinding and pressing the olives using heavy granite millstones or modern stainless steel presses, percolation or centrifugation (Figure 1.4). Although the pressing process produces heat through friction, the temperature must not rise above 27^{o}C for the oil to be considered cold pressed virgin olive oil. Cold pressed oils retain all of their flavor, aroma and nutritional value of olives.

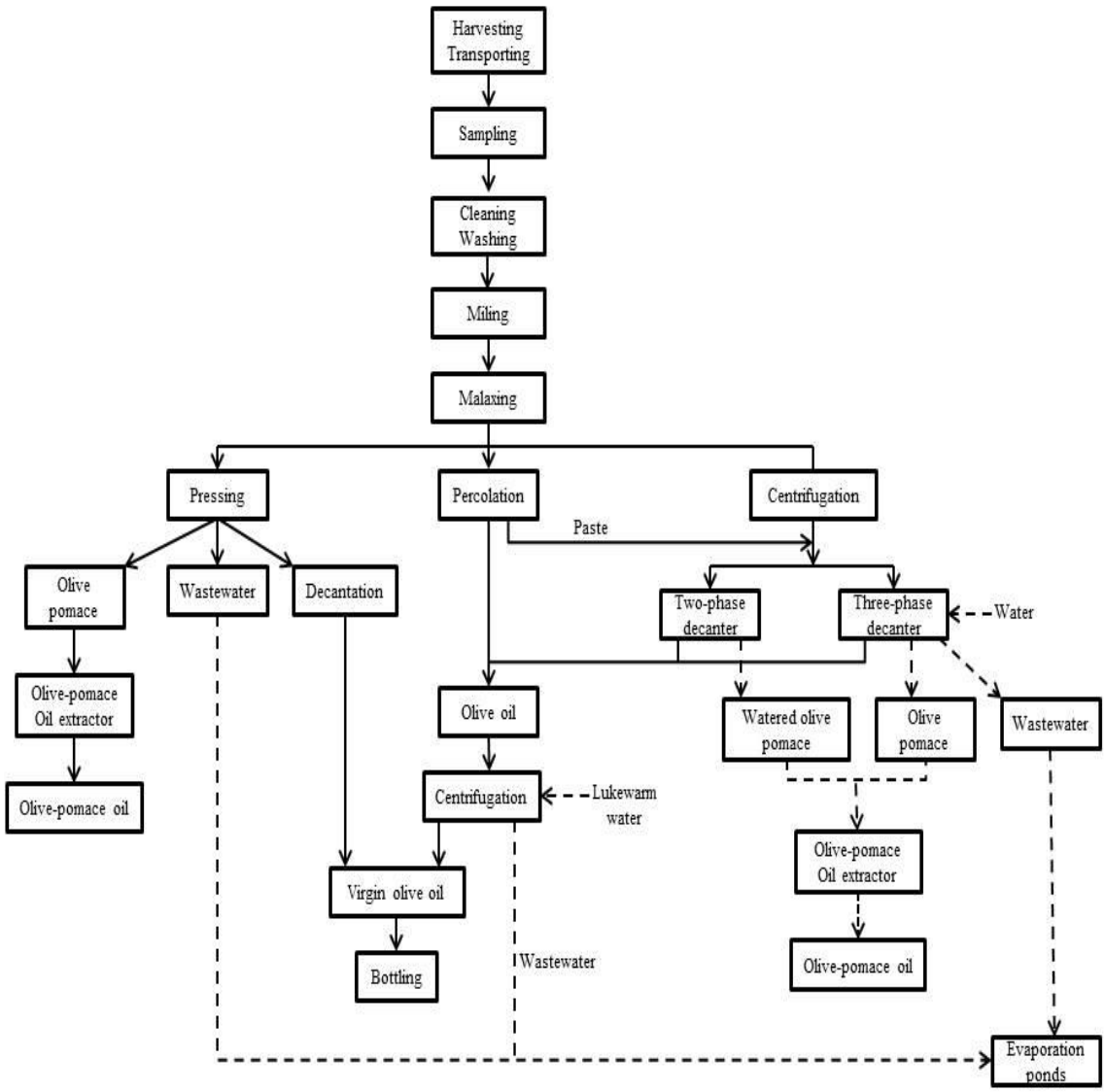


Figure 1.4: Flow diagram of olive oil production

Olives are harvested from tree and categorized by ripeness which is determinant variable in virgin olive oil quality. Then olives are transported to the oil mills. The time between harvesting and transportation is important to avoid significant changes in the oil profile and optimally ripe olives. When olives arrive to the olive mill, experts collect samples randomly from olive batches and classify olives as their maturity levels, quality and sanitary conditions by yield, sensory assessment, humidity and free acidity analyses. Then, olives are washed to remove foreign materials and dirt by machinery which has powerful

fans and pipes with forced water circulation. Cleaned and washed olives are moved to the crusher and they are crushed to assure the taste and aroma of the end product and the yield of the extraction process. Crushing process is followed with the malaxation process. The mixing or malaxation process reduces emulsions that lower yield which could happen in crushing process, and causes the olive droplets to coalesce, thus percentage of available oil increases. Malaxation process provides optimal oil extraction, antioxidants and flavor value. Olive paste is mainly made up of olive oil and olive mill wastewater which are liquid and small pieces of kernel and tissues which are solid. They need to be separated in order to obtain olive oil. Pressure, percolation and centrifugation could be used for extraction process.

Centrifugation is a continuous process that is able to separate olive oil from water and solid parts by centrifugal force. Separation is performed inside a decanter, a cylindrical bowl with co-rotating scroll with helical blades that rotate. Liquid (oil and water) and solid (olive pomace) constituents of olive paste moves to different ends of decanter centrifuge by rotation. The paste removes from the bottom of the malaxing vats by means of a mono pump feeding the paste to the decanter centrifuge. In the three-phase decanter centrifuge, lukewarm water is added to increase the fluidity of the pumped paste and to assist in separating the liquid and solid phases by centrifugal force. In two-phase decanter centrifuge, addition of water do not need so they are more environmental friendly due to decreasing the amount of wastewater.

After the separation of olive oil and other constituents by three-phase decanter centrifuge or two-phase decanter centrifuge, final centrifugation is applied to remove water and small solids from the oil. This centrifugation is performed in vertical centrifuges which rotate at high speed and lukewarm water is added to clean the oil of fine solids. Virgin olive oil after those processes is stored in sealed stainless steel tanks which protects olive oil from oxidation and by-product formation (“Olive Oil”, 2019).

Virgin olive oils are divided into several groups such as extra virgin olive oil, virgin olive oil, ordinary virgin olive oil and lampante virgin olive oil. Extra virgin olive oil (EVOO) definition is only related to free acidity level which is below 0.8%, if free acidity level of oils are not more than 2%, they are considered as virgin olive oil and free acidity level not more than 3.3% considered as ordinary virgin olive oil. Lampante virgin olive oil is not fit for consumption has free acidity level more than 3.3% and intended for refining or technical use according to International Olive Council (IOC) standards and Turkish Food Codex.

Refined olive oil obtained from virgin olive oil by refining methods which do not lead alterations in the initial glyceridic structure and has free acidity level not more that 0.3%.

Riviera olive oil is the oil consisting of blend of refined olive oil and virgin olive oils fit for human or culinary consumption as they are and has a free acidity level not more than 1%.

Olive-pomace oils are obtained by treating olive pomace with solvents, to the exclusion of oils obtained by re-esterification processes and of any mixture with oils of other kinds. They are defined as crude olive-pomace oil, refined olive-pomace oil and olive-pomace oil. Crude olive-pomace oil is intended for refining for use for human consumption or for technical use. Refined olive-pomace oil obtained from crude olive-pomace oil by refining methods which do not lead to alterations in the initial glyceridic structure and has free acidity level not more than 0.3%. Olive-pomace oil is the oil contains the blend of refined olive-pomace oil and virgin olive oils which fit for consumption as they are and has free acidity level not more than 1% (“Designations and definitions of olive oils”, 2015).

1.5 Rafination of olive oil and other vegetable oils

The traditional refining process of crude vegetable oils generally includes the steps of degumming, neutralization, bleaching and deodorization. However, during these processes, high amounts of the micronutrients and antioxidants such as, polyphenols, tocopherols, sterols, carotenoids are lost by chemicals used and high temperatures which reduce substantially the nutritional value and quality of vegetable oils (Szydlowska-Czerniak, 2013). Thus, refined olive oil has the same glyceridic composition as virgin olive oil, but contains less alpha-tocopherol and squalene (Boskou, 2009).

1.6 Adulteration

As olive oil is usually much more expensive than other edible oils, it has historically been one of the most frequently adulterated products not only in European Union and USA, but also almost all over the world. Food adulteration can be defined as lowering the quality of food by intentional, inclusion of poor quality of substances which have similar properties to the foods they are added, or unintentional, inclusion of unwanted substances during process because of lack of proper facilities and hygiene, carelessness or ignorance, substitution of food with some inferior foreign particle or by removal of some value added food substitute from main food item. Adulterated food is dangerous, as it may be toxic and effect health, it could deprive nutrients required to maintain proper health, and it may cause intoxication or problems such as allergy in sensitized individuals (Bansal et al., 2017).

The increasing number of food adulterants or contaminants in food has raised alarms about food safety and has resulted in tremendous improvements in analytical methodologies to analyze contaminants and adulterants. Nowadays food laboratories are forced to replace their classical procedures with modern analytical techniques that allow them to provide an adequate answer to global demands on food safety, quality and traceability leading to development of more convincing analytical methodologies including molecular methodologies for easy and low cost adulterant detection in food (Wright, 2009). Three basic strategies can be followed for demonstrating adulteration by the presence of foreign

substance or a marker in the commodity, that a component is deviated from its normal level and that a profile is unlikely to occur (Wilhelmsen, 2004; 2006).

The aim of our study was to develop an easy, quick, cost effective and environmentally friendly screening method to determine adulteration in cold pressed olive oil in the presence of other cheaper oils such as rapeseed oil, sunflower oil and corn oil based on their α-tocopherol content as tocopherols can be determined by electrooxidation. For this purpose, cyclic voltammetry and differential pulse voltammetry were applied to the oil mixtures directly adsorbed on pencil graphite electrode (PGE).

CHAPTER 2

THEORETICAL FRAMEWORK

Detection of olive oil adulteration is a challenging analytical problem, as olive oil consists of complex mixtures of triacylglycerols (TAGs), free glycerides, hydrocarbons, tocopherols, pigments, sterols, alcohols, triterpene acids, volatile compounds, phenolic compounds and phospholipids. There are various methodologies developed over the decades for detection of other vegetable oils in olive oil. There are three main methods for detection of adulteration.

2.1 Detection methods

2.1.1 Spectroscopic methods

Spectroscopic methods are commonly used due to their rapid and nondestructive advantages, such as total synchoronous fluorescence (TSyF) spectra, mid-infrared, fourier transform infrared (FT-IR), nuclear magnetic resonance (NMR) and Raman (Yang et al., 2013). Mass spectrometry (MS) techniques include the formation of ions from atoms or molecules, separation according to the mass to charge ratio, and then detection. MS gives qualitative and quantitative information about the atomic or molecular composition of organic or inorganic materials. Optical spectroscopic techniques involve the interaction of electromagnetic radiation with atoms or molecules where matter absorbs, emits, or scatters electromagnetic radiation. In recent years, some mass spectrophotometric methods coupled with chromatographic separations such as direct analysis in real time-high resolution mass spectrometry (DART-HRMS), solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC/MS), liquid chromatography-mass spectrometry (LC-MS), quadrupole time of flight mass spectrometer-mass spectrometry (QTOFMS-MS) are utilized to determine and compare triacylglycerol (TAG) composition of olive oils.

Chromatographic methods are currently used, such as supercritical fluid chromatography (SFC) leverages to improve separation efficiency, speed and selectivity by using super critical fluids which has dual characteristics of gas and liquid (Jiang et al., 2018). SFC is an environmentally friend technique, consuming less organic solvents than HPLC. HPLC is the most commonly used method. Variable detectors have been used for HPLC such as electrochemical detector (ED), fluorescence detector (FLD), ultraviolet detector (UVD) and mass spectrometric detector (MSD) and mass/mass spectrometric detector. In addition, HPLC includes normal-phase (NP) and reverse-phase (RP) systems (Lu et al., 2015). Stable isotope ratio analysis (GC-IRMS) is also applied to determine the origin of virgin olive oils. However, all these techniques are tedious, time-consuming and need instrumentation with high cost; it is essential to have highly educated/trained analysts in such sophisticated methods and instruments.

