NEJAT SHIFAMUSSA HAMED
ELECTROCHEMICAL BEHAVIOR OF SOME COMMERCIAL OLIVE OIL SOLD IN NORTH CYPRUS
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A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF APPLIED SCIENCES

OF
NEAR EAST UNIVERSITY

By
NEJAT SHIFAMUSSA HAMED

In Partial Fulfillment of the Requirements 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 degree of Master of Science in Food Engineering

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Name, Last name:

Signature:

Date:

To my parents…

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ABSTRACT

The aim of this study is to investigate electrochemical behavior of various olive oil samples sold in market based on tocopherols content by using bare PGE. A total of 11 commercial olive oils were purchased from Northern Cyprus and included in the study; five of them were extra virgin olive oil (EVOO) samples, adding to that five were virgin olive oil (VOO) samples and one riviera sample.

The PGE showed the good electrocatalytic activity to the oxidation of tocopherols, a wide potential range and reliability. Conventional three electrode system, consisting of the PGE as the working electrode, (Ag/AgCl) as a reference electrode and a platinum wire as a counter electrode are engaged in connection with the Nova2.12 software. DPV was performed with a potential range from 0 to +1.2 V, with 0.005 V step potential, 10 mV/s scan rate, 119 s duration time.

When we compare tocopherol levels of extra virgin olive oil (EVOO) samples and virgin olive oil (VOO) samples, α-tocopherol level (as peak height) might be considered distinctive parameter for screening purposes in adulteration. Alpha tocopherol content of extra virgin olive oil (EVOO) samples were changing in arrange of 5.95×10^{-8} A to 1.2×10^{-7}A, while 1.33×10^{-8} A to 7.8×10^{-11}A in virgin olive oil (VOO) samples.

Tocopherol content of Riviera oil was found just close to virgin olive oil Tocopherol content.

Keywords: electrochemical; olive oil; pencil graphite; DPV; tocopherols

ÖZET

Bu çalışmanın amacı, markette satılan çeşitli zeytinyağı örneklerinin elektrokimyasal davranışlarını tokoferol içeriğine dayalı olarak PGE kullanarak araştırmaktır. Kuzey Kıbrıs'ta markette satılan toplam 11 ticari zeytinyağı satın alınmış ve çalışmaya dahil edilmiştir; Bunlardan beşi, ekstra sızma zeytinyağı (EVOO) numuneleri; diğer beşi, sızma zeytinyağı (VOO) numuneleri ve bir tanesi de riviera numunesidir.

Çalışma elektrotu olarak PGE, referans elektrotu olarak (Ag / AgCl) ve karşı elektrot olarak bir platin telden oluşan konvansiyonel üç elektrot sistemi Nova2.12 yazılımı ile bağlantılı olarak birleştirilmiştir. DPV, 0.005 V adım potansiyeli, 10 mV / s tarama hızı, 119 s süresi ile 0 ila +1,2 V aralığında gerçekleştirilmiştir.

Ekstra sızma zeytinyağı (EVOO) numuneleri ve sızma zeytinyağı (VOO) numunelerinin tokoferol seviyelerini karşılaştırdığımızda, a-tokoferol seviyesi (en yüksek yükseklik olarak), tağşişte tarama amaçlı parametre olarak kabul edilebilmektedir. Ekstra sızma zeytinyağı (EVOO) numunelerinin alfa tokoferol içeriği 5.95 × 10-8 A ila 1.2 × 10-7A, 1.33 × 10-8 A ila 7.8 × 10-11A (VOO) düzenlenerek değişiyordu.

Riviera yağının tokoferol içeriği, sızma zeytinyağı Tokoferol içeriğinin hemen yakınında bulunmuştur.

Anahtar kelimeler: elektrokimyasal, zeytinyağı, kalem grafit, DPV, tokoferoller

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LIST OF ABBREVIATIONS

CE:	Counter electrode
CV	Cyclic voltammetry
DC:	Consistent potential detection
DPV:	Differential pulse voltammetry
EU:	European Union
EVOO:	Extra virgin olive oil
FFA:	Free fat
FT-IR:	Fourier transform infrared spectroscopy
GCE:	glassy carbon electrode
GPL:	Graphite pencil leads
IOC:	The International Olive Council
LOD:	low detection limit
MIR:	Mid-infrared spectroscopy
NMR:	Nuclear magnetic resonance
OO:	Olive Oil
OPO:	olive pomace oil
Pad:	Pulsed amperometric detection
PGE:	Pencil Graphite Electrode
PV:	peroxide value

RE:	Reference electrode
ROO:	refined olive oil
VOO:	Virgin olive oil
WE:	Working electrode

CHAPTER 1

INTRODUCTION

Cyprus, the third biggest Mediterranean Island, located in the north eastern portion of the Mediterranean Ocean, 33° east of Greenwich and 35° north of the Equator and has a zone of 9,251 Km^2, of which 1,733 are forested. Separate from the terrain ranges from 75 km from Turkey within the north to 150 km from Syria within the east and 380 km from Egypt within the south, whereas within the west the closest shores are the Greek islands of Karpathos and Rhodes at 380 km (Delipetrou et al., 2008).

Olive oil production has been really imperative labor for Cyprus since the ancient years. The development of olive tree dates back to 4800 B.C. at Fyllia town in Northern Cyprus, whereas archeological prove appeared that olive domestication began at past time for numerous ranges of the Mediterranean countries (Weiss et al., 2012).

Olive oil, a vital component within the diet of Mediterranean individuals, is gotten by mechanical extraction from the natural product of *Olea europaea L* tree, which has a place to the Olive family. It comprises 400 species and flourishes in calm and tropical climates (Firestone, 2005).

The biology and therapeutic value of olive oil are specifically related to its chemical composition. In most cases, health and dietary benefits initiated by consumption of virgin olive oil (VOO) have been related to its a few minor constituents such as vitamins, phytosterols, pigments, terpenic acids, squalene and phenolic compounds. Although these compounds are found at levels of mg/kg, many of them are incredibly responsible not only for healthy properties, but also for the high oxidative stability and its desirable organoleptic characteristics and nutritional qualities in comparison with the rest of consumable vegetable oils.

Two olive genetic varieties, Cypriot (ladoelia) and Koroneiki (lianolia or psiloelia) are the predominant cultivars on the island. Cypriot cultivar is autochthonous, adapted to dry season and hot conditions of the island, and produces fruits of medium measure, fitting for table

olives and olive oil of sensitive taste (Gregoriou, 2006). Cypriot cultivar is considered as one of the foremost aromatic varieties of olives within the world since of its characteristic flavor. The development of the olive at the lowlands happens in October- early November, whereas at sloping zones in late November - early December (Angelina et al., 2018).

There are 31 local increases of the Cypriot assortment developed at distinctive ranges, most of them check very little populace, getting their names from the names of villages at which the particular increases were distinguished (Gregoriou, 1996).

There are many varieties in the olive tree that have major or minor phenotypic and genetic differences. Today, in the main olive growing countries, most of the differences in size, color, oil content, fatty acid composition, and other properties were recorded. Fontanazza (1996) discussed the most important varieties. Some of them are of local interest only, others are distributed more widely (Boskou, 2006).

Olive maturation is a slow and long process that lasts several months and varies depending on the latitude of the growing area, the variety, the availability of water, temperature and cultural practices. The first stage of maturation is called the "green" stage. This corresponds to the mature green fruit that has reached its final dimensions. Anthocyanins gradually replace chlorophylls in the skin. This is the transition to a stage called "spotted," "purple" and "black.". The olives have the highest phenolic content at the stage between the yellow green and purple skin (veraison). (Boskou, 2006).

The reliance of olive oil quality on the cultivar, geographic and pedoclimatic conditions comes about within the generation of monovarietal olive oils applying special chemical composition and organoleptic properties.

Olive oils are classified by various international organization according to their quality, based on certain parameters. These parameters verify the hydrolytic and oxidative processes that occur in the fruit and during the extraction and technological refining procedures as well as during the preservation of the oil (Angerosa,2006).

The overall quality of olive oil, from production to consumption, is strongly linked to oxidative stability and its impact on the evolution of flavor, taste, color and content of endogenous and the EEC10 defined the quality of olive oil on the basis of parameters including free fat (FFA) content, peroxide value (PV), Ultraviolet (UV)-specific extinction coefficients (K232 and K270) and sensory score .Commercial quality is based in particular on

FFA as an important factor in the classification of olive oil into commercial grades and the nutritional quality of oil is defined by high levels of oleic acid and antioxidant components such as phenolic compounds, tocopherols, chlorophyll and carotenoids (Tietel et al., 2018). Electrochemical detection is a powerful analytical method that can detect electrical currents in test compounds generated by oxidative or reductive reactions (Song et al., 2018).

Biosensors can be classified in accordance with the type of active biological component involved in the mechanism or the way in which signal transmitted or combination of these two aspects depending on the properties of each sample of interest and the type of physical magnitude to be measured, the choice of the biological material and the adjusted transducer will be done. The bio component type determines the biosensor's degree of selectivity or specificity (Kuralay, 2009).

The most commonly used biosensors were electrochemical biosensors. The advantages of electrochemical techniques are their simplicity, low cost and speed (Ostojić, J et al., 2017). Also, electrochemical sensors have more advantage over the others because; in these, the electrodes can sense the materials present within the host without harming the host system (Yogeswaran et al., 2018).

The advantage of biosensors based on the electrochemical transducer is that they are economical and have a fast response. They can be operated in turbid media, have comparable instrumental sensitivity, and are more likely to be miniaturized. A wide range of samples can also be used for automation (Malhotra and Chaubey, 2003).

A challenge is the selection and development of an active material. The active sensing materials may act as a catalyst to detect a specific analyte or a set of analytes of any kind. The recent development of nanotechnology has paved the way for a large number of new materials and devices with desirable properties that have useful functions for numerous applications of electrochemical sensors and biosensors (Yogeswaran et al., 2018).

The aim of this study is to investigate electrochemical behavior of various olive oil samples sold in market based on tocopherols content by using bare PGE .