2.1.2 Capillary Electrophoresis

Capillary electrophoresis (CE) methods are used due to their high efficiency, cost effectiveness and insignificant environmental impact and play an important role in the analysis of constituents in different matrix. Although capillary electrophoresis system has very wide application ranges, it could not separate vitamin E isomers (tocopherols and tocotrienols) which are fat soluble. So, scientists developed non-aqueous capillary electrophoresis (NACE), capillary electrochromatography (CEC) and microemulsion electrokinetic chromatography (MEEKC) to improve the separation efficieny (Lu et al., 2015).

2.1.3 Electrochemical methods

In recent years, substantial efforts have been focused on development of simplified, fast and cost effective approaches. In this aspect, electrochemical techniques are very promising with their high sensitivity, simplicity, miniaturization and low cost. These techniques can provide a non-specific fingerprint of oil samples. The employment of voltammetric techniques for the detection of olive oil adulteration is very rarely described in the literature (Apetrei et al., 2014). Apetrei et al. (2014) used chemically modified

carbon paste electrodes using three virgin olive oils of different quality, a refined olive oil and two seed oils. Based on their previous work on chemically modified electrodes (Apetrei et al., 2005; 2006), Apetrei and Apetrei developed also voltammetric e-tongues for the detection of olive oil adulteration with seed oils (Apetreiand Apetrei, 2014). Oliveri et al. also achieved a discrimination of olive from maize oils as well as classification of olive oils according to their geographical origin (Oliveri et al., 2009). Tsopelas et al. were used glassy carbon electrode for voltammetric fingerprinting of extra virgin olive oil with olive pomace oil as well as the most common seed oils, such as sunflower, soybean and corn oil, by either direct analysis of diluted oils or using methanolic extracts of them (Tsopelas et al., 2018).

2.2 Theory of electrochemistry and some electrochemical techniques

Electrochemical techniques are related to the interaction between electricity and chemistry, expressly the measurements of electrical quantities, such as current, potential, or charge, and their relationship to chemical parameters. There are variable applications of using electrochemical measurements such as industrial quality control, and biomedical analysis.

Electrochemical processes take place at the electrode-solution interface. Potentiometric and potentiostatic measurements are the two principal types of electrochemistry. Both of them need at least two electrodes as conductors and a contacting sample (electrolyte) solution, which compose the electrochemical cell. One of these electrodes which called working electrode responds to the target analyte. Other one called reference electrode which is of constant potential due to independent of the properties of the solution.

Potentiometry is a static (zero current) technique in which information about the sample composition is obtained from measuring the potential through a membrane. Potentiostatic also known as controlled potential, techniques relate to the study of charge transfer processes in the electrode-solution interface and are based on dynamic (no zero current) conditions. The electrode potential is used to derive an electron transfer reaction and the

resulting current is measured while chemical species gaining or losing an electron. The resulting current demonstrates the rate at which electrons move along the electrode solution interface. Any chemical species that is electroactive (reduce or oxidize) can be measured by potentiostatic techniques.

The purpose of controlled-potential electrochemical experiments is to obtain a current response which is related to the concentration of the target analyte. This accomplished by monitoring the electron transfers during the redox process of the analyte.

$$O + ne^- \leftrightharpoons R \qquad (2.1)$$

Where O is oxidized and R is reduced forms of redox couple. Such a reaction occurs in a potential region which makes the transfer of electrons thermodynamically or kinetically suitable. The potential of the electrode can be used to determine the concentration of the electroactive species at the surface [$C_O(0,t)$ and $C_R(0,t)$] according to the Nernst equation for systems controlled by the laws of thermodynamics.

Nernst equation:

$$E = E^o + \frac{2.3RT}{nF} \log \frac{C_O(0,t)}{C_R(0,t)} \qquad (2.2)$$

Where E^o is the standard potential for the redox reaction, R is the universal gas constant (8.314 J K^{-1} mol^{-1}), T is the temperature (Kelvin), n is the number of electrons transferred in the reaction and F is the Faraday constant (96,487 coulombs). The current caused by a change in the oxidation state of the electroactive species is called the faradaic current. Faradaic current is a direct measure of the rate of the redox reaction. Voltammogram is the resulting current-potential plot and it is a display of current signal on vertical axis versus the excitation potential on horizontal axis. The exact shape and magnitude of the

voltammetric response is governed by the processes involved in the electrode reaction. The total current is the summation of the faradaic currents for the sample and blank solutions.

The path of the electrode reaction can be quite complex and is carried out in a row containing several steps. The rate of such reactions is determined by the slowest step in the sequence. Mass transport of the electroactive species to the electrode surface, the electron transfer across the interface and the transport of the product back to the bulk solution are the simple reactions that involve. Mass transport occurs by diffusion, convection and migration. The spontaneous movement under the influence of concentration gradient which aimed at minimizing concentration differences called diffusion. Transportation of the electrode by a gross physical movement (stirring, rotating or vibration of electrode) called convection. Movement of charged particles along an electrical field called migration.

When three modes of mass transport occur simultaneously, it becomes complicated to relate the current to the analyte concentration. This condition can be substantially simplified by suppression of electromigration or convection by the addition of excess inert salt or by the use of a quiescent solution, respectively and the movement of the electroactive species is limited by diffusion. The reaction on the surface of the electrode produces a concentration gradient adjacent to the surface which causes a diffusional flux (Wang, 2000).

2.2.1 Cyclic voltammetry

Cyclic voltammetry used to acquire qualitative information on electrochemical reactions. Significant information on the thermodynamics of redox processes, on the kinetics of heterogeneous electron transfer reaction, and on coupled chemical reactions or adsorption processes can rapidly provided by cyclic voltammetry results. Cyclic voltammetry is usually the first experiment performed in an electrochemical study due to offering a rapid location of redox potentials of the electroactive species, and convenient evaluation of the effect of media upon the redox process.

Cyclic voltammetry is a linear scan of the potential of a stationary working electrode (in an unstirred solution) using a triangular potential waveform as shown in the Figure 2.1. During the sweep potential, the resulting current is measured from the applied potential by the potentiostat. Cyclic voltammogram is the resulting plot of current versus potential.

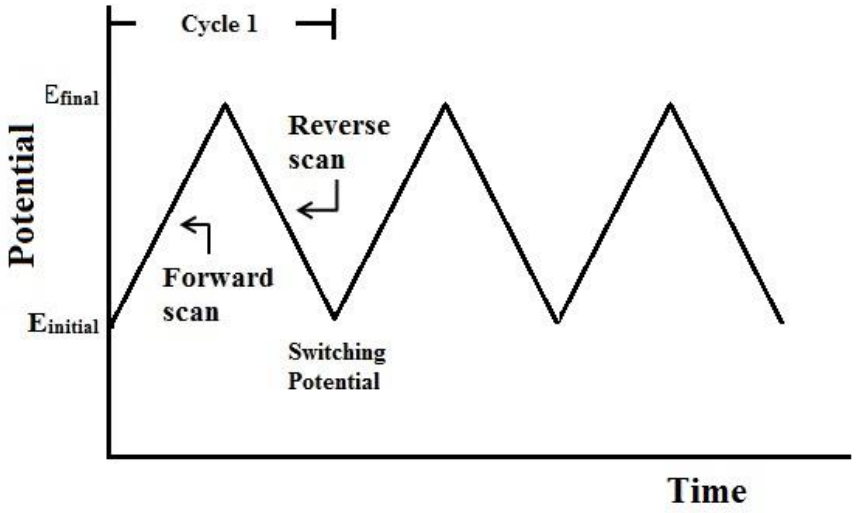


Figure 2.1: Potential-time excitation signal of cv experiments

The expected response of a reversible redox couple ($O + ne^- \leftrightharpoons R$) during a single potential cycle is shown in Figure 2.2.

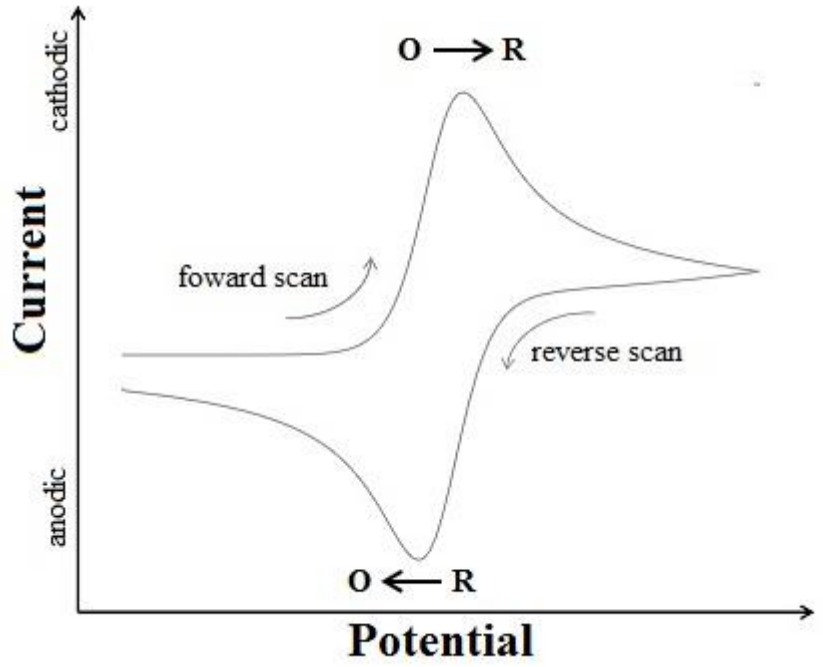


Figure 2.2: Cyclic voltammogram of reversible redox process

Initially it is assumed that only the oxidized O form is present. For the first half a negative-going potential scan is chosen, starting from a value where no reduction occurs. A cathodic current begins to increase until a peak is reached as the potential approaches the characteristic E^o for the redox process. The direction of the potential sweep is reversed when the potential region where the reduction process has taken place traversed. R which is generated in the forward half cycle is reoxidized back to O and an anodic peak results during the reverse scan. Formation of the diffusion layer near the electron surface causes the characteristic peaks of cyclic voltammogram (Wang, 2000).

2.2.2 Differential pulse voltammetry

The aim of pulse voltammetric techniques is lowering the detection limits of voltammetric measurements. Measuring trace levels of organic and inorganic species makes the differential pulse voltammetry very useful technique. Fixed magnitude pulses which are superimposed on a linear potential ramp, are applied to the working electrode at a time just before the end of the drop in differential pulse voltammetry as shown in Figure 2.3.

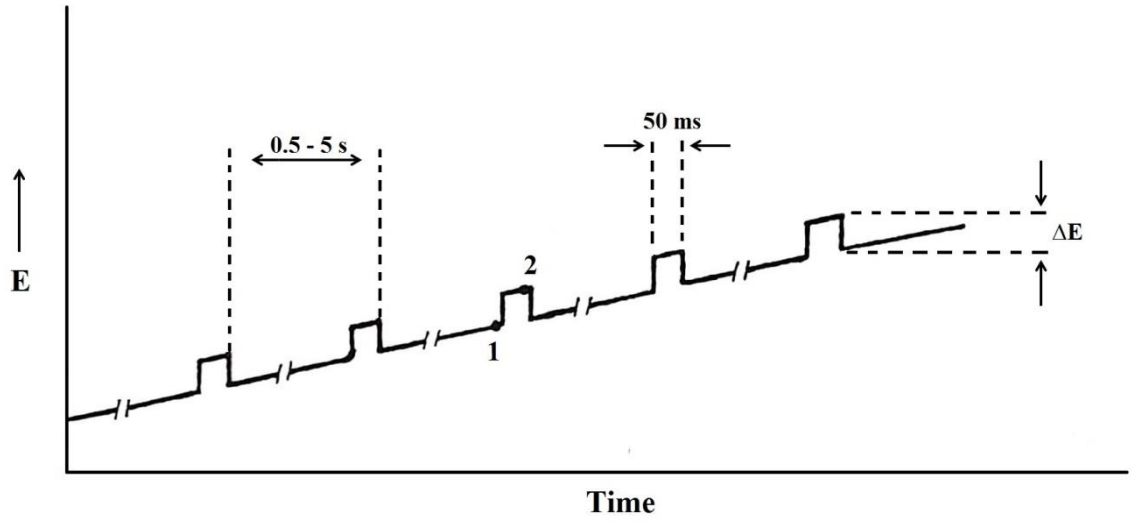


Figure 2.3: Excitation signal of differential pulse voltammetry

In differential pulse voltammetry, the current is sampled twice. One is just before the pulse application (at point 1 in Figure 2.3) and other one late in the pulse life (at point 2 in Figure 2.3) when the charging current has decayed. The first current is instrumentally subtracted from the second current. This current difference [$\Delta i = i(t_2) - i(t_1)$] is plotted versus the applied potential. The resulting differential pulse voltammogram consists of the current peaks whose height is directly proportional to the concentration of the corresponding analytes:

$$i_p = \frac{nFAD^{1/2}C}{\sqrt{\pi t_m}} \left(\frac{1-\sigma}{1+\sigma}\right) \quad (2.3)$$

Where σ = exp[(*nF/RT*)(Δ*E*/2)] and Δ*E* is the pulse amplitude. Large pulse amplitudes obtained from the maximum value of the quotient (1 – σ)/(1 + σ) which is unity. If species occur near the polarographic half wave potential, they can be identified by using the peak potential (E_p):

$$E_p = E_{1/2} - \Delta E/2 \quad (2.4)$$

The differential pulse process results in a very efficient correction of the charge background current. The charging current contribution to the differential current is negligible. It can be described as:

$$\Delta i_c \simeq -0.00567 C_i \, \Delta E m^{2/3} t^{-1/3} \quad (2.5)$$

Where C_i is the integral capacitance.

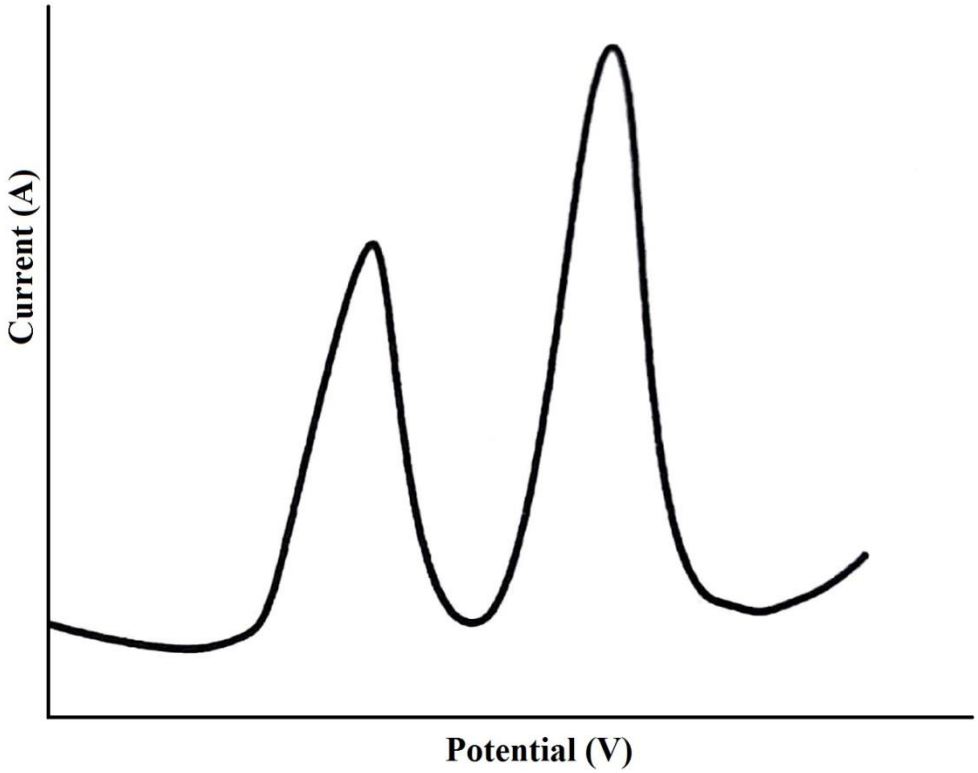


Figure 2.4: A typical differential pulse voltammogram

The selection of pulse amplitude and potential scan rate generally requires a balance between sensitivity, resolution and speed. Large pulse amplitudes cause larger and wider peaks. Pulse amplitudes of 0.025V coupled with a 0.01V/s scan rate are commonly used. Besides the improvement of sensitivity and resolution, information about the chemical form of analyte appear can be provided by differential pulse technique (Wang, 2000).

CHAPTER 3

RELEATED RESEARCH

Olive oil is a healthy food due to its nutritional value and has important positive effects on human health. Other edible oils are cheaper than olive oil. Therefore, olive oil the most frequently adulterated products. Adulteration foods may effects human health negatively as they could be toxic, cause allergy problems and deprive the nutrients needed to maintain proper health. For this reason, some researchers study on detection of olive oil adulteration by using various methods. Some of these researches will be summarized below.

Yang et al (2013) carried out a study on detection and identification of extra virgin olive oil adulteration with corn, peanut, rapeseed and sunflower oils by using GC-MS combined with chemometrics. They used 22 fatty acids and 6 parameters such as ratio of oleic/linoleic acid, linoleic/linolenic acid, total saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, MUFAs/PUFAs as variables for evaluation. For detecting adulteration, they performed univariate analyses and multivariate analyses. They observed higher contents of tetracosanoic acid, eicosanoic acid, docosanoic acid, and total saturated fatty acids were the peculiarities of peanut adulteration and higher levels of linolenic acid, 11-eicosenoic acid, nervonic acid and erucic acid as the characteristics of rapeseed adulteration as a result of the univariate analyses. They found 1% detection limit and 90% prediction ability by PLS-LDA and identified the type of adulteration with 85% prediction ability by Monte Carlo tree.

Bakre et al. (2014) studied to determine the presence of sunflower oil in olive oil. They selected α-tocopherol as discriminating parameter for detecting sunflower oil adulterant in olive oil. They prepared admixtures of olive oil and sunflower oil as 5%, 10%, 15% and 20% of sunflower oil in olive oil and analysed them by using reversed phase high pressure liquid chromatography coupled with fluorescence detector. The chromatographic system

made up of a C18 column with methanol: acetonitrile (50:50) mobile phase. They set the fluorescence detector at 290 nm the excitation wavelength and the emission wavelength at 330 nm. They observed that the concentration of α-tocopherol increased linearly in olive oil adulterated with sunflower oil. Their method was simple, selective, sensitive and precise (RSD=2.65 %) for α-tocopherol and precisely detected 5 % sunflower oil in olive oil.

Apetrei and Apetrei (2014) carried out a study on detection of virgin olive oil adulteration by using voltammetric e-tongue. They prepared different percentages of sunflower oil, soybean oil and corn oil with extra virgin olive oil. They used Ag/AgCl with 3M KCl as reference electrode, platinum wire as counter electrode and carbon paste electrodes modified with oils (carbon paste based sensor) as working electrode. They used kernel method to process the square wave voltammetric signals. The applied chemometric methods allowed the discrimination and classification of oils according to botanical origin. They obtained PLS regression from the correlation between voltammetric signals and polyphenolic content. They observe PLS-DA and PLS regression showed that it was possible to detect the adulterations of olive oil with less than 10% of sunflower, soybean and corn oil.

Diaz et al. (2004) studied voltammetric behavior of Vitamin E in the olive oil to the determination of the tocopherols in different vegetable oil samples. They used Ag/AgCl with 3M KCl as reference electrode, platinum wire as counter electrode and glassy carbon electrode as working electrode in a hexane–ethanol medium and applied direct current (DC), differential pulse voltammetry (DPV) and square wave voltammetry (SWV). They investigated the effect of variables such as hexane-ethanol ratio, sulfuric acid concentration and instrumental parameters. They obtained separate voltammetric peaks of α-tocopherol and δ-tocopherol while peak of β-tocopherol and γ-tocopherol were overlapped. They used PLS multivariate calibration method and compared the results of DC and DPV voltammograms. They obversed DPV voltammograms give best data set.

Sys et al (2017) studied on total content of tocopherols to determination of vitamin E in margarines and edible oils. Their system was consist of glassy carbon paste electrode as working electrode, Ag/AgCl with 3M KCl salt bridge as reference electrode and platinum wire as counter electrode and they used square wave anodic stripping voltammetry (SWASV) in 0.1 M HNO_3 for detection. Their method was based on extraction of biologically active compounds into silicone oil, acted as lipophilic binder of glassy carbon paste electrode. They determined the linear ranges for α-tocopherol 5×10^{-7} - 4×10^{-5} and 5×10^{-8} - 1×10^{-5} mol/L with 1×10^{-7} and 3.3×10^{-9} mol/L detection limits for 5 and 15 minutes respectively.

Tsopelas et al. (2018) studied on voltammetric fingerprinting of oils and their combination with chemometrics to detect the adulteration of extra virgin olive oil with olive pomace, sunflower oil, soybean oil and corn oil. They use 0.1 M of LiClO4 in EtOH as electrolyte, platinum wire as counter electrode, Ag/AgCl with 3M KCl as reference electrode and glassy carbon as working electrode. They applied cyclic voltammetry in presence of dichloromethane to the diluted extra virgin olive oils, regular olive oils (mixture of refined olive oils with virgin olive oils), olive pomace oils and seed oils. They also applied cyclic voltammetry to the methanolic extracts of olive and seed oils. They exported and submitted datas of cyclic voltammograms to Principal Component Analysis (PCA), Partial Least Square Discriminant Analysis (PLS-DA) and soft independent modeling of class analogy (SIMCA). They observed in diluted oils that PLS-DA ensure a clear discrimination between olive oils (extra virgin and regular) and olive pomace/ seed oils, while SIMCA provided a clear discrimination of extra virgin olive oil in regard to all other samples. They gathered more information with PLS-DA than SIMCA, from extra virgin olive oils, regular olive oils and seed/ olive pomace oils when they used methanolic extracts and considered datapoints recorded between 0.6 and 1.3 V. They developed a model based on Partial Least Square (PLS) analysis for quantification of extra virgin olive adulteration with olive pomace oil or seed oils and found detection limit of 2% and linearity range up to 33%.

CHAPTER 4

MATERIALS AND METHOD

4.1 Materials

4.1.1 Preparation of oil mixtures

Cold pressed olive oil obtained from local producer in Güzelyurt and kept in dark at room temperature, other adulterant vegetable oils, rapeseed, sunflower and corn (maize) oil, obtained from local markets. All adulterant vegetable oils were refined oils. Combined oil samples were prepared on weight basis by the addition of adulterant oil to cold pressed olive oil in different proportions (from 0 to 100% with 10% increments). Thus three sets of adulterated cold pressed olive oil were used for electrochemical measurements.

4.1.2 Tocopherol standards

As a source of α-tocopherol standard Evicap natural vitamin E – soft capsules of 200 I.U. corresponding to 134 mg/capsule obtained from local pharmacy was used. γ-tocopherol was pure standard by Supelco (USA,47785 LB61002). 0.025 g γ-tocopherol was dissolved in 2000 µl refined sunflower oil. Final concentration of γ-tocopherol was $1.2x10^{-7}$ molar.

4.1.3 Apparatus and electrodes

Electrochemical cyclic voltammetric and differential pulse voltammetric measurements were performed using AUTOLAB PGSTAT 204 (Utrecht, The Netherlands) potentiostat with NOVA 2.1.2 software. Reference electrode (RE) was an Ag/AgCl with 3M KCl, counter electrode (CE) was platinum wire and working electrode (WE) was pencil graphite lead. 0.5mm Pentel P205 model pencil and Tombow HB 0.5mm pencil leads obtained from bookstore in Cyprus were used as pencil graphite electrode (PGE). Pencil leads are made up of ~65% graphite, ~30% clay, and a binder (wax, resins, or high polymer).

4.1.4 Reagents

All electrochemical measurements were carried out in an electrochemical cell filled with pH 4.80, 0.5M acetate buffer solution (ABS).

Preparation of 0.5M acetate buffer solution:

20 mg of NaOH was dissolved in distilled water and made up volume to 500 ml for 1 N NaOH solution. Then 14.45 ml of glacial acetic acid was dissolved in 250 ml of distilled water and its ph was adjusted to 4.8 by addition of 1 N NaOH. After that 0.84 g NaCl was added to pH-adjusted acetic acid solution and made up volume to 500 ml. All aqueous solutions were prepared with deionized water.