CHAPTER 2

THEORETICAL FRAMEWORK

2.1 The Types of Olive Oil

2.1.1 Virgin Olive Oil

Virgin olive oil (VOO) is a fat known around the world for its useful properties for human health. The utilization of olive oil within the Mediterranean diet is related with low mortality from cardiovascular disease. Several health benefits have been related with certain antioxidant compounds such as phenols, the effect of bioactive phenolic compounds on the protection of blood lipids against oxidati. High nutritional quality arises from huge amounts of unsaturated fatty acids within the composition of oil, such as oleic acid and linolenic acid (Vidal et al., 2019).

Chemical composition of virgin olive oil is impacted by different variables such as botanical origin, climatic conditions, soil, degree of maturing of olives and the way of extraction (Tapp et al., 2003).

Scientific evidences suggest that both unsaturated fatty acids together with VOOs phenolic compounds are responsible for reduction of cardiovascular diseases risk. A wide number of compelling reports have been actually published including evidences of VOOs phenolic compounds effects on health (Garcíaa et al., 2018).

It must be taken into consideration that the distinctive smell of virgin olive oil is credited to an expensive number of chemical compounds of diverse chemical classes, i.e., aldehydes, alcohols, esters, hydrocarbons, ketones and likely, to other unidentified unstable compounds, but phenolic compounds significantly impact to the sensory properties of the same. In particular, these compounds have been related to the severe and astringent taste.

On the other hand, and in spite of its high steadiness, virgin olive oil is additionally susceptible to lipid oxidative processes, enzymatic oxidation, photo-oxidation and autoxidation. During these reactions, an arrangement of compounds is formed in virgin olive oil (VOO) whereas

minor components are degraded. The secondary oxidation items have an unpleasant flavor and odor and may adversely influence the nutritional value of the oil, causing at last customer rejection. It has been found that phenolic compounds and carotenoids decrease autoxidation in oil, whereas tocopherols, chlorophylls and phospholipids demonstrate both antioxidant and prooxidant action depending on the oil framework and storage conditions. However, the vitamin E is in general critical natural antioxidants in foods since they have the capacity to block the propagation of radical reactions in spite of the fact that the different vitamins vary widely in activity. The oil antioxidant substance in VOO depends on the cultivar, fruit maturing stage, agroclimatic conditions and olive growing procedures, and gives data about the oil's oxidative status (Franco et al., 2013).

The main endogenous factors responsible for the high oxidative stability of virgin olive oil (VOO) is the characteristic content in fatty acids, and, as recognized in many studies, the presence of certain minor components, such as phenolic compounds (Bendini et al., 2007).

2.1.2 Extra Virgin Olive Oil

Extra virgin olive oil (EVOO) is extricated from high-quality olives that can be freshly consumed without any assisted treatment. Olive oil steadiness is related to preservation of so-called dynamic parameters during the valuable life of the item. During the autoxidation process a series of compounds are shaped, causing off-flavors, rancidity, loss of nutritional value and customer rejection of the food item (Adreou et al., 2017).

It has been reported within the literature that the stability of EVOO is impacted by the presence of suspended solids and vegetative water that stay within the item after the extraction handle, which can lead to fermentation and off-flavors, such as fusty-muddy sediments or winey, that declassify the item (Bendini et al., 2013).

In addition, exogenous variables can strongly influence the shelf-life of EVOOs, such as the availability of oxygen, temperature and light during the storage. These last mentioned variables impact the oxidative deterioration of triglycerides, in this way shaping peroxide compounds that evolve into secondary oxidation items driving to the rancid off-flavor (García et al., 2003).

In order to decrease the negative impacts connected to the presence of suspended or emulsified compounds, filtration is a process permitted by European Community (EEC Reg. 1638/1998) as pre-treatment before bottling to improve the quality and appearance of olive oil during storage (Sánchez et al., 2012) (Vallia et al., 2019).

2.1.3 Cold Pressed Extra Virgin Olive Oil

Over a long time, expanded the interest in cold-pressed plant oils has been observed as these oils have superior nutritive properties than those after refining. Cold pressing is basic biological system that does not require much energy. The disadvantage of this process is low productivity and troubles in getting a product of constant quality (Rotkiewicz et al. 1999). Such variables as geographical area, species and processing method may impact the final chemical composition of plant oils (Siger et al., 2008).

Phenolic compounds have much impact on the stability, sensory and nutritional characteristics of the product and may avoid deterioration through quenching of radical responses responsible for lipid oxidation (Ruth et al.2001; Quites et al.2002; Koski et al. 2003).

Cold-pressed oils contain phenols show within the seed, and they may have the potential for applications in the advancement of health and anticipation of oxidative damages caused by radicals. Variables affecting the antioxidant activity of phenolic compounds include position and number of hydroxyl groups, polarity, solubility and stability of phenolic compounds during preparing (Decker, 1998) (Siger et al., 2008).

Olive oil is accepted to be most steady because of its high amount of phenols . As a result of mechanical pressing at low temperatures, olive surrender cold-pressed high-quality oil that can be consumed unrefined. Olive oil is outstandingly rich in oleic acid (18:1). Moreover, it contain significant sums of polyunsaturated fatty acids such as linoleic acid (18:2) (Kosk et al., 2002).

The consumers' current tendency to favor the slightest processed foods and to maintain a strategic distance from manufactured additives make cold-pressed oils an appealing choice since no solvents and no advance processing other than filtering are included. Within the cold-pressed items, minor components influencing the color, flavor and keeping qualities of the oil are in this way protected. These minor constituents can have either pro-oxidative (free greasy

acids, hydroperoxides, chlorophylls, carotenoids) or antioxidative (tocopherols, phenols, phospholipids) impacts (Kosk et al., 2002).

2.2 Olive Oil Processing

Olive is the common title for around 35 species of evergreen bushes and trees of the genus Olea within the olive family, the Oleaceae, local to tropical and warm temperate regions. The title is particularly utilized for Olea Europaea, the well-known olive which is grown for its consumable natural products (Boskou, 2006).

It is possible to use the same olive cultivars for table olives and oil production, but generally olives for oil production have a lower pulp-to-kernel ratio (4:1-7:1) compared to the same olive ratio for table olives (7:1-10:1). The task of identifying and classifying olive varieties is very complex, as Essadki and Ouazzani (2003) emphasize (Dimitrios Boskou, 2006). To get a characteristically fragrant but delicately flavored oil, it is basic that it is appropriately extricated from develop, undamaged olives. In this manner, the degree of maturity is an imperative quality factor (Boskou, 2006).

The maturity stage has a 30 percent contribution, according to Montedoro and his colleagues (1986). Other factors contribute by the following percentage: 20% variety; 5% harvest; 15% transport and pre-milling storage; 30% extraction system (Boskou, 2006).

The International Olive Council (IOC) distinguishes between extra virgin olive oil (EVOO) and virgin olive oil (VOO). In both cases, olive oil is gotten from the natural product of the olive tree *Olea europaea L* exclusively by means of mechanical, such as pressure, or other physical methods under conditions, especially warm conditions, not driving to critical changes within the oil (Tsopelas et al.,2018).

These oils are not submitted to any treatments other than washing, decantation, centrifugation and filtration, it differs between EVOO and VOO basically lie in their free acidity. Within the case of EVOO, free acidity isn't higher than 0.8 g per 100 g, whereas for VOO it uses to be below 2 g per 100 g. Other commercially available olive oils are: refined olive oil (ROO); olive oil (OO); and olive pomace oil (OPO). Refined olive oil is obtained from virgin olive oils submitted to several refining strategies, which don't lead to modifications within the initial glyceridic structure (Tsopelas et al., 2018).

Olive oil production is steeped in tradition, although some degree of automation and ease of handling improvements have inevitably occurred over time. The initial step is to wash the olives to remove the fruit's dirt, stones. In a hammer mill, the olives are then crushed and the pomace (the mixture of crushed olive pericarp and stones) is finely homogenized (malaxation) before pressing. The pomace is fed directly to the hydraulic press plates when traditional methods are used, each of which is covered by a filtering diaphragm before the plate pile is loaded into the press for extraction. The oil is produced at pressures of up to 39 300 kN / m2 and is separated from the water in the pomace by a centrifugal clarifier after passing through a filter press, resulting in a brilliant clear oil. A continuous horizontal centrifuge rotating at 1200–1500 rpm separates the oil from the pomace water and the vegetation water when using modern methods. After further washing with about the same proportion of clean water, the oil is again centrifuged and then harvested, yielding extra virgin olive oil (VOQ).High-quality oils are bottled directly, but low-quality oils (high acidity) are processed once more, yielding refined virgin oil (RVO). Oil extracted with organic solvents such as hexane from the residual pomace or husk produces a low-quality refined husk oil (RHO). Usually mixing with VOQ improves the quality of both RVO and RHO (Owen et al., 2000).

The concentration of volatile compounds and polyphenols in olive oils depends on the type of grinding machines, conditions of malaxation, and system of extraction. Metallic crushers are used to observe a greater recovery of phenolic compounds. In contrast, the volatile compounds in oils obtained with a mill stone are significantly higher (Angerosa and Di Giacinto, 1995).

Time of malaxation and particularly temperature adversely affect the composition of metabolites resulting from the lipoxygenase pathway, reduce volatile compounds with pleasant odors and increase them giving less attractive perceptions (Morales et al., 1999; Angerosa et al., 2001).

Oils extracted by pressure are much more stable and have more intense notes of grass and bitter taste compared to oils extracted by the three phase decanters. This is attributed to a higher phenol and volatile compound concentration (Aparicio et al., 1994b; Di Giovacchino et al., 1994; Angerosa et al., 2000b).

The ideal objective of any extraction technique is to extract the most important potential amount of oil without altering its original quality. However, if quality isn't to be modified, it's essential to use only mechanical or physical ways for extracting the oil, avoiding chemical and enzymatic reactions which may change its natural composition. When treating the olive as prime material, one should consider two groups of phases: the solid components of the skin, pulp, and kernel, and also the liquid phases made up of the oil and the vegetable water (Petrakis, 2006).

The preparation of oil is an industrial process, the purpose of that is to separate one of the liquid phases—the oil—from the other constituents of the fruit. Thus, starting with healthy, whole, clean fruit, harvested at optimum maturity, a paste preparation must be made by breaking the vegetable structure; the oil must be released from the cells and finally solid and liquid phases must be formed. The solid and liquid phases are then separated by pressure, percolation, or centrifugation. Lastly, the liquid phases are separated into oil and vegetable water by decantation and/or vertical centrifugation (Petrakis, 2006).