4.2 Methods

4.2.1 Electrochemical activation of PGEs

Graphite pencil leads were cut in 3 cm length and 1.5 cm of pencil lead placed into the Pentel P205 model pencil which was used as a holder for each graphite lead. Chrono Amperometry was applied to bare PGE by applying +1.4V for 30 seconds in 0.5M acetate buffer solution (ABS) for activation and cleaning its surface.

4.2.2 Adsoption of oil matrix on PGE

Preliminary experiments were conducted to reveal optimum adsorption of oil on PGE by using various adsorption and drying periods. Application of 30 minutes for adsorption and 30 minutes for drying was optimum for voltammetric analysis of oils.

200 µl of oil mixtures were poured into the 200 µl eppendorf tubes and 1.5 cm of pretreated PGEs were immersed into the oil mixtures and kept at room temperature for 30 minutes for adsorption. After that PGEs were taken out and turned upside down and dried

for 30 minutes on a stand and then replaced into pencil (Figure 4.1 and 4.2). Electrochemical measurements were then performed in ABS (pH 4.80).

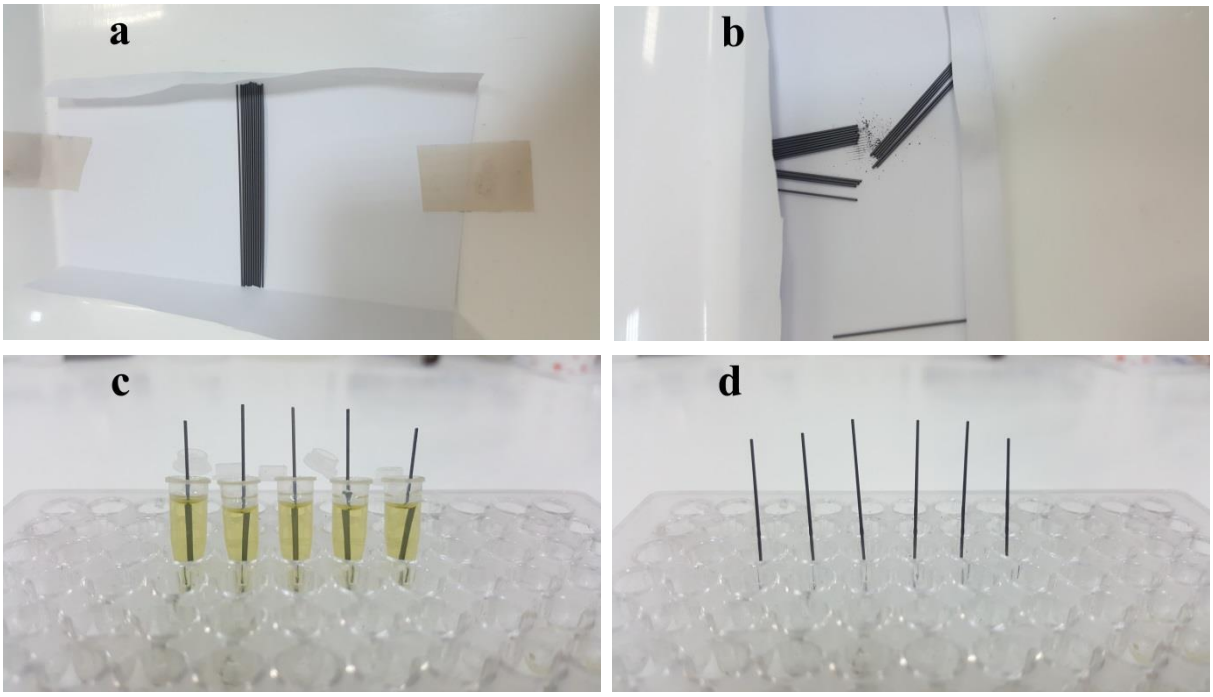


Figure 4.1: (a) Pencil graphite leads, (b) leads cut in 3 cm length, (c) adsorption of oil on graphite pencil lead, (d) Drying period of leads after adsorption

Figure 4.2: Pencil graphite electrode (PGE)

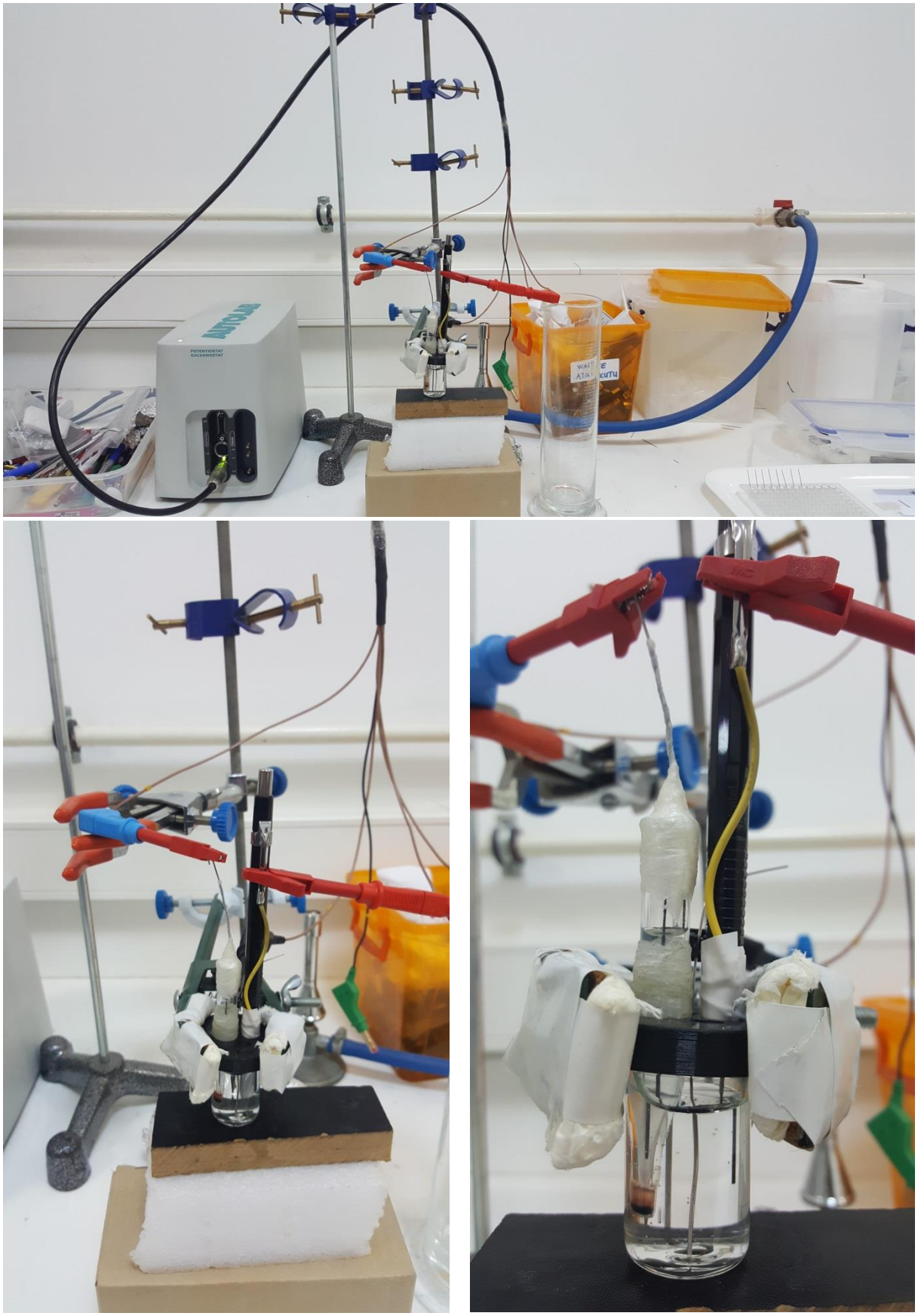


Figure 4.3: AUTOLAB PGSTAT 204 potentiostat

4.2.3 Cyclic voltammetric and differential pulse voltammetric measurements

The electrochemical behavior of α-tocopherol and γ-tocopherol in various oils was studied using cyclic voltammetry and differential pulse voltammetry techniques in acetate buffer system.

Cyclic voltammetry applied to olive oil, rapeseed oil, sunflower oil and corn (maize) oil separately to acquire qualitative information on electrochemical behaviours of various oils, i.e rapid location of redox potentials of electroactive species (tocopherols) and convenient evaluation of the effect of media upon the redox potentials and then differential pulse voltammetry applied to all oil samples and mixtures.

Oil-adsorbed PGE was connected to the potentiostat and immersed vertically into the ABS in the electrochemical cell (Figure 4.3).

Cyclic voltammetry (CV) measurements were performed with start potential of 0V, stop potential of 0V, upper vertex potential of 1.5V, lower vertex potential of -0.5V, scan rate of 0.1V/s and step of 0,00244V.

Differential pulse voltammetry (DPV) was applied to above mentioned oils used for these experiments separately and also for oil mixtures. Differential pulse voltammograms were scanned from 0V to 1.2V with a scan rate of 0.01V/s, voltage step of 0.005V and pulse amplitude of 0.025V. The raw data that obtained from differential pulse voltammetry measurements was smoothed by using Savitzky and Golay filter (level 2) and then followed by the moving average baseline correction.

4.2.4 Calculation of cut off value

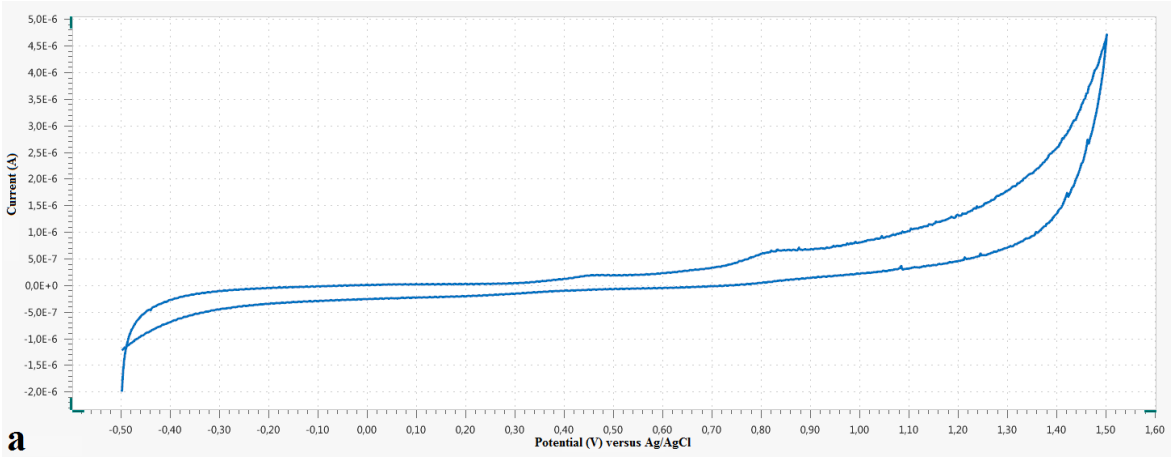
In order to determine the confidence level of a screening method, cut off concentration is defined as the concentration corresponding to the mean ± 2SD (Olivas, 2018; Singh, 2007).

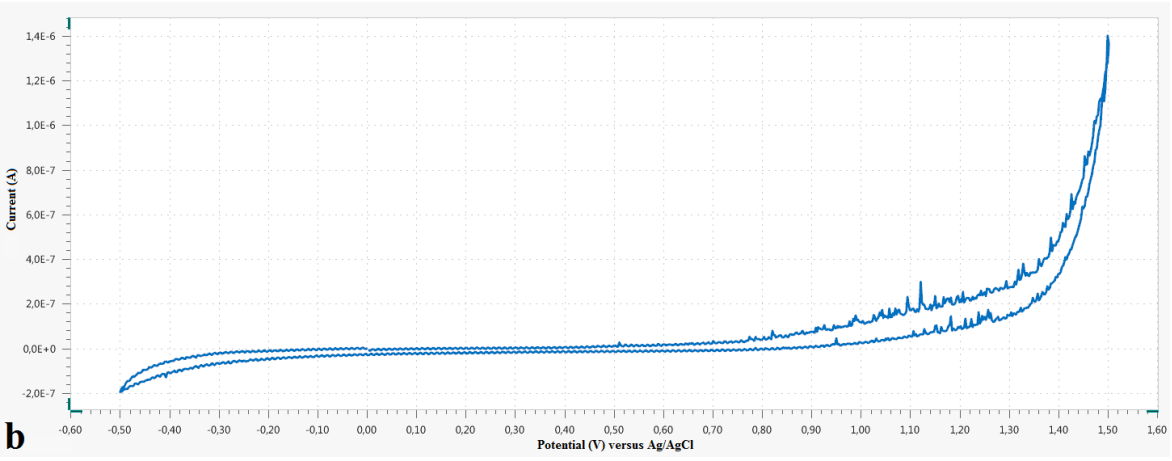
CHAPTER 5

RESULTS AND DISCUSSION

5.1 Direct cyclic voltammetric measurements of various oils

Inıtıally, cyclic voltammetry was directly employed to each oil i.e. cold pressed olive oil, rapeseed oil, sunflower oil and corn (maize) oil to obtain their electrochemical fingerprint involving redox peaks.