The separation between solid and liquid phases is not complete the mass of solids with varying percentages of moisture and oil content make up the olive pomace by-product and the liquids with varying percentages of fine solid material make up the oily must. With the use of hydraulic presses and transmission mechanisms, extraction methods became more efficient. They have become increasingly mechanized over the years, driven by the need to spare labor expenses to reduce costs, but the entire process has been discontinuous (Petrakis, 2006).

Olive oil extraction starts from the olive tree and ends with product storage. Before the extraction process, there are limitations in a number of factors that affect the quantity and quality of the oils. The main factors are: olive varieties, microclimatic conditions, soil variability, cultivation systems that regulate the soil's absorption capacity and retain rain or irrigation water (Montedoro et al., 1989, 1992; Inglese et al., 1996; Reiners et al., 1998; Gutierrez et al., 1999; Tovar et al., 2001; Romero et al, 2002; Morello et al., 2003; El Antari et al., 2003; Servili et al., 2004; Royo et al., 2005); and pest monitoring and control (Zunin et al., 1993).

Most olives are harvested with shakers and/or by hand. It is more likely that newly planted orchards will be harvested mechanically. Some varieties ' high trees are harvested with the help of nets after the fruit's natural drop. Precautions should be taken to prevent fruit breakage due to mechanical damage and soil material contamination of the fruit. For controlling mechanical damage and temperature, olive transport and storage should be considered as critical phases. Improper handling during these phases can lead to unwanted enzymatic reactions and yeasts and mold growth (Petrakis, 2006).

The best way to transport the olives is in open-mesh plastic crates, to allow air to circulate and to prevent harmful heating caused by fruit catabolic activity (Kiritsakis, 1998). The olives must be spread in shallow layers when stored before processing and kept in well ventilated, cool, dry areas. It is necessary to avoid storing the olives in jute sacks.To ensure that the olives retain the quality characteristics they had when they were harvested, they must be delivered to the extraction plant immediately for processing (Petrakis, 2006).

There are two operations involved in fruit cleaning: leaf removal and washing. Defoliators use a powerful airflow generated by an exhaust fan to suck the leaves, twigs and dirt. After that, the olives are washed in a stream of water. After decanting, this water is recycled and clean water is constantly mixed in pre-set proportions. The washing vat is equipped with a shaker that shakes any impurities via screens as well as an air injection system to create turbulence in the mass to improve washer efficiency (Petrakis, 2006).

Pressing is based on the principle that when a combined solid / liquid mass, such as olive paste, is subjected to pressure, the mass volume decreases because the liquid phase the oily must is forced out using the drainage effect of the mats and the stone fragments and is separated from the solid phase. It's an operation that can be compared with filtration, and it actually shares the same kinetic properties, but it's more complex (Petrakis, 2006).

Continuous centrifugation involves the following steps: removal and washing of the leaves, crushing of the olives, mixing of the olive paste and centrifuging with or without water in the "three-phase" or "two-phase" mode (Petrakis, 2006).

Three-phase Centrifugation. Olive pastes undergoing centrifugal extraction had to be quite fluid for many years to facilitate the separation of fractions with different specific weights; this

was done by adding lukewarm water, equivalent to about 40-60% of the olive fruit's weight. In the decanter, the water-thin paste is centrifuged. The disadvantages of this process include increased quantities of wastewater produced as a result of increased water use (1,25 to 1,75 times more water than press extraction), loss of valuable components (e.g. natural antioxidants) in the water phase, and problems with the disposal of wastewater from the Oil Mill. to reduce this problem, the water phase can be recycled as soon as it leaves the decanter, thinning the olive paste by injection into the pump that delivers the paste into the decanter.

This technique has made it possible to reduce the volume of wastewater by approximately 35% and to improve the total polyphenol content of the oil by approximately 30% (Khlif et al., 2003). However, the practice negatively affects the quality of the produced oil and it is hardly used anymore.

Two-phase Centrifugation. The failure to develop an appropriate end-of-pipe wastewater treatment technology gave technology manufacturers the opportunity to develop a two-phase process that uses no water process, delivers oil as the liquid phase, and a very wet olive pomace (humidity 60 ± 5 %) as the solid phase using a more efficient centrifugation technology. This technology has attracted particular interest in restricting water supply and/or reducing aqueous effluent.

The paste is produced without adding water when fresh olives are used, while a small amount of water is added when dried olives are used. In the decanter, from which two phases are obtained, the disrupted paste is centrifuged: oily must and a solid / water mixture (pomace).

Several companies have developed decanters based on the two-phase process. Compared to the traditional three-phase extraction process, the performance of the two-phase decanters was evaluated and olive oil was produced in similar yields to the three-phase process but of superior quality in terms of the content and retention of polyphenols and o-diphenols. Furthermore, during oil extraction, the two-phase process did not produce wastewater. Decanting in two phases reduces the water requirements.

It creates, however, a high humidity pomace called "Alperujo" in Spain, which is difficult to handle. After malaxing and proper dilution of the paste obtained from the first two-phase centrifugation pomace, applying a second three-phase centrifugation decreases the humidity of

the final pomace, but only a small percentage of oil is recovered. This oil is green with a higher content of aliphatic alcohols, waxes and triterpene alcohol.

Pressure extraction is often carried out in hydraulic super-presses with a service pressure up to 400 atm (which refers to the area of the piston). Super-presses working single press mode with gradual increase of the pressure up to the maximum value within 45-60 min, remaining at that high pressure for an additional 10-20 minutes.

After pressing, a little amount of water is used to rinse the stuck material off the mats and transfer the oily should for clarification. In practice, a processing yield of 86-90% is achieved and the pomace's humidity is around 28%. This method therefore ensures a high-quality oil due to the short beating time and low temperatures throughout the operation, provided that the quality of the olives and the condition of the mats are also good (Petrakis, 2006).

There is plenty of research work related to the level of polar as well as non-polar phenols and oxidative stability with the milling conditions. Salvador et al. (2003) examined samples from the three main extraction systems: pressure, dual-phase, and triple-phase. Total phenols and o-diphenols were found to be present at higher levels in the oil obtained by the two phase decanters. Due to the addition of water, the phenol content of the oil extracted is lower in three phase centrifuges which reduce the concentration of polar phenolic compounds (Cert et al., 1996; Di Giovacchino et al., 2001).

2.3 Adultration and Detection Methods:

Authenticity studies have been reported for the classification of olive oils according to their botanical or geographical origin based on determinations of variables count for their major or minor constituents, such as: fatty acids profile of olive oils (D'Imperio et al., 2007; GarcíaGonzález, Luna, Morales, & Aparicio, 2009; Mannina et al., 2003; Stefanoudaki, Kotsifaki, & Koutsaftakis, 1999), phytosterols (Matos et al., 2007), phenols (Alonso-Salces et al., 2010; Petrakis, Agiomyrgianaki, Christophoridou, Spyros, & Dais, 2008), squalene (D'Imperio et al., 2007), volatiles (Luna et al., 2006) or a combination of two or more components (Aparicio, Morales, Aparicio-Ruiz, Tena, & García-González, 2013; Karabagias

et al., 2013; Longobardi et al., 2012; Matos et al., 2007; Merchak et al., 2017; Ollivier, Artaud, Pinatel, Durbec, & Guérere, 2006; Ollivier, Artaud, Pinatel, Durbec, & Guérère, 2003). In olive oil, the major hydrophilic phenolic constituents are tyrosol and hydroxytyrosol, along with benzoic, cinnamic, and phenylacetic acid derivatives and other more complex phenols (Kosk et al., 2002).

As olive oil is ordinarily sold at a higher cost than other vegetable oils, it is frequently adulterated with seed oils and olive oils of lower grade. The confirmation of olive oil authenticity is of vital significance to protect the image of olive oil, to progress its competitiveness and increase the consumers' believe as expressed within the last Horizon 2020 call.

The detection of virgin olive oil adulteration may be a complex examination and it can be basically accomplished by getting its fingerprint, which reflects its complex chemical composition and exploits the variability caused by differences of samples utilizing chemometric procedures. For this reason, two distinctive approaches can be followed. The primary is based on particular chemical examination, counting quantification of fatty acids, sterols, and triterpene alcohols. In this case, gas and fluid chromatography are the strategies of choice.

The most disadvantage of this approach is the need for sample pretreatments, frequently resulting in a long turnaround time. The elective approach depends on the implementation of instrumental strategies not to confirm or to measure particular compounds, but to get a comprehensive and multivariate description of the chemical composition of the sample. These nonspecific fingerprints can be gotten by Fourier transform infrared spectroscopy (FT-IR), mid-infrared spectroscopy (MIR), Raman spectrometry, nuclear magnetic resonance (NMR) and differential scanning calorimetry.

Most of such approaches require extensive analytical resources and they can barely be utilized for rapid investigations under field conditions. In recent years, considerable efforts have been situated towards the development of simplified, quick and cheap approaches with the possibility to be utilized in portable analytical devices. In this viewpoint, electrochemical strategies are exceptionally attractive owing to their high sensitivity, characteristic simplicity,

miniaturization and low cost. These procedures can give a non-specific unique mark of oil samples, reflecting the redox properties of the electroactive species show within the oils. In any case, electroanalytical techniques have been once in a while connected to direct measurements in eatable oils basically due to the very poor conductivity of the framework.

Extra virgin olive oil is the one having the highest quality in terms of aromaticity and flavor, but its generation is very limited, and the high request from shoppers makes it susceptible to be adulterated with cheaper seed oils or indeed with other olive oils of lower quality. In this sense, and due to their low costs, refined olive oil, olive oil or olive pomace oil are some of the time utilized to adulterate olive oil of way better quality, such as virgin and extra virgin olive oil. Essentially, due to lower showcase costs, other eatable vegetable seed oils such as soybean, corn, canola, cotton, sunflower, shelled nut, and almond is likely to be utilized as unlawful adulterants of olive oil.

In this situation, a fast and strong analytical technique able to distinguish adulteration is critical and very welcome for purposes of quality control and labeling olive oils of high quality. Different strategies have been described within the literature dealing with the discovery of olive oils adulterated with other consumable oils. Within the past decade, analytical methods based on liquid and gas chromatography, capillary electrophoresis, and spectroscopic methods such as Fourier change infrared spectrometry (FTIR) and FT-Raman were published. In any case, few papers have been published addressing the issue of adulteration of high- quality olive oils with other low- quality olive oils, such as ROO, OO or OPO.