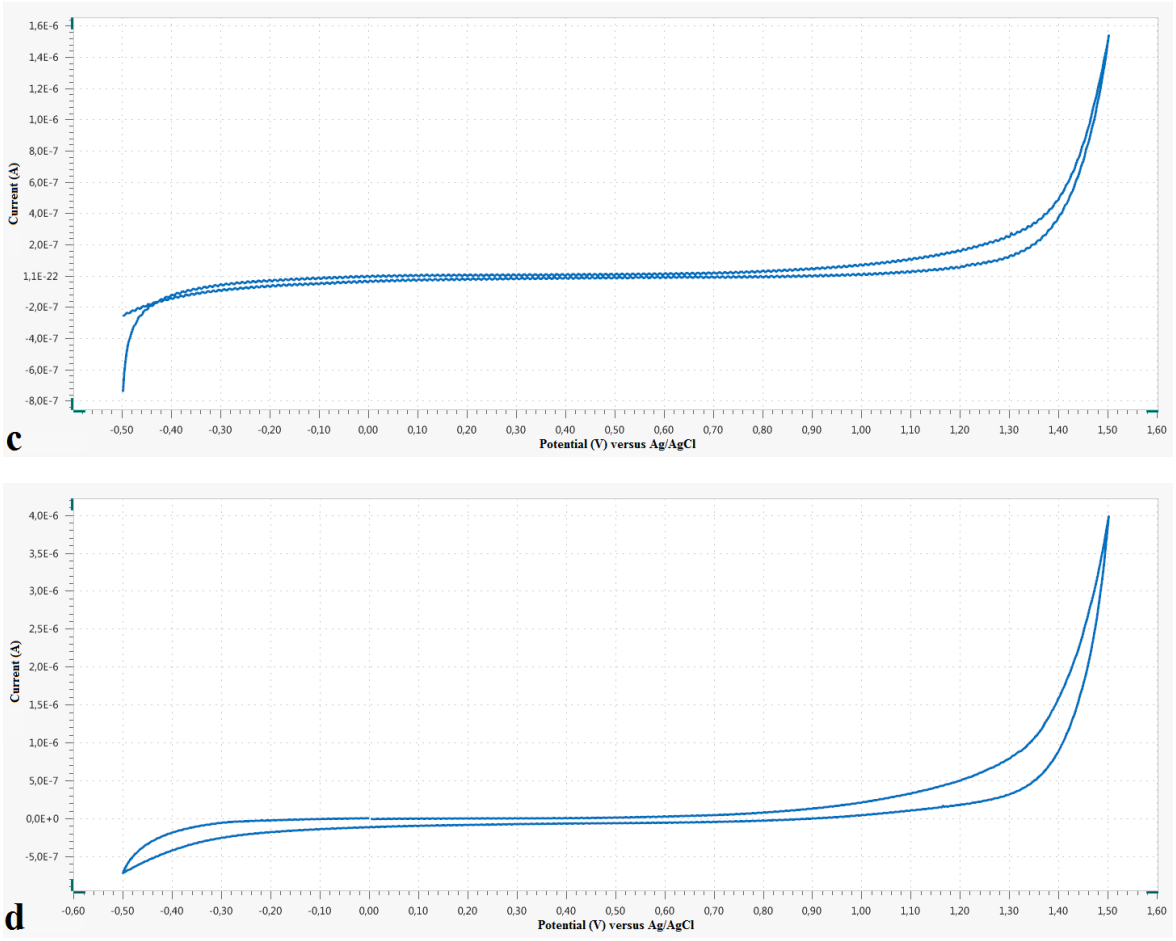


Figure 5.1: Cyclic voltammograms of cold pressed olive oil (a), rapeseed oil (b), sunflower oil (c), and corn (maize) oil (d)

No oxidation signal in the absence of tocopherol was recorded in ABS medium. Characteristic cyclic voltammograms obtained for each tested oils are represented in Figure 5.1. As can be seen in the voltammograms, no any oxidation or reduction peaks were found in sunflower, rapeseed and corn oil, whereas cold pressed olive oil gave two oxidation peaks in the range of 0.36-0.55V and 0.70-0.94V.

Tocopherols are likely to be oxidized in the range of 0.4-0.8V (Diaz et al., 2004).

5.2 Differential pulse voltammetric measurements

Based on cyclic voltammetry studies of various oils discussed above, differential pulse voltammetry measurements were performed in the range of 0-1.2V.

5.2.1 DPV measurements of α- and γ-tocopherol

Typical voltammograms of α- and γ-tocopherol were represented in Figure 5.2.

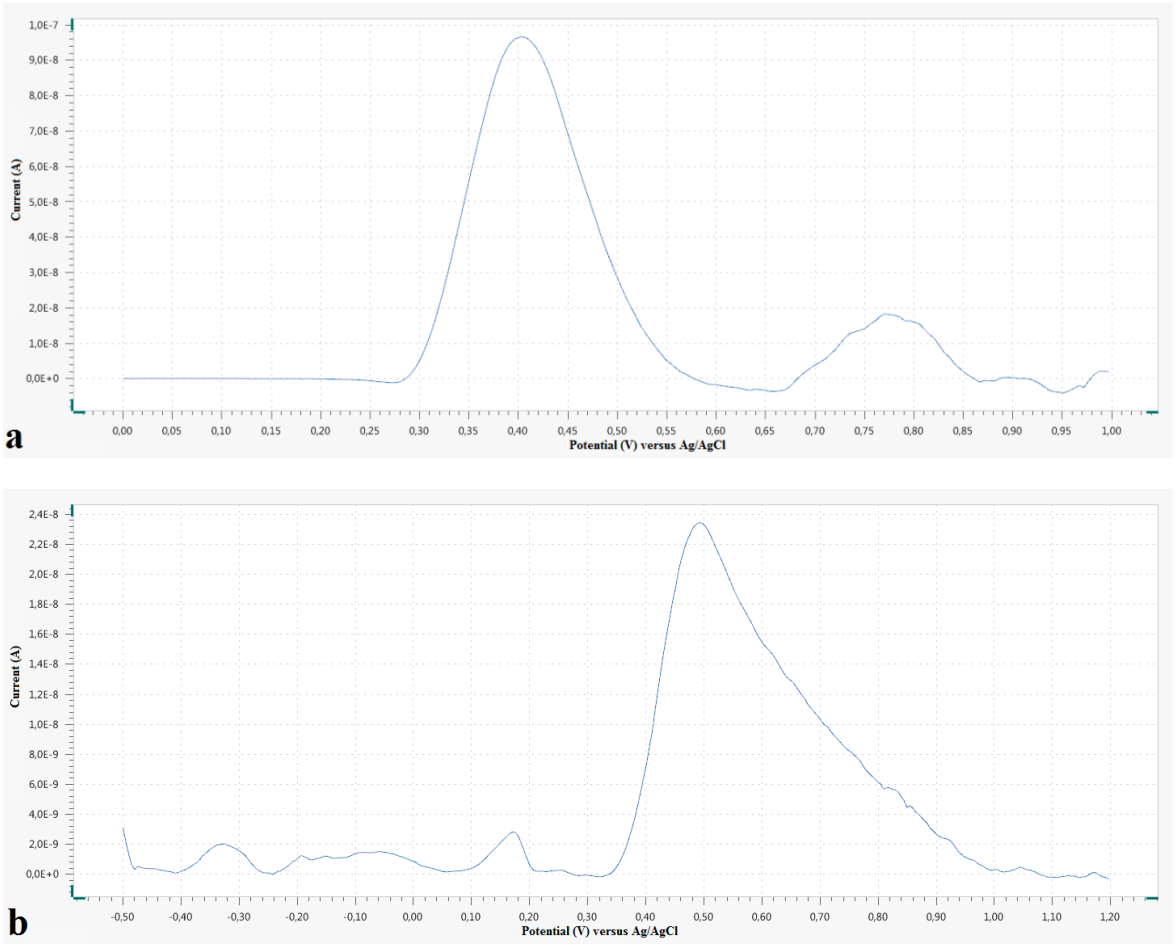


Figure 5.2: Differential pulse voltammograms of α-tocopherol (a) and γ-tocopherol (b)

α-tocopherol gave oxidation peak in the range of 0.28-0.60V, peak position of 0.40V and average peak height of $9.8x10^{-8}$A, where γ-tocopherol gave oxidation peak in the range of 0.34-1.00V, peak position of 0.49V and average peak height of $2.35x10^{-8}$A.

5.2.2 Differential pulse voltammetry of individual oils used

Cold pressed olive oil contains high amounts of α-tocopherol because no chemical processes or heat applied to it while production. Cold pressed olive oil gave two oxidation peaks in the range of 0.24-0.52V and 0.67-0.90V, peak positions of 0.41V and 0.77V, and average peak height of $3.34x10^{-8}$A and $3.07x10^{-8}$A, respectively as shown in Figure 5.3. By comparing oxidation peak range and position with α-tocopherol, it is obvious that cold pressed olive oil contains α-tocopherol at 0.4V.

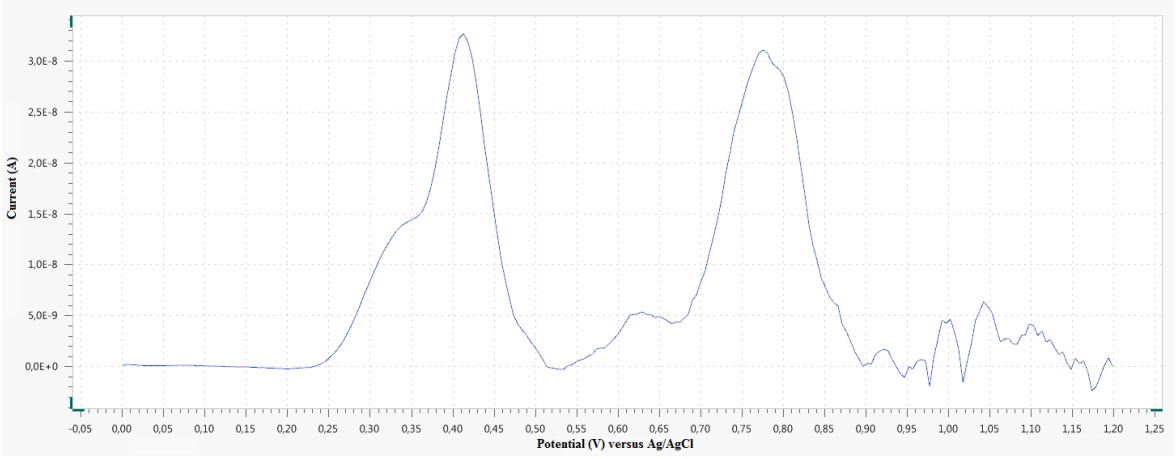


Figure 5.3: Differential pulse voltammogram of cold pressed olive oil

Rapeseed oil contains only γ-tocopherol due to its oxidation peak range and position. Rapeseed oil gave oxidation peak in the range of 0.40-0.73V, peak position of 0.48V and average peak height of $1.75x10^{-9}$A, where peak position of γ-tocopherol is 0.49V. Voltammogram of rapeseed oil is represented in Figure 5.4.