Spectroscopic parameters, as the particular termination coefficients at 232 and 270 nm (K232 and K270), the variety between both (ΔK), and the content of trans fatty decide EVOO adulteration with 2% of refined olive oil and 0.4% of pomace olive acids and stigmasta3,5-diene, both analyzed by gas chromatography, were utilized to oil. A direct model calculation, based on chaotic parameters from UV–vis scans of adulterated EVOO tests, has been proposed to evaluate adulterations with low-grade olive oils. An artificial neural network model in combination with absorption spectral data have been utilized to recognize the adulteration of EVOO with olive pomace oils or with olive oils. FTIR data and DA-UPLS were utilized to segregate between olive oils gotten from entirety olive and stone olive pastes.

Other spectroscopic methods, such as synchronous or conventional fluorescence combined with chemometric methods, have been moreover proposed to distinguish adulteration of high-quality olive oil with olive oils of lower quality. Right now, one of the most promising progress is the profiling approach, which typically isn't able to distinguish between analytes and neither evaluate them, but it permits the quick determination of the validity of olive oils based on data from multi-target screening techniques.

2.4 The Determination of Phenolic Compounds

Phenolic compounds represent one of the most various and ubiquitous groups of plant metabolites and are a necessary portion of both human and animal diets. They are a heterogeneous family of chemical compounds comprising, among others, phenolic acids, flavonoids, tannins, stilbenes, coumarins, and lignans. Polyphenols are synthesized by plants during the development and in response to stress conditions, such as infection, injuring, UV radiation, etc. Traditionally, their relevance has been basically related to the organoleptic properties, such as color (e.g., anthocyanins and curcumin), astringency (tannins), bitterness (flavanols) and taste In any case, within the last decades they are progressively being recognized for their nutritional value, since they can decrease the chance of chronic disease and, in general, have a positive impact on wellbeing, appearing anti-carcinogenic, anti-atherogenic, anti-ulcer, anti-thrombotic, anti-inflammatory, immune modulating, antimicrobial, vasodilatory, and analgesic impacts. Hence, there is an expanding request of polyphenols from low-cost materials (e.g., vegetable by-products) that are very vital in food innovation, since they represent a financially attractive resource of high-value components (Natale et al., 2015).

The utilization of vegetable by-products as a common source of antioxidant compounds may be appreciated to avoid oxidation during food processing and storage, in substitution of manufactured additives. As a result, particular analytical methods for the characterization and quantification of polyphenolic compounds, and after that for their extraction from vegetable and food-industrial byproducts, are fundamental to get innovative items, representing a challenge for an eco-innovation. Polyphenols are well known to be electroactive due to the

presence of hydroxyl groups as substituents of aromatic rings that experience electrochemical oxidation reactions (Natale et al., 2015).

Hence, the amperometric detection following LC division can be considered a valuable procedure giving great results in terms of sensitivity, selectivity, instrumental costs and simplicity. Very recently, electroanalytical methods based on voltammetric approaches and their coupling to flow-injection analysis, high-performance liquid chromatography or capillary electrophoresis for the analysis of polyphenols in wine have been checked on. In addition, several methodologies for the determination of phenolic compounds in tea, alcoholic beverages, and pharmaceutical details have been reported including amperometric biosensors, the anodic discovery at chemically modified electrodes or extended graphite-epoxy composite, and rotating spectral graphite disk electrodes. However, these approaches refer to the determination of total phenolic content or to a limited number of polyphenols (Natale et al., 2015).

Among the different amperometric methods, consistent potential detection (DC) is the simplest, but limitations due to the electrode poisoning from test matrices and analyte oxidation items are generally watched. Consequently, to induce a reproducible electrochemical signal over time, the fouled terminal surface got to be occasionally cleaned, earlier to each measurement session. In pulsed amperometric detection (Pad) the electrode surface is renewed within a pulsed potential waveform that continuously cleans and reactivates the working electrode with a repeated arrangement performed at a frequency of 0.5–2.0 Hz (Anna Natale et al., 2015).

2.5. Voltametric Determination

Several potential waveforms have been proposed for the electrochemical detection of a wide extent of analytes (carbohydrates, nitrogen, sulfur compounds, etc.) at gold or platinum working electrodes. Compared to noble-metal electrodes, glassy carbon electrode (GCE) is very resistant to fouling, in spite of the fact that poisoning phenomena at constant potential happen, owing to the adsorption of the oxidation products at the cathode surface, which causes a decrease of sensitivity and a time-dependent deterioration of the reaction. In the literature, different activation/polishing techniques are reported to obtain sensitive and steady electrode responses at GCE. Very as of late in alternative to the off-line preactivation or in situ laser

irradiation, the development of new approaches based on well-performing potential waveforms has been proposed for sensitive and reproducible detection of aryl ethanolamine and phenolic moiety based compounds. The potential-time profile was outlined to avoid the carbon electrode fouling following repeated examinations, assuring a reproducible and sensitive quantitative determination without the need for mechanical polishing. The electrochemical characterization studies of carbon electrode surface by cyclic voltammetry and flow injection analysis has recommended the formation of an oxygen-rich surface film, consisting of quinone functionalities that show up to be likely candidates as mediators of electrons between the electrode and the electroactive species. When the electrochemical detection is coupled with liquid chromatography, the proper choice of the mobile phase may be an essential aspect to upgrade the electrode performance and sensitivity. On the other hand, the selection and optimization of the eluent composition is a basic factor in accomplishing great chromatographic behavior as peak shape and resolution. Retention behaviors of phenolic compounds in LC by reversed phase column have received far less consideration, and poor resolution and efficiency were generally obtained for polyphenol isomers unless the particular detection in multiple-stage mass spectrometry is applied (Natale et al., 2015).

2.6 Electrochemical detection of olive oil

The first endeavor for eatable oil separation according to their voltammetric reaction on chemically altered carbon paste electrodes was made by Apetrei in 2014 (Tsopelas et al., 2018).

Utilizing three virgin olive oils of different quality, refined olive oil and two seed oils based on their past work on chemically altered electrodes, Apetrei created moreover voltammetric e-tongues for the detection of olive oil adulteration with seed oil, Also accomplished a separation of olive from maize oils as well as classification of olive oils according to their geological origin. However, more orderly examinations are still required, particularly utilizing expanded datasets (oil samples) in order to discover the appropriate conditions for the exact quantification of olive oil adulteration, freely of the adulterant oil (Tsopelas et al.,2018).

Electrochemical cells activity cell is that the place wherever all the electrochemical reactions and measurements happens. Electrochemical cell can be designed in several volumes and shapes looking on the character of the study. principally cells are made of glass, however if a

spectrochemical experiment is required to be studied the cell material should be able to transfer the light simply considering the fact that a light reflection may be observed due to electrochemical. Electrode types in electrochemical are counter-electrode, working electrode reference electrode (Kaplan, 2015).

It is desired that one of the electrodes in the system is not affected by the solution in many electro analytical studies. It's called an electrode of reference (RE). There are some factors to consider when using an RE ion concentrations must stay the same should not be affected by the experimental solution, ions, potential, current change and RE should be polarized .The reference electrode reaction should be reversible. Reference electrodes of Silver / Silver Chloride (Ag / AgCl) are frequently used. Ag / AgCl reference electrodes consist of a saturated potassium chloride (KCl) solution with an Ag wire (percent 99. 999 purity).

There are different types of working electrodes in electrochemical studies. The functioning electrode (WE) is the electrode studied by the analyte. WE must have certain characteristics such as stability and easy preparation. The WE can be solid or liquid. For example, Pencil Graphite Electrode (PGE)is a solid working electrode with easy preparation and low cost and hanging ,mercury drop electrode is a liquid working electrode with ineffectiveness of depositing metals. Different studies have been carried out with different working graphite electrodes, carbon paste, glass carbon, gold, microarrays (Kaplan, 2015).

Counter (auxiliary) electrodes Potential is controlled in electrochemical measurements and the objective is to observe the current, generally WE signals form in a wrong way. In order to overcome this problem, another third electrode (counter electrode, CE) is needed. Current flows from WE to CE. The potential for WE is measured under almost zero. CE also does not affect the reaction in the electrochemical cell that must be larger than WE and maintained close to the WE. CE platinum, tantalum, tungsten, carbon wires have been used (Kaplan, 2015).

Graphite pencil electrodes (GPEs) are carbon-based electrodes that are recognized by their low price, simplicity, commercial accessibility, simple modification and disposability. Mechanically rigid GPEs are simple to change and reduce. GPEs are attractive substrates for electrochemical sensing due to their distinctive feature of “Disposability” compared to

different usually used carbon-based electrodes. The sensitivity and selectivity of GPE toward certain analytes are often increased by applying totally different modification materials (Abdel-Nasser Kawde et al., 2016).

As compared to the other electrodes like glassy carbon electrode, the renewal of surface plays a vital role for later analysis due to chemical reactions of the molecule might cause a modification in surface properties of the electrode. Thus consequent renewal of surface of the PGE for the every trial could result in the selective and sensitive electrochemical investigation of analyte (Purushothama et al., 2018).

2.6.1. Electrochemical Measurement Techniques

The most widely used technique for obtaining qualitative information on electrochemical reactions is cyclic voltammetry. The power of cyclic voltammetry results from its ability to provide significant information on the thermo-dynamics of redox processes, on the kinetics of heterogeneous electron-transfer reactions, and on combined chemical reactions or processes of adsorption. In an electroanalytical study, cyclic voltammetry is often the first experiment performed. It provides a rapid location of the electroactive species ' redox potential and a convenient evaluation of the media's effect on the redox process (Wang, 2000).

Cyclic voltammetry consists of linear scanning using a triangular potential wave form of a stationary working electrode (in an unstirred solution). Single or multiple cycles can be used depending on the information sought. The potentionstat measures the current resulting from the applied potential during the potential sweep. The cyclic voltammogram is a complicated function of a large number of physical and chemical parameters that depend on time (Wang, 2000).

Cyclic voltammetry starts in 1938. Usually used to understand the nature of the surfaces of the electrode. Scanning takes place in these techniques with the linearly changing potential in two different potential ranges. The electrode is scanned back and forth. After you have scanned There are two peaks: the 12 peak of oxidation and the peak of reduction. By using CV, the following features can be found: .Diffusion controlled or not and adsorption , If cycle number is more than one it is important to select initial potential where there is no redox reaction. The electrode is first scanned forward and second scanned Potential backward limits with a certain

scanning rate. The scanning ends at the stop potential. All these potentialities and scanning rates differ depending on the purpose of material use (Kaplan, 2015).

One of the parameters, scan rate affects the results. Generally, The scan rate is between 1 mV/s and 1 V/s. But when scan rate get close to the 1 V/s there might be some problems such as double layer capacitance. In reversible reactions anodic and cathodic peak potential difference needs to be 59 mV and it shows that it is adsorbtion controlled reaction This difference of more or less than 59 mV and peak shapes is not well defined in reversible reactions. The height of anodic and cathodic peaks is different. If a reaction is completely irreversible, it is not possible to observe a reduction peak (Kaplan. 2015).

Characteristic peaks are caused by the formation of the diffusion layer near the electrode surface in the cyclic voltammogram. These can best be understood by carefully examining the profiles of concentration-distance during the potential sweep (Wang, 2000).

CV is also used to adsorb and desorb. If the current of anodoic and cationic peaks is gradually increased, it is an adsorption sign. If the product gathers around the surface of the electrode, the forward peak at potential is observed (Kaplan. 2015).

The most important applications of cyclic voltammetry are the qualitative diagnosis of chemical reactions preceding or following the redox process. Such mechanisms of reaction are commonly classified using letters E and C (for redox and chemical steps, respectively) in the order of steps in the reaction scheme. The occurrence of such chemical reactions, which directly affect the electroactive species' available surface concentration, is common to redox processes of many important organic and inorganic compounds. Changes in the form of the cyclic voltarnmogram resulting from chemical competition for the electrochemical reactant or product can be extremely useful in the clarification of these reaction pathways and in the provision of reliable chemical information on reactive intermediates (Wang, 2000).

Differential pulse voltammetry is an especially useful technique for measure trace levels of organic and inorganic species. In differential pulse voltammetry, fixed-magnitude pulses are applied to the working electrode just before the end of the drop, superimposed on a linear potential ramp (Wang, 2000).

The current is sampled twice, just before the pulse application (at 1) and again late in the pulse life. The resulting differential pulse voltammogram consists of current peaks, the height of which is directly proportional to the corresponding analyte concentration (Wang, 2000).

During linear scanning, DPV signals occur. For a while, consistently high pulses are applied to the working electrode. The current is twice measured. First, at the same time as the pulse starts without increasing(16.7ms), second right before the pulse end(16.7ms). Difference between two pulses is named as ΔI pulse. DPV has a relatively low detection limit (LOD) and it is possible to measure even the smallest difference between the peak of two currents. Measurement takes place at a time when faradaic current is high and capasive current is lowest (Kaplan, 2015).

2.7 Selection of the Working Electrode

It is well known from the literature that phenolic compounds can foul the electrode surface during their electrooxidation by covering it with a non-conductive polymer film. Therefore, a tedious time-consuming electrode surface cleaning step is necessary before each measurement to ensure reproducibility of determination. The use of a disposable and renewable electrode, such as the PGE is an excellent alternative to traditional electrodes**.** Similarly, the nature of the active surface of the working electrode influences the voltammetric behavior of the analyte and therefore the shapes of the voltammograms could differ significantly**.** Graphite pencil leads (GPL) are composites consisting of three components, i.e. graphite, lead and binder. The GPL's hardness and names depend on the graphite: lead ratio. The GPL referred to as B (from blackness) contains more graphite and is softer, whereas the tougher H (from hardness) pencils have lead as the main component. HB pencils have the same graphite-lead ratio. The GPL type can influence an analyte's voltammetric behavior. HB graphite pencil leads obtained the highest electrochemical signal. Thus, for further studies HB GPL was selected as the working electrode (Gomez et al.,2017).

CHAPTER 3

RELATED RESEARCH

There are various researches about electrochemical determination of olive oil some of researches are summarized below:

Cecilia et al. (2000) the aim of the work was to compare different techniques in assessing the phenolic content of an extra virgin olive oil with different storage time and storage conditions. A disposable screen-printed sensor (SPE) was combined with DPV to determine the phenolic fractions after extraction with a glycine buffer; DPV parameters were selected to study the oxidation peak of oleuropein used as a reference compound. In glycine buffer10 mM, pH=2, NaCl 10 mM (D.L.=0.25 ppm oleuropein, RSD=7 %), a calibration curve of oleuropein was performed. In addition, a tyrosinase based biosensor operating in organic solvent (hexane) was also assembled, using an amperometric oxygen probe as transducer. The calibration curves were carried out using an analysis flow injection (FIA) with phenol as a substrate (D.L.=4.0 ppm phenol, RSD=2 %). Both of these methods are easy to operate, do not require extraction (biosensor) or rapid extraction, and short analytical time (min). Using Folin±Ciocalteau reagent and HPLC analysis, the results obtained with these two innovative procedures were compared with a conventional spectrophotometric assay. Other parameters of the quality of extra virgin olive oil were investigated using traditional methods to improve the alteration process and results were reported. For Differential pulse voltammetry (DPV) analysis conditions were: potential range 100±700 mV vs.screen-printed reference electrode, pulse amplitude 50 mV, scan rate 50 mV/s, pulse width 60 ms. a calibration curve of oleuropein used as standard compound is reported (y=-33+201x (y=current, µA; x=oleuropein, ppm], r^2=0.996, LOD=0.25 ppm oleuropein, RSD=7%) Screen-printed three-electrode strips (a carbon working electrode and silver counter and reference electrodes) were employed for all experiments. To eliminate surface fouling of the working electrode, a new electrode was used for each sample. Using a calibration curve, quantitative sample analysis was performed. The detection limit (LOD) was calculated as 3 times the blank standard deviation. It seems

from the reported results that the DPV analysis measures polyphenol compounds with an oleur-like structure. As per the authors compared to conventional methods for polyphenol analysis, the two methods proposed are faster and more inexpensive and can be considered promising systems for assessing this class of compounds in oil samples (Cecilia et al.2000).

Gomez et al. (2017) a methodology was presented for enhanced electrochemical detection of oleuropein by Graphene Oxide Pencil Grahite Electrode (GOPGE) in complex plant matrices in combination with a Natural Deep Eutectic Solvent buffer containing 10% (v/v) of lactic acid, glucose and H_2O (LGH) . Using differential pulse voltammetry, the electrochemical behavior of oleuropein in the modified-work buffer was examined. The electrochemical behavior of oleuropein in working buffer was examined using differential pulse voltammetry (DPV). DPV was performed with a potential range from −0.5 to +1.0 V, with 5 mV step potential, 25 mV pulse potential, 20 mV/s scan rate, 0.01 s pulse time and 3 s equilibration time.The combination of both modifications, NADES modified buffer and LGH-GOPGE modified nanomaterial electrode, resulted in a 5.3 times higher signal enhancement than the bare electrode with unmodified buffer. A calibration curve of oleuropein was performed between 0.10 to 37µM and a good linearity was obtained with a correlation coefficient of 0.989. Method limits for detection and quantification were obtained as 30 and 102 nM respectively. Furthermore, precision studies indicated that the voltammetric method was sufficiently repeatable, with a percentage of RSD 0.01 and 3.16 (n=5) respectively for potential and intensity. An electrochemical sensor is presented and evaluated in this work, based on the combination of natural deep eutectic solvent modified buffer and nanomaterial pencil graphite electrode. As per the authors the proposed electrochemical sensor was successfully applied to the determination of oleuropein in an olive leaf extract prepared by ultrasound-assistedextraction. The results obtained with the proposed electrochemical sensor were compared with Capillary Zone. The sensor's electrochemical response has been greatly enhanced and has demonstrated exceptional benefits such as single use, disposability, high analytical performance and extremely low cost. In addition, the system's portability makes it very valuable without the need for skilled personnel for wide-spread use. All these features make these electrochemical approaches very valuable before using more sophisticated analytical techniques for polyphenol screening tools (Gomez et al. 2017).

Apetrei et al. (2006) A method for evaluating the bitterness of extra virgin olive oils has been developed. The method uses electrodes of carbon paste where olive oils are used as the material of the electroactive binder. Voltammetric experiments were conducted with an EG&G Model 263A potentiostat/galvanostat, connected to a desktop computer for data acquisition and experiment control. A standard three-electrode cell was used, using the modified carbon paste electrodes as the working electrode, Pt wire as the counter electrode, and Ag / KCl sat. electrode as the electrode of reference. Electrodes have been fabricated with nine extra virgin olive oils differing in their degree of bitterness. The characteristics observed in the voltammograms reflect the reactions of electroactive compounds (such as polyphenols) found in the virgin olive oils mixed with the matrix of carbon paste. For this reason, the electrodes voltammetric responses are specific to each oil type. Furthermore, each electrode displays a variety of responses immersed in various electrolytic solutions. Such response pattern can be considered as the oil's characteristic fingerprint. The Principal Component Analysis (PCA) and the Partial Least Squares Discriminant Analysis (PLS-DA) of the electrochemical signals obtained by immersing the electrodes in various solutions has allowed a clear discrimination of the nine virgin olive oils according to their degree of bitterness. Good correlations have been found between the redox processes observed in the electrodes and the analytical and sensory characteristics of the studied virgin olive oil (Apetrei et al. 2006).