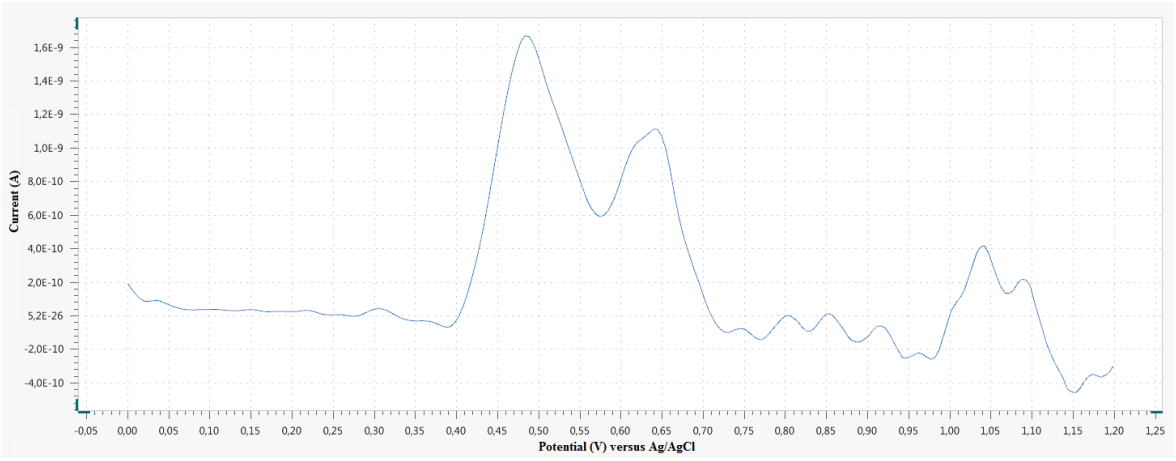


Figure 5.4: Differential pulse voltammogram of rapeseed oil

Sunflower oil and corn oil contain neither α-tocopherol nor γ-tocopherol because they are refined oils and lost almost all tocopherol content during refining process. Both sunflower and corn oil gave approximately 10^{-10}A at peak position of α-tocopherol and γ-tocopherol, and it was considered as noise as it shown in the Figure 5.5.

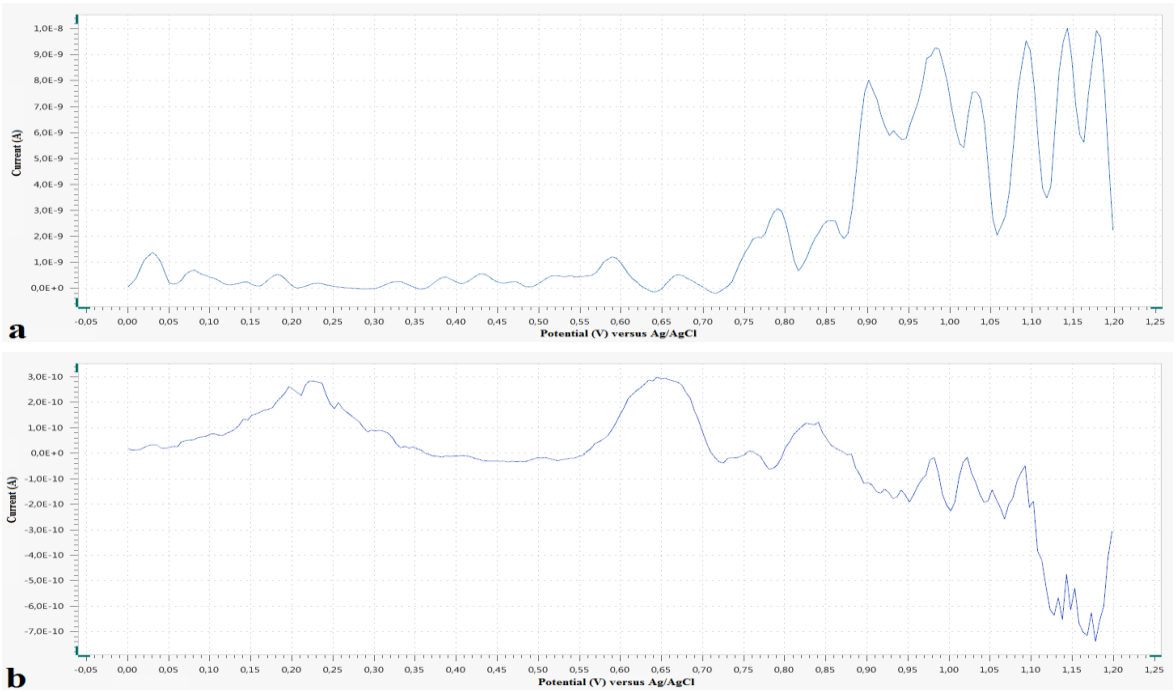


Figure 5.5: Differential pulse voltammograms of sunflower oil (a) and corn oil (b)

5.2.3 Differential pulse voltammetry of oil mixtures

Oil mixtures were prepared on weight basis by the addition of adulterant refined oils (rapeseed oil, sunflower oil and corn oil) to cold pressed olive oil separately, in different proportions (from 0 to 100% with 10% increments) as mentioned before. Decreasing trend of oxidation peak of α-tocopherol (at the potential of 0.40 ± 0.01V) was observed in all oil mixtures as the concentration of olive oil decreases as shown in Table 1, 2 and 3, and Figure 5.6, 5.7 and 5.8. Height of oxidation peak equals to amount of that substance.

Table 5.1: Electrochemical measurements of mixtures of cold pressed olive oil (CPOO) and rapeseed oil (RO) (from 0 to 100% with 10% increments)

Measurement of oxidation signal (10^{-9}A) of α-tocopherol at 0.40V											
	100% CPOO	90% CPOO + 10% RO	80% CPOO + 20% RO	70% CPOO + 30% RO	60% CPOO + 40% RO	50% CPOO + 50% RO	40% CPOO + 60% RO	30% CPOO + 70% RO	20% CPOO + 80% RO	10% CPOO + 90% RO	100% RO
Replication 1	32.9	23.2	13.4	13.0	15.1	7.47	7.12	2.83	1.43	0.685	0
Replication 2	33.4	18.1	17.6	12.9	14.2	6.88	5.11	3.26	1.71	0.553	0
Replication 3	33.9	23.5	21.3	16	13.4	6.61	6.21	3.08	1.66	0.636	0
Average Peak Height	33.4	21.60	17.43	13.97	14.23	6.99	6.15	3.06	1.60	0.625	0
SD	0.50	3.03	3.95	1.76	0.85	0.43	1.01	0.22	0.15	0.0667	0
RSD (%)	1.50	14.05	22.67	12.61	5.98	6.29	16.37	7.07	9.33	10.68	0

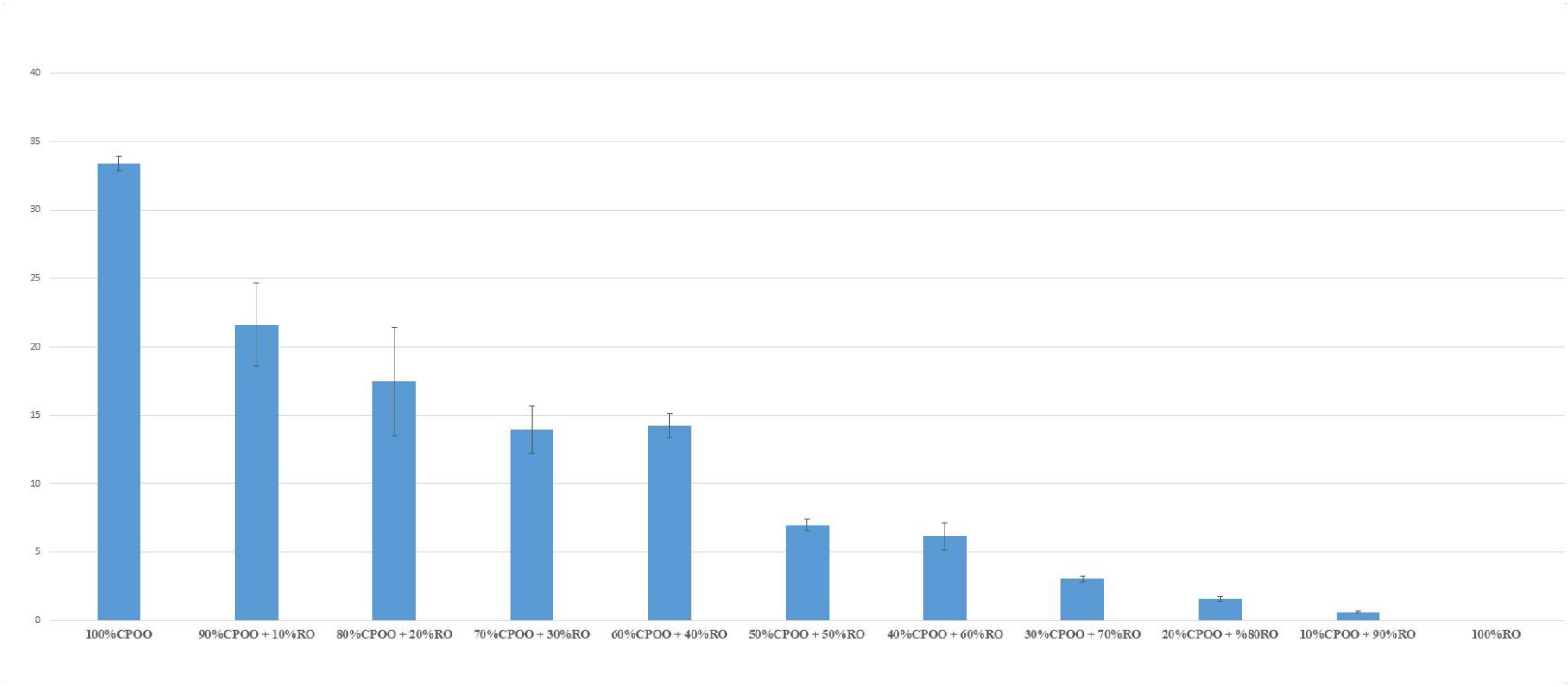


Figure 5.6: Histogram of representing decline of oxidation signal of α-tocopherol in the mixtures of cold pressed olive oil (CPOO) and rapeseed oil (RO)

Table 5.2: Electrochemical measurements of mixtures of cold pressed olive oil (CPOO) and sunflower oil (SFO) (from 0 to 100% with 10% increments)

	Measurement of oxidation signal (10^{-9}A) of α-tocopherol										
	100% CPOO	90% CPOO + 10% SFO	80% CPOO + 20% SFO	70% CPOO + 30% SFO	60% CPOO + 40% SFO	50% CPOO + 50% SFO	40% CPOO + 60% SFO	30% CPOO + 70% SFO	20% CPOO + 80% SFO	10% CPOO + 90% SFO	100% SFO
Replication 1	32.9	18.4	8.23	5.18	8.24	8.76	5.17	1.83	0.674	0.625	0
Replication 2	33.4	17.6	8.93	4.72	9.21	6.56	5.53	1.53	0.584	0.642	0
Replication 3	33.9	17.5	12.6	5.01	8.24	7.31	6.42	1.38	0.936	0.646	0
Average Peak Height	33.4	17.83	9.92	4.97	8.56	7.54	5.71	1.58	0.731	0.638	0
SD	0.50	0.49	2.35	0.23	0.56	1.12	0.64	0.23	0.183	0.011	0
RSD (%)	1.50	2.77	23.66	4.68	6.54	14.83	11.28	14.50	25.00	1.75	0