Fernández et al. (2018) The approach is presented to determine hydrophilic phenols in olive oil samples, using vortex-assisted reversed-phase dispersive liquid-liquid microextraction (RP-DLLME) for sample preparation and screen-printed carbon electrodes for voltammetric analysis. A vortex mixer from Heidolph (Swabach, Germany) was used to support RP-DLLME. A centrifuge from Selecta (Barcelona, Spain) was used for phase separation. A Multi Autolab/M101 Potentiostat/Galvanostat from Metrohm Autolab B.V. (Utrecht, The Netherlands) controlled by NOVA software version 1.10 was used for electrochemical experiments. The working disk-shaped electrode was made of carbon ink, 4 mm in diameter, also same to the counter electrode, while the pseudo-reference electrode was made of silver. For connecting SPCEs to the potentiostat, specific connectors obtained from DropSens (ref. DRP-DSC) were used. An ultraviolet-visible spectrophotometer from Thermo Scientific (Waltham, MA, USA) was used in Folin-Ciocalteu assays. In linear discriminant analysis, the concentrations of a fifteen oil samples were used as input variables to distinguish between

olive oils of different quality. To investigate the electrochemical behavior of hydrophilic phenols with screen-printed carbon electrodes (SPCEs), cyclic voltammetry (CV) was used. Potential was recorded at 100 mV scan rate from 0.0 V to + 1.2 V. DPV was used as electroanalytical technique after reversed-phase dispersive liquid-liquid microextraction (RP-DLLME). After a single use, SPCEs were always discarded. All experiments were carried out in triplicate and at room temperature. As per authors In order to determine hydrophilic phenols in olive oil samples, RP-DLLME was successfully combined with SPCEs for the first time. Thus, the advantages of miniaturized systems were exploited synergistically, both in the sample preparation and detection phase. RP-DLLME, on the one hand, involves a fast and easy-to-handle process with significantly low organic solvent consumption compared to SPE technique, making it environmentally friendly. Unmodified and commercially available SPCEs, on the other hand, provide a quick and sensitive response with affordable and portable instrumentation (Fernández et al. 2018).

Nadifiyine et al. (2013) A biosensor was developed based on the immobilization on carbon black paste electrode of commercially available tyrosinase. Compared to enzymatic sensors based on traditional graphite paste electrodes, this device showed significantly reduced noise. Also, peroxidase and laccase biosensors were prepared using catechol as the substrate. These three enzymatic biosensors' responses to twenty different phenolic compounds were investigated, taking into account their molecular structure and their specific relationship to enzyme activity. Furthermore, another sensor based on semi-purified tyrosinase was reported. A good statistical correlation existed between the results obtained with the biosensor of tyrosinase and the spectrophotometric methods of Folin-Ciocalteu for determining phenol in olive oils. All experiments were conducted using a portable electrochemical analyzer from PalmSens (PalmSens BV, Houten, Netherlands) with a current resolution of 0.1 percent at the lowest current range (1 pA). This device is connected with PC Software. The three-electrode system consisted of an electrode (carbon paste), an electrode of reference Ag/AgCl (3 M NaCl) and an auxiliary electrode of stainless steel. A good statistical correlation existed between the results obtained with the biosensor of tyrosinase and the spectrophotometric methods of Folin-Ciocalteu for determining phenol in olive oils. Folin-Ciocalteu Method Folin-Ciocalteu is a widely used method of evaluating phenol content (Capannesi et al. 2000; Mello, Sotomayor, and Kubota 2003). This method has been used as a guide in this study to

compare the results obtained with amperometric analysis. Contrary to peroxidase and laccase, the tyrosinase biosensor reacted with a wide range of phenolic compounds and showed a good correlation coefficient with the olive oil colorimetric Folin-Ciocalteau method (Nadifiyine et al. 2013).

Gomez et al. (2017) Graphene Oxide Pencil Grahite Electrode (GOPGE) in combination with a buffer modified with a Natural Deep Eutectic Solvent containing 10% (v/v) of lactic acid, glucose and H2O (LGH) is presented with a methodology for enhanced electrochemical detection of oleuropein in complex plant matrices. The combination of both modifications, NADES modified buffer and LGH-GOPGE modified nanomaterial electrode, resulted in a 5.3 times higher signal enhancement than the unmodified buffered bare electrode. A calibration curve of oleuropein wasperformed between 0.10 to 37μM and a good linearity was obtained with a correlation coefficient of 0.989. Method limits for detection and quantification were obtained as 30 and 102 nM respectively. Furthermore, precise studies showed that the voltammetric method was sufficiently repeatable %RSD 0.01 and 3.16 (n=5) for potential and inten-sity, respectively the electrochemical behavior of oleuropein within the modified-working buffer was examined using differential pulse voltammetry. At room temperature, all electrochemical measurements were performed on a USB-based portable electrochemical station with DropView 200 software controlled bipotentiostat (Dropsens, Oviedo, Spain).

A conventional electrode system consisted of a disposable pencil graphite electrode (PGE) as the working electrode, with an Ag / AgCl/3 M KCl as a reference electrode and a platinum wire an auxiliary electrode. A mechanical pencil from Plantec, Model 9512 (Argentina), has been used as a pencil lead holder. Measurements were performed in a 5 ml solution glass cell. Stirring was achieved with a magnetic stirring bar during DPV measurements. The proposed electrochemical sensor was successfully applied to the determination of oleuropein in an olive leaf extract prepared by ultrasound-assisted extraction. The results obtained with the proposed electrochemical sensor were compared with Capillary Zone electrophoresis analysis with satisfactory results (Gomez et al.2017).

Tsopelas et al. (2018) In order to detect the adulteration of extra virgin olive oil with olive pomace oil and the most common seed oils, namely sunflower, soybean and corn oil, two approaches for the voltammetric fingerprinting of oils and their combination with

chemometrics were investigated. In particular, cyclic voltammograms of diluted extra virgin olive oils, regular (pure) olive oils (mixtures of refined olive oils with virgin olive oils), olive pomace oils and seed oils were recorded in the presence of dichloromethane and 0.1 M LiClO4 in EtOH as electrolytes in a glassy carbon working electrode. Cyclic voltammetry was also used in olive and seed oils methanolic extracts. Cyclic voltammogram data points have been exported and submitted to Principal Component Analysis (PCA), Partial Least Square Discriminant Analysis (PLS-DA) and Soft Independent Class Analogy Modeling (SIMCA). In diluted oils, PLS-DA clearly discriminated between olive oils (extra virgin and regular) and olive pomace / seed oils, whereas SIMCA showed a clear discrimination of extra virgin olive oil in regard to all other samples. For extra olive oil three minor broadened anodic peaks are evident in the range of 0.52-0.65, 0.77-0.95 and 1.08-1.22 V. Using methanol extracts and considering data points recorded between 0.6 and 1.3 V, more information was provided by PLS-DA, resulting in three clusters of extra virgin olive oils, regular olive oils and seed / olive pomace oils, while SIMCA showed inferior performance.

For the quantification of extra virgin olive oil adulteration with olive pomace oil or seed oils, a model based on Partial Least Square (PLS) analysis was develop. A suitable test set has been used to prove validation and applicability of all models. For PLS, synthetic oil mixtures with 4 known levels of adulteration ranging from 4% to 26% were also used as a blind test set. All electroanalytical measurements were carried out using the 797 VA Computrace Stand (Metrohm) electrical analytical systems connected to a PC through a USB port. A glassy carbon one was used as a working electrode. The reference electrode was an electrode Ag / AgCl in High Purity Water filled with 3 M KCl and the auxiliary a Pt wire. At the beginning of each measurement, the working electrode was polished with alumina powder (0.3 μm) using a polishing cloth, rinsed with acetone for 10 minutes in distilled water. The working electrode was also polished between measurements in various samples with alumina powder. Voltammetric analysis was performed at 24 ± 1 ° C temperature controlled. Mid-term repeatability of the obtained voltammograms for the oils under investigation was evaluated by performing the analyses of the same oil in different days. The techniques used are easy, quick, low-cost and require minimal sample preparation before analyzing. In addition, the developed voltammetric fingerprinting approaches can be applied or used to determine electrochemically active species in fatty matrices (Tsopelas et al.2018).

Robledo et al. (2014) has describe the use of square wave voltammetry in ultramicroelectrodes to determine natural antioxidants (tocopherols) and tert-butyl hydroxytoluene in edible vegetable oils such us olive oil. Tocopherol determinations were made in benzene / ethanol (1:2) + 0.1 mol L−1 H2SO4 + oil samples on a carbon fiber disk ultramicroelectrode, and tert-butyl hydroxytoluene was determined in acetonitrile (ACN) + 0.1 mol L−1 $(C_4H_9)4NF_6P$ on a Pt band ultramicroelectrode after extraction from the oil sample using ACN. The concentrations of antioxidants calculated using this methodology were in good agreement with the values declared by the manufacturers (Robledo et al. 2014).

Pella et al. (2017) for class-selective electrochemical detection of ortho-diphenols (o-diphenols) and mono-phenols (m-phenols) antioxidants, carbon black nanoparticles (CBNPs) printed films are proposed. A conventional three-electrode cell system was used in all electrochemical experiments with CBNPs electrode as working electrode, Ag/AgCl as reference and a platinum wire as counter electrode. To investigate their electrical properties, the electrodes were characterized by Electrochemical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV). . Olive oil polyphenolic extracts were assayed using DPV.

For Electrochemical characterisation and detection of olive oil phenolic standard compounds the response of the CBNPs electrodes to the polyphenols standards and extracts was investigated using CV and DPV. The standards were analyzed in phosphate buffer (PB; pH 7.40, 0.05 M) individually and in mixture. The cyclic voltammetry was carried in the potential range of −0.20V and+ 1.0V (vs. Ag / AgCl) with a scan rate of 0.050Vs−1. Pulse amplitude; 50 mV / s, scan rate 10 mVs−1 were the best conditions found for DPV. The anodic peaks of o-diphenols and m-phenols (n = 5) peaked in the range 0.120–0.160V and 0.590–0.610V, respectively .All measurements were carried out at room temperature in triplicate. According to the study considering the ease of operation, speed, automation, sensitivity and selectivity of the assay, CBNPs have been shown to be a useful material for relevant food analysis applications nowadays (Della Pella et al. 2017).

Grossi et al. (2014) a technique based on electrical impedance spectroscopy is presented and implemented in this study as a low-cost, mobile tool to be used everywhere and by anyone, with substantial improvements to the current quality control of olive oil producers of any

dimension. The method presented is validated on 39 samples of olive oil with varying acidity levels, peroxide index and total phenolic content. The results show that the conductance with a hydro-alcoholic solution of an emulsion of olive oil is correlated with the acidity of oil. In addition, since emulsion conductance for the same oil varies with the conditions of oil storage (because of increases in the peroxide index), the technique presented could also be used to evaluate product ageing.

The sensor hosting the emulsion's "measurement head" is a 50 ml tube modified to include a few cap-shaped stainless steel electrodes (6 mm in diameter, 12 mm apart from each other) needed for electrical characterization. To ensure that all samples are tested at the same temperature, the thermal incubator Binder APT KB 53 is set to 20 C (Grossi et al. 2014).

Apetrei et al. (2014) the article presents the use of an e-tongue voltammetric to detect virgin olive oil adulteration. Using modified carbon paste based sensors, adulterations of an extra virgin olive oil with different percentages of sunflower oil, soybean oil and maize oil were measured. The voltammetric square wave signals were processed using the method of the kernel. Chemometric methods used make it possible to discriminate and classify oils according to botanical origins. Correlation obtained between voltammetric signals and polyphenolic content. Carbon paste electrodes (CPE) modified with edible oils were prepared for electronic tongue measurements. Voltammetric measurements were performed using a conventional three-electrode cell in a Biologic Science Instruments SP 150 potentiostat / galvanostat (EC-Lab Express software). The modified EO carbon paste-based sensors were used as working electrodes. The reference electrode was an Ag / AgCl KCl 3 M and a platinum wire was the counter electrode. Measurements of the e-tongue were carried out using the EO carbon paste-based sensors. Cyclic voltammetry (CV) performed preliminary studies and stabilization of the sensor signals. CV has been used to study qualitative information on electrochemical processes, reversibility of reactions, and sensor response stability. E-tongue consisting of voltammetric sensors together with appropriate chemometrics, presents itself as a powerful tool for the detection of adulteration of extra virgin olive oil. E-tongue application combined with e-nose to detect olive oil adulteration could improve the system's ability even at lower levels of adulteration (Apetrei et al. 2014).

Talarico et al. (2015) has report the detection of phenolic compounds by a miniaturized and disposable electrochemical sensor. The sensor was built by modifying the working electrode surface of screen-printed electrode (SPE) with carbon black (CB) dispersion. This new probe showed higher sensitivity and better fouling resistance than the bare SPE, showing CB's suitability as an excellent SPE nano modifier for the detection of phenolic compounds.

Square wave voltammetry with a detection limit of 0.1 μM, 1 μM, 0.8 μM, and 2 μM respectively detected catechol, gallic acid, caffeic acid, and tyrosol. Measurements of Cyclic Voltammetry (CV) and Square Wave Voltammetry (SWV) were carried out using a portable PalmSens instrument (Netherlands). The sensor was able to selectively discriminate with rapid and easy measurement of mono-phenols and ortho-diphenols, helping to use of a cost-effective device for quality control of phenolic compound foods and beverages. CB-SPE showed better electrochemical properties than bare SPE, in terms of reducing peak-to-peak separation and intensity of the peak for compounds characterized by a reversible behavior like catechol. In addition, in the case of compounds characterized by an irreversible behavior as tyrosol, a decrease in the required applied potential was observed.

The SWV analysis using a portable device (PalmSens) highlighted the advantages of using CB-SPE, enabling this sensor to detect phenolic compounds at lower potential, with higher sensitivity without fouling problem at μM level compared to the bare SPE. In addition, CB-SPE is able to distinguish between mono-phenols and ortho-diphenols with the optical methods advantages. CB's relevant electrochemical properties confirm the high potential of this cost-effective nanomaterial, which makes it increasingly competitive with the graphene and carbon nanotubes that are most used (Talarico et al.2015).

Enache et al. (2012) To determine the total ortho-phenol content of virgin olive oil (VOO) with high sensitivity and reproducibility, an electroanalytical methodology was developed. The electroanalytical methodology developed was used to determine fresh and old VOO ortho-phenol content. The content of VOO ortho-phenol depends on its freshness and is usually expressed as equivalent to HT. To investigate the oxidation of catechol, phenol, hydroxytyrosol (HT), tyrosol, caffeic acid and ferulic acid, screen-printed electrodes were used with cyclic voltammetry. Oxidation of ortho-phenols and mono-phenols occurs at

different mechanisms and potentials. An HT detection limit of 0.40µM was obtained using screen-printed electrodes and square wave voltammetry. To determine the ortho-phenol content in fresh and old VOO, the electroanalytical methodology developed was applied.

Voltammetric experiments were conducted using a portable electrochemical analyzer PalmSens running PalmSensePC 2.6, Palmsens BV, Houten, Netherlands. Cyclic voltammetry (CV) used a scan rate 30 or 50 mV s1 . For square wave (SW) voltammetry, the parameters were: pulse 25 mV, frequency 12 Hz and potential increment 2 mV, corresponding to an effective scan rate of 50 mV s1. The SPEs consisted of three electrodes: two carbon electrodes as working and counter electrodes, and a silver pseudo-reference electrode. The aim of the work was to provide an indicator of olive oil freshness, so SPEs studied in detail the selectivity of voltammetric methods for electrochemical detection of ortho-phenols with respect to mono-phenols. As per the study the benefits of SW voltammetry are higher analytical speed, lower electroactive species consumption in relation to differential pulse voltammetry, and reduced electrode surface poisoning problems, which is a major limitation in the direct electrochemical detection of polyphenols in real samples (Enache et al.2012).

Fernández et al. (2018) was conducted a study to determine hydrophilic phenols in olive oil samples using vortex-assisted reversed-phase dispersive liquid-liquid microextractionRP-DLLME for sample preparation and screen-printed carbon electrodes for voltammetric analysis. The applicability of the proposed method was tested in olive oil samples of different quality (i.e., refined olive oil, virgin olive oil and extra virgin olive oil). The proposed method was used to analyze fifteen samples and a high correlation was obtained with the traditional Folin-Ciocalteu spectrophotometric method. Thereafter, the concentrations of the fifteen oil samples were employed as input variables in linear discriminant analysis in order to distinguish between olive oils of different types. To investigate the electrochemical behavior of hydrophilic phenols with SPCEs, cyclic voltammetry was used. Potential was recorded at 100 mV s−1 scan rate from 0.0 V to + 1.2 V. Following RP-DLLME, DPV was used as an electroanalytical technique. SPCEs were always discarded after a single use. The lowest hydrophilic phenols content, as expected, corresponded to ROO samples while the highest concentrations were found in EVOO samples. The proposed method combines a simple, fast

and environmentally friendly sample preparation technique with electrochemical detection using unmodified, low-cost and commercially available SPCEs, offering unique benefits.

Finally, the proposed method has resulted in an appropriate strategy to discriminate between ROO and higher quality olive oils in combination with LDA.RP-DLLME coupled with SPCEs is promising alternative to detecting hydrophilic phenols in olive oil samples, is affordable to any laboratory, and has a potential application for rapid assessment of olive oil quality and detection of fraudulent practices (e.g., adulteration) (Fernández et al. 2018).

CHAPTER 4

MATERIAL AND METHODS

4.1 Oil Samples

Total of eleven commercial olive oils were purchased from Northern Cyprus and included in the study; five of them are extra virgin olive oil (EVOO) which are coded as (A,B,C,D,E) samples, five are of virgin oil (VOO) samples which are coded as (F,G,H,I,J) and one Sample is for Riviera.

4.2 Apparatus and Reagents

Conventional three electrode system, consisting of the PGE as the working electrode, (Ag/AgCl) as a reference electrode and a platinum wire as a counter electrode are engaged in connection with the Nova2.12 software (Figure4.1). The potential-controlled cyclic voltammetric and differential pulse voltammetric**.** All the experiments were carried out at a constant temperature of 25 ◦C.

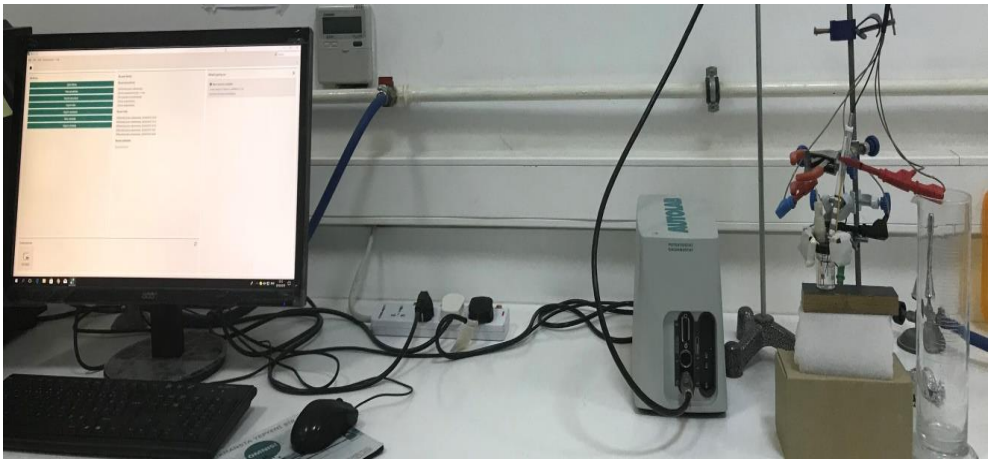

Figure 4.1: Electrodes connecting with Nova2.12

4.2.1 Preparation of buffer solutions

Acetate buffer solution (ABS) 0.5 M :

Acetate buffer solution is prepared by measuring 14.45 ml of concentrated acetic acid and complete to 250 ml with distilled water. 1M NaOH volume of 250 ml should be enough to add in to solution and 0.02 M for NaCL. The pH is 4.8.

4.2.2 Preparation of Pencil graphite

Various lengths of pencil lead can be extruded to yield different surface areas. As expected, pencil lead length (exposed to the sample) affects the response deeply. Thus, the graphite leads were cut in half and inserted into a mechanical pencil holder to keep out 1.5 cm of the pencil lead (Figure 4.2). The PGE was connected to the instrument by soldering a metal wire at the pencil holder's metallic top (Figure 4.3). During measurements, 1 cm of the graphite lead was inserted into the solution to be analyzed while the holder was kept upright.