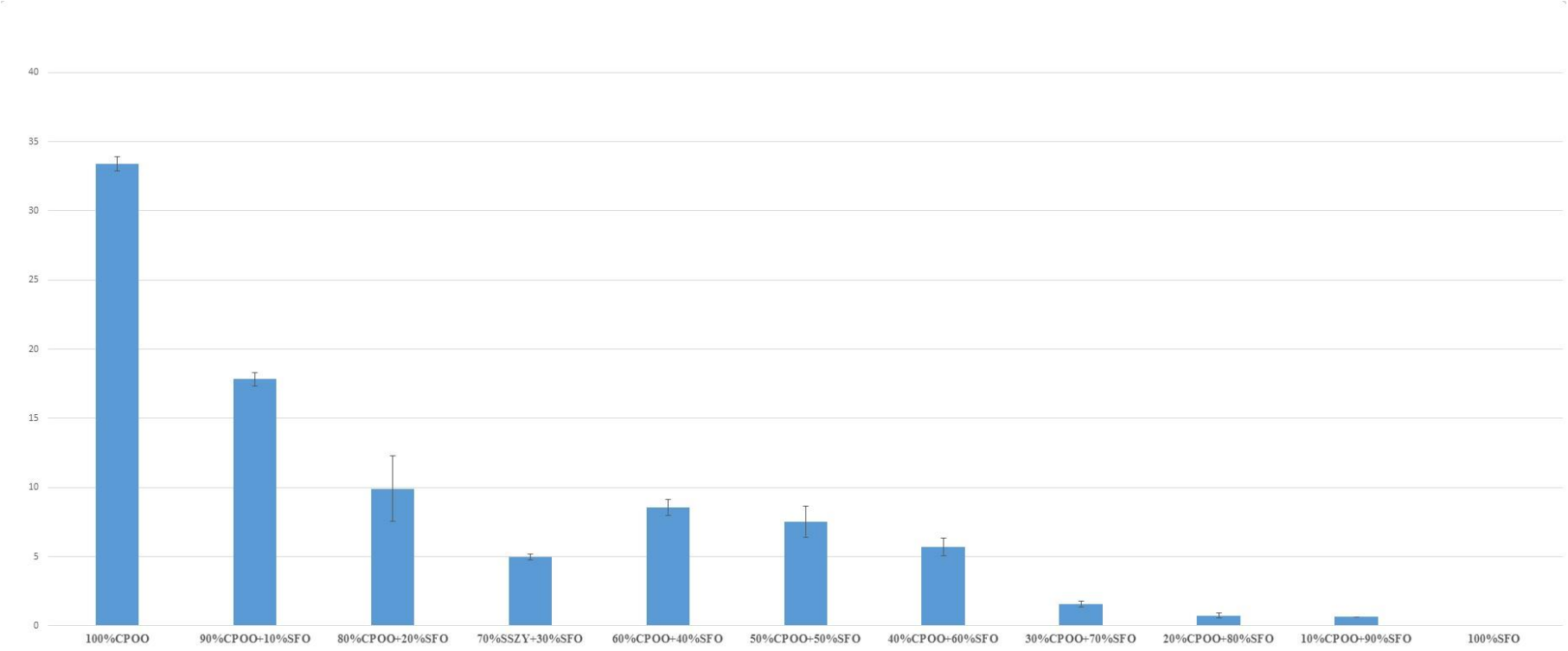


Figure 5.7: Histogram of representing decline of oxidation signal of α-tocopherol in the mixtures of cold pressed olive oil (CPOO) and sunflower oil (SFO)

Table 5.3: Electrochemical measurements of mixtures of cold pressed olive oil (CPOO) and corn oil (CO) (from 0 to 100% with 10% increments)

Measurement of oxidation signal (10^{-9}A) of α-tocopherol											
	100% CPOO	90% CPOO + 10% CO	80% CPOO + 20% CO	70% CPOO + 30% CO	60% CPOO + 40% CO	50% CPOO + 50% CO	40% CPOO + 60% CO	30% CPOO + 70% CO	20% CPOO + 80% CO	10% CPOO + 90% CO	100% CO
Replication 1	32.9	11.2	16.0	7.17	4.7	2.14	1.27	1.16	0.948	0.474	0
Replication 2	33.4	19.6	10.1	4.86	4.23	2.48	0.871	1.23	1.15	0.453	0
Replication 3	33.9	16.6	5.86	6.27	3.88	2.66	1.74	1.24	1.19	0.249	0
Average Peak Height	33.4	15.8	10.65	6.1	4.27	2.43	1.29	1.21	1.10	0.392	0
SD	0.50	4.26	5.09	1.16	0.41	0.26	0.43	0.04	0.13	0.12	0
RSD (%)	1.50	26.94	47.80	19.09	9.64	10.88	33.62	3.60	11.84	31.71	0

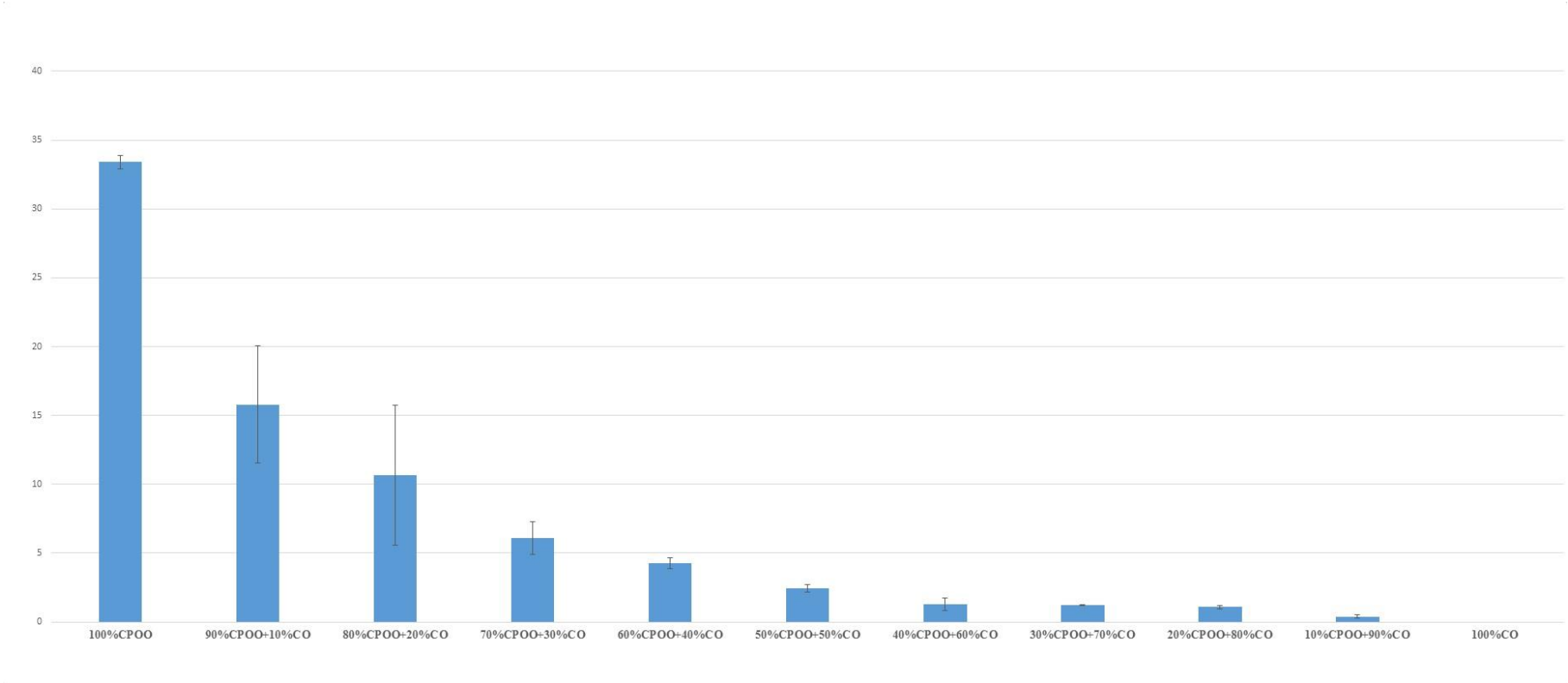


Figure 5.8: Histogram of representing decline of oxidation signal of α-tocopherol in the mixtures of cold pressed olive oil (CPOO) and corn oil (CO)

Results of mixtures of cold pressed olive oil (CPOO) and rapeseed oil (RO) are determined as below.

90% CPOO + 10% RO measured as 21.60 ± 3.03 nA (RSD%, 14.05%, n=3) at the potential of 0.40V. 80% CPOO + 20% RO measured as 17.43 ± 3.95 nA (RSD%, 22.67%, n=3) at the potential of 0.40V. 70% CPOO + 30% RO measured as 13.97 ± 1.76 nA (RSD%, 12.61, n=3) at the potential of 0.41V. 60% CPOO + 40% RO measured as 14.23 ± 0.85 nA (RSD%, 5.98, n=3) at the potential of 0.41V. 50% CPOO + 50% RO measured as 6.99 ± 0.43 nA (RSD%, 6.29, n=3) at the potential of 0.40V. 40% CPOO + 60% RO measured as 6.15 ± 1.01 nA (RSD%, 16.37, n=3) at the potential of 0.40V. 30% CPOO + 70% RO measured as 3.06 ± 0.22 nA (RSD%, 7.07, n=3) at the potential of 0.40V. 20% CPOO + 80% RO measured as 1.60 ± 0.15 nA (RSD%, 9.33, n=3) at the potential of 0.41V. 10% CPOO + 90% RO measured as 0.625 ± 0.0667 nA (RSD%, 10.68, n=3) at the potential of 0.42V.

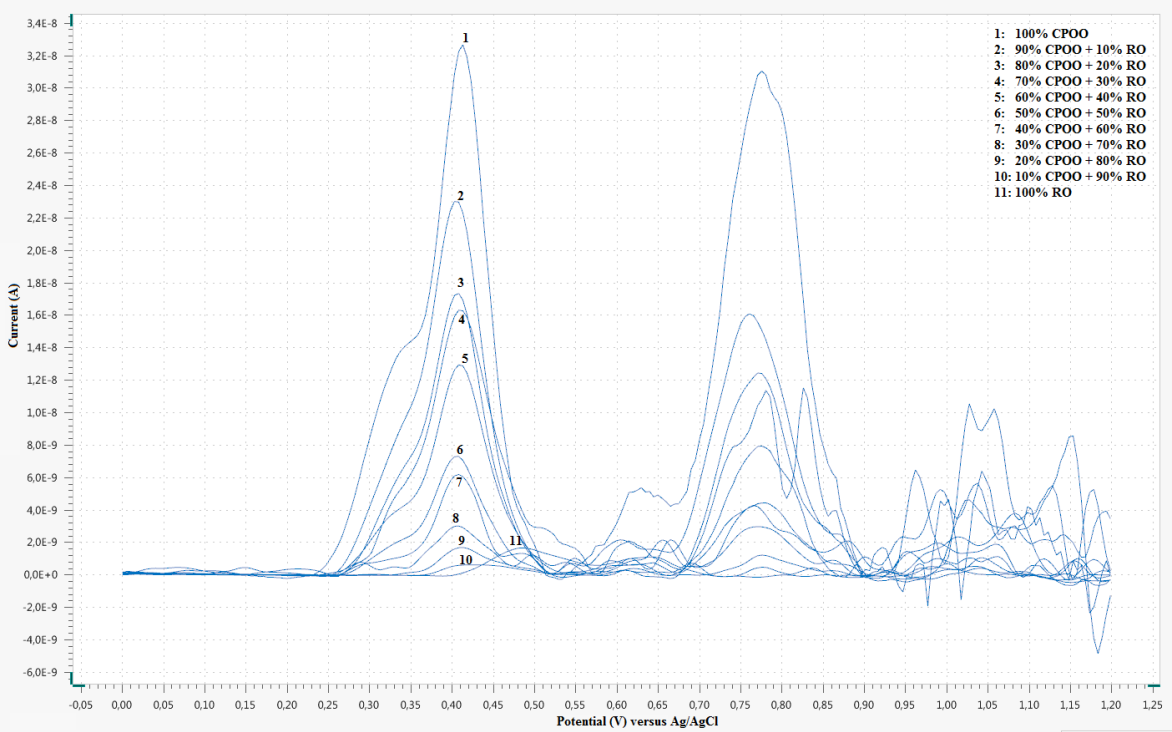


Figure 5.9: Differential pulse voltammogram of mixtures of cold pressed olive oil and rapeseed oil from 0 to 100% with 10% increments

Results of mixtures of cold pressed olive oil (CPOO) and sunflower oil (SFO) are determined as below.

90% CPOO + 10% SFO measured as 17.83 ± 0.49 nA (RSD%, 2.77%, n=3) at the potential of 0.42V. 80% CPOO + 20% SFO measured as 9.92 ± 2.35 nA (RSD%, 23.66%, n=3) at the potential of 0.42V. 70% CPOO + 30% SFO measured as 4.97 ± 0.23 nA (RSD%, 4.68, n=3) at the potential of 0.41V. 60% CPOO + 40% SFO measured as 8.56 ± 0.56 nA (RSD%, 6.54, n=3) at the potential of 0.41V. 50% CPOO + 50% SFO measured as 7.54 ± 1.12 nA (RSD%, 14.83, n=3) at the potential of 0.41V. 40% CPOO + 60% SFO measured as 5.71 ± 0.64 nA (RSD%, 11.28, n=3) at the potential of 0.41V. 30% CPOO + 70% SFO measured as 1.58 ± 0.23 nA (RSD%, 14.50, n=3) at the potential of 0.41V. 20% CPOO + 80% SFO measured as 0.731 ± 0.183 nA (RSD%, 25.00, n=3) at the potential of 0.41V. 10% CPOO + 90% SFO measured as 0.638 ± 0.011 nA (RSD%, 1.75, n=3) at the potential of 0.41V.

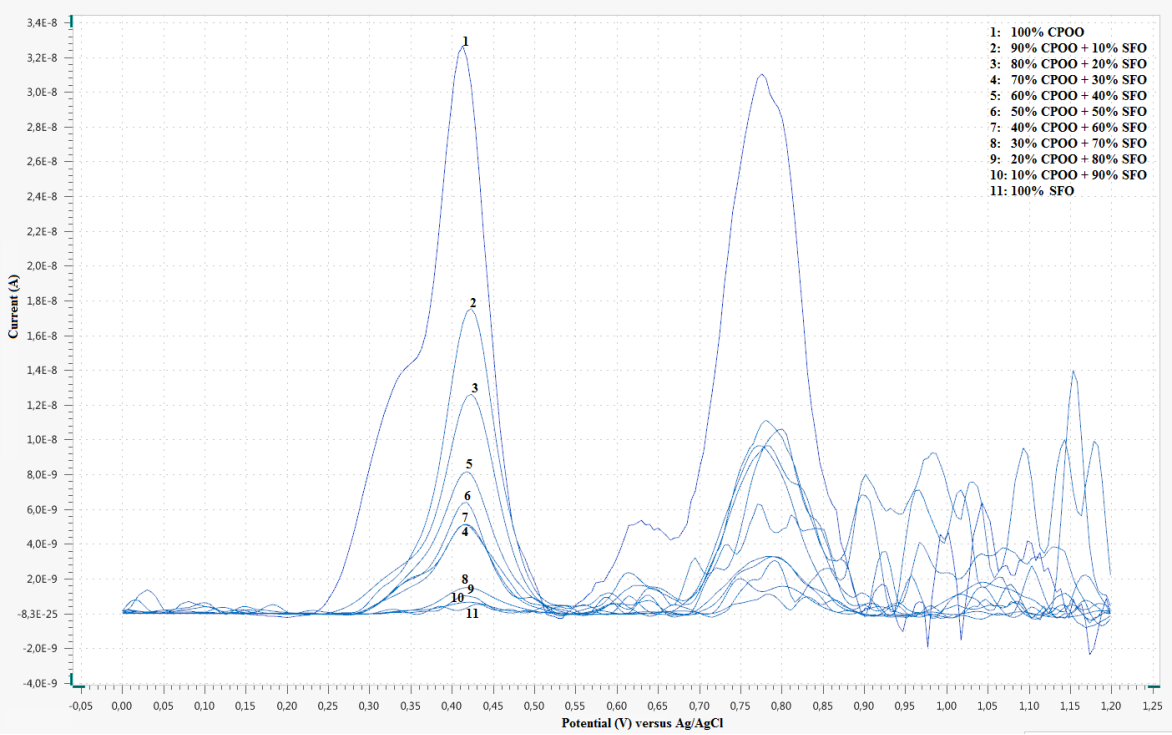


Figure 5.10: Differential pulse voltammogram of mixtures of cold pressed olive oil and sunflower oil from 0 to 100% with 10% increments

Results of mixtures of cold pressed olive oil (CPOO) and corn oil (CO) are determined as below.

90% CPOO + 10% CO measured as 15.80 ± 4.26 nA (RSD%, 26.94%, n=3) at the potential of 0.41V. 80% CPOO + 20% CO measured as 10.65 ± 5.09 nA (RSD%, 47.80%, n=3) at the potential of 0.41V. 70% CPOO + 30% CO measured as 6.10 ± 1.16 nA (RSD%, 19.09, n=3) at the potential of 0.41V. 60% CPOO + 40% CO measured as 4.27 ± 0.41 nA (RSD%, 9.64, n=3) at the potential of 0.41V. 50% CPOO + 50% CO measured as 2.43 ± 0.26 nA (RSD%, 10.88, n=3) at the potential of 0.42V. 40% CPOO + 60% CO measured as 1.29 ± 0.43 nA (RSD%, 33.62, n=3) at the potential of 0.41V. 30% CPOO + 70% CO measured as 1.21 ± 0.04 nA (RSD%, 3.60, n=3) at the potential of 0.40V. 20% CPOO + 80% CO measured as 1.10 ± 0.13 nA (RSD%, 11.84, n=3) at the potential of 0.41V. 10% CPOO + 90% CO measured as 0.392 ± 0.12 nA (RSD%, 31.71, n=3) at the potential of 0.41V.

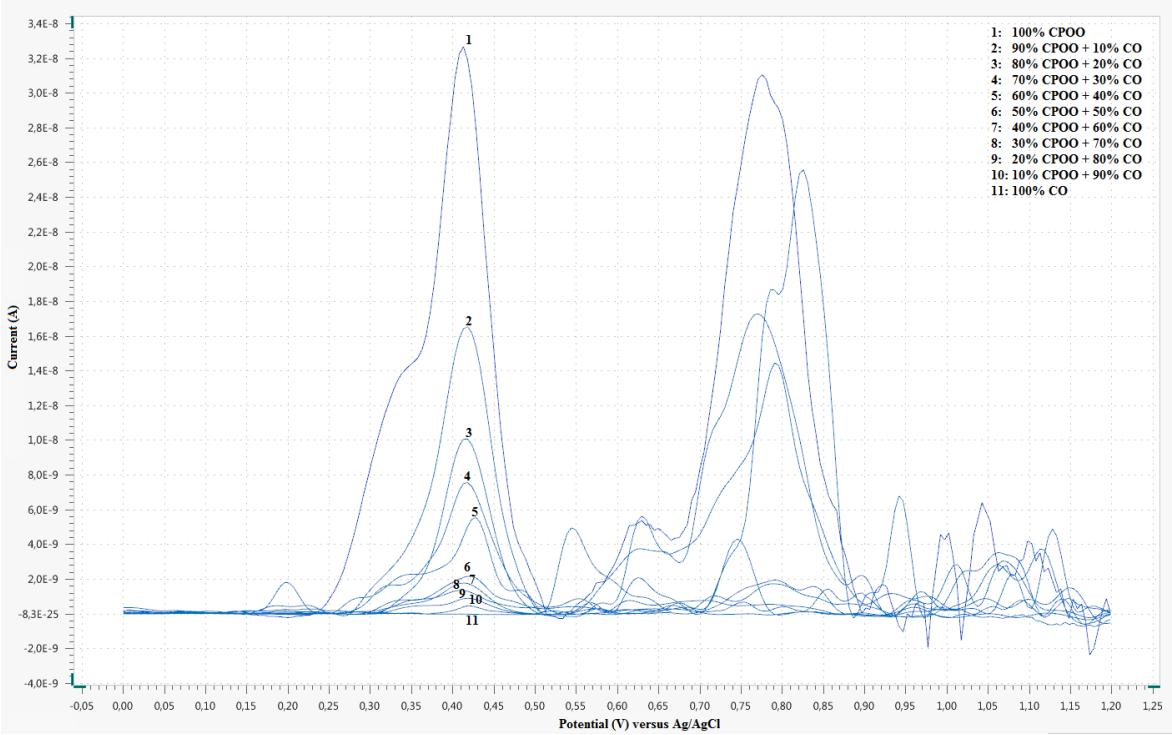


Figure 5.11: Differential pulse voltammogram of mixtures of cold pressed olive oil and corn oil from 0 to 100% with 10% increments

5.2.4 Cut off value of the measurement

Oxidation signal of cold pressed olive oil have been measured 8 times and results were $23.2x10^{-9}$A, $24.9x10^{-9}$A, $32.9x10^{-9}$A, $33.4x10^{-9}$A, $29.6x10^{-9}$A, $42.9x10^{-9}$A, $33.9x10^{-9}$A and $27.0x10^{-9}$A at the potential of 0.41V. Average of these measurements was $30.98x10^{-9}$ and standard deviation was 6.27. In order to determine confidence level of a screening method, cut off value was calculated as the following:

Cut off value = mean ± 2SD

Cut off value = $30.98x10^{-9}$ ± 2(6.27)

Cut off value = $30.98x10^{-9}$ ± 12.54 A

CHAPTER 6

CONCLUSION

In this study, single-use pencil graphite lead sensors used for screening adulteration. It is proved that adulteration of cold pressed olive oil in the presence of other cheaper oils (rapeseed, sunflower and corn oils) with different proportions can be detected as its α-tocopherol content by differential pulse voltammetric technique. As the amount of other oils in cold pressed olive oil increases, oxidation signal of α-tocopherol decreases (Figure 6.1). Utilizing electrochemical analysis of cold pressed olive oil and the mixtures of other vegetable oils has presented a potential for fast acquisition about cold pressed olive oil adulteration. The method is quick, cost-effective, easy and also environmentally friendly as only water has been used as solvent in the method, while other conventional methods use organic solvents.

Overall relative standard deviation of the measurement was 14%. Cut off value of cold pressed olive oil for these measurements was $30.98 \times 10^{-9} \pm 12.54$ A. It means test value is somewhere in the range of 18.44 - 43.52 $\times 10^{-9}$ A. Lower limit of this interval maybe taken as a cut off score. Since proposed method tends to have qualitative rather than quantitative emphasis, results require confirmation by other conventional techniques.

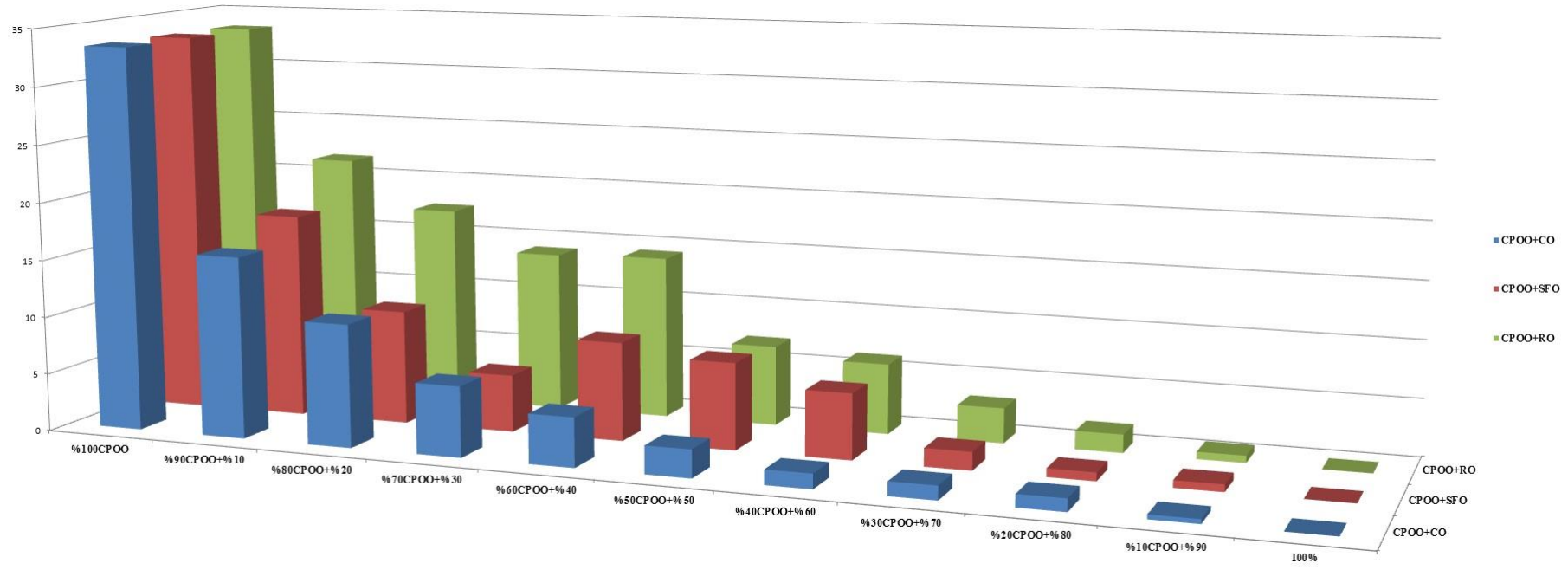


Figure 6.1: Histogram of representing decline of oxidation signal of α-tocopherol in the mixtures of cold pressed olive oil (CPOO) in the presence of other vegetable oils (rapeseed oil (RO), sunflower oil (SFO) and corn oil (CO))

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