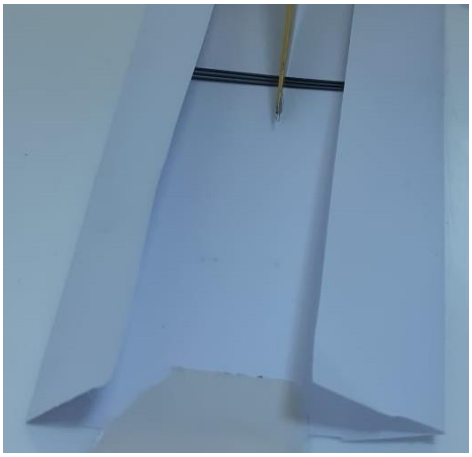

Figure 4.2: Graphite leads cut in half

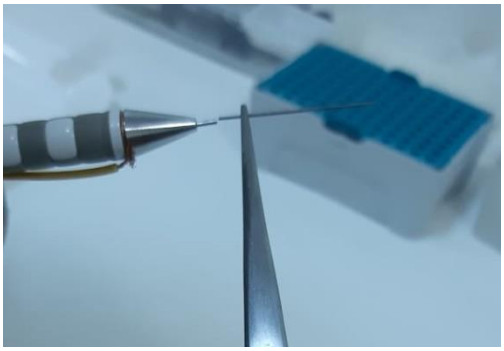

Figure 4.3: Replacing leads into pencil to form PGE

4.3 Methods

4.3.1 Electrochemical activation of PGE

Chronoamperometry involves stepping the working electrode's (Pencil graphite) potential from a value where there is no faradaic reaction to the potential where the electroactive species ' surface concentration is effectively zero. A Pencil graphite a is used, along with an unstirred acetate buffer solution. Chronoamperometry was performed under the constant potential +1.40 V for 30s**.**

4.3.2 Differential Pulse Voltammetry of olive oil samples

The electrochemical behavior was examined using differential pulse voltammetry (DPV). Pencil tips were kept in 200µl olive oil Eppendorf tubes for 30minutes (Figure4.4), then dried for 30 minutes to go later under differential pulse voltammetry. These steps were done to the 11 different olive oil samples and 3 pencil tips were used in each sample. The reference and the counter electrodes were washed with distilled water after each experiment, DPV was performed with a potential range from 0 to +1.2 V, with 0.005 V step potential, 10 mV/s scan rate, 119 s duration time.

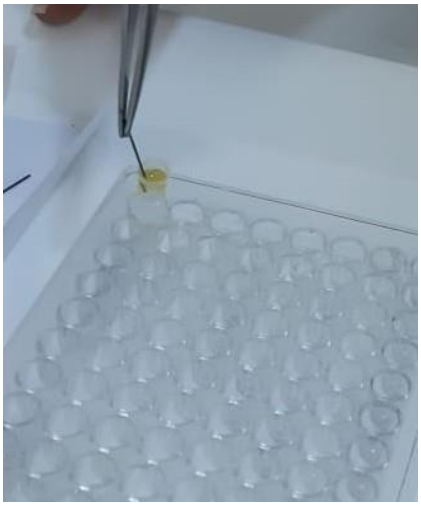

Figure 4.4: Pencile tip kept in Eppendrof tubes

CHAPTER 5

RESULTS AND DISCUSSION

5.1 DPV Voltammograms of Olive Oils

The determination of tocopherols in olive oil samples was carried out without pretreatment of samples, taking 200µl of oil and applying the proposed method as described before.

The DPV responses of different extra virgin olive oil samples and virgin olive oil samples were recorded as shown in Figure 5.1 , Figure 5.2 and Figure 5.3 respectively. Table 5.1 the peak positions and peak heights in all brands of EVOOs and Table5.2 summarizes the peak positions and peak heights in all brands of VOOs.

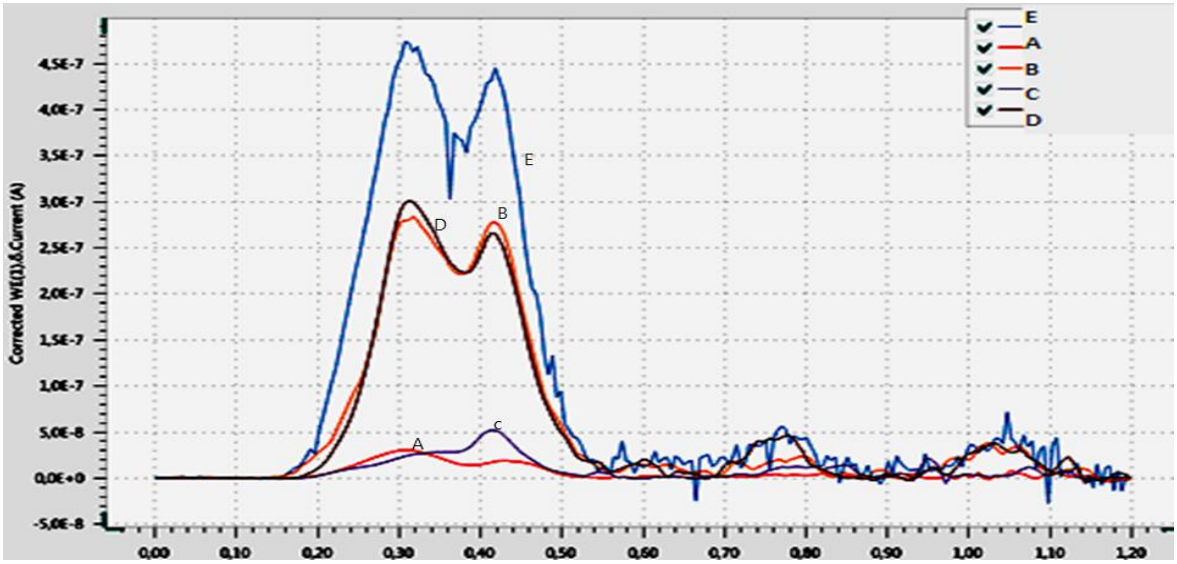

Figure 5.1: The voltammograms of all brands of EVOO which are coded as (A,B,C,D,E). In 0.5 M (ABS), pH is 4.8 and E vs Ag/Agcl 0 – 1.2 (V).

Table 5.1: The peak positions and peak heights in all brands of EVOOs**.**

Samples (EVOO)	Peak position (V)	Peak Height (A)
A	0.45	5.95×10^{-8}
B	0.42	1.2×10^{-7}
C	0.42	3.3×10^{-8}
D	0.42	1.04×10^{-7}
E	0.42	6.165×10^{-7}

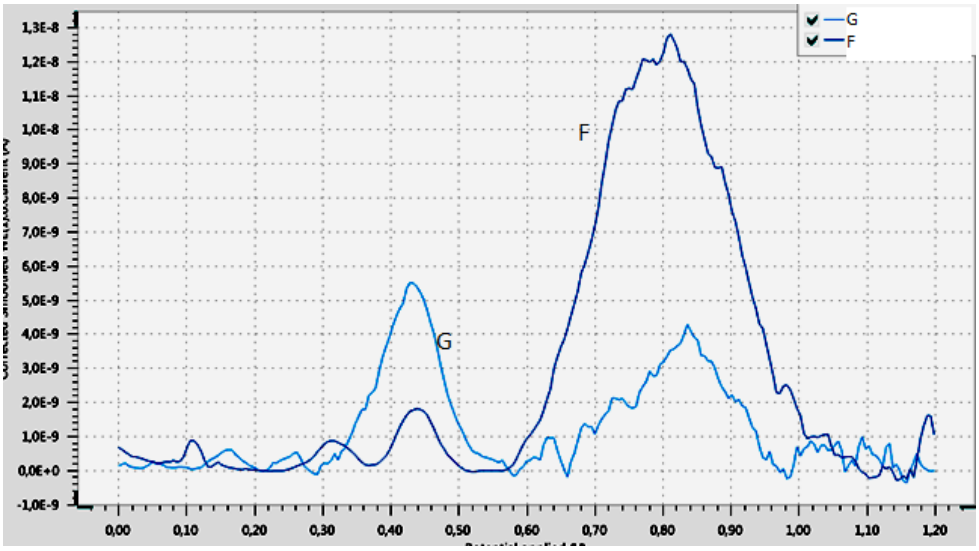

Figure 5.2: The voltammograms of brands F and G of VOOs. In 0.5 M (ABS), pH is 4.8 and E vs Ag/Agcl 0 – 1.2 (V).

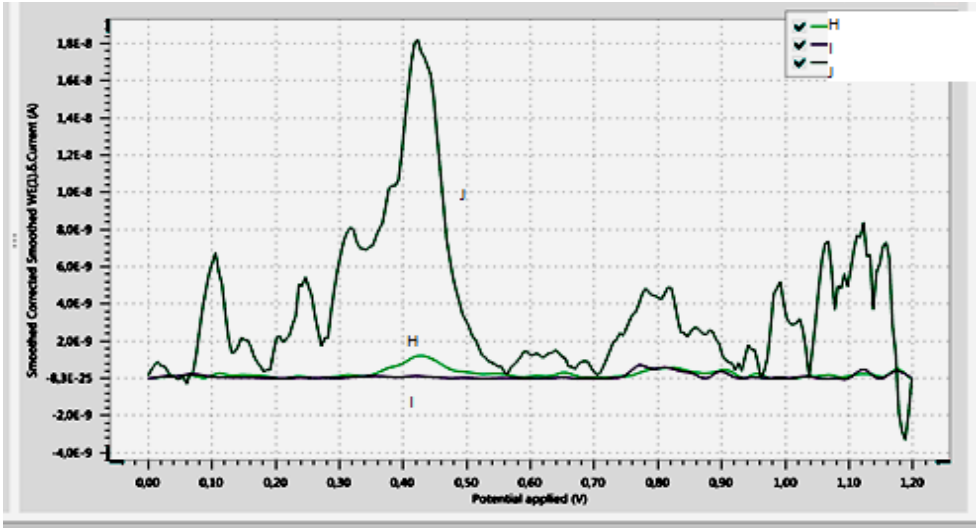

Figure 5.3: The voltammograms of brands H,I and J of VOOs**.** In 0.5 M (ABS), pH is 4.8 and E vs Ag/Agcl 0 – 1.2 (V).

Table 5.2: The peak positions and peak heights in all brands of VOOs

.

Samples (VOO)	**Peak position (V)**	**Peak Height (A)**
F	0.44	1.76×10^{-9}
G	0.43	5.2×10^{-10}
H	0.42	1.05×10^{-9}
I	0.42	7.8×10^{-11}
J	0.43	1.33×10^{-8}

5.2 Confirmation of α-tocopherol peak position with standard addition

First, we obtained olive oil voltammograms. It was observed that there were two adjacent peaks at peak position of α-tocopherol in olive oil. Then to be sure we added some α-tocopherol standard into olive oil and re-analyzed. Figure 5.4 shows voltammograms of these measurements in olive oil. As a result, α-tocopherol peak was found as second peak on sample matrix.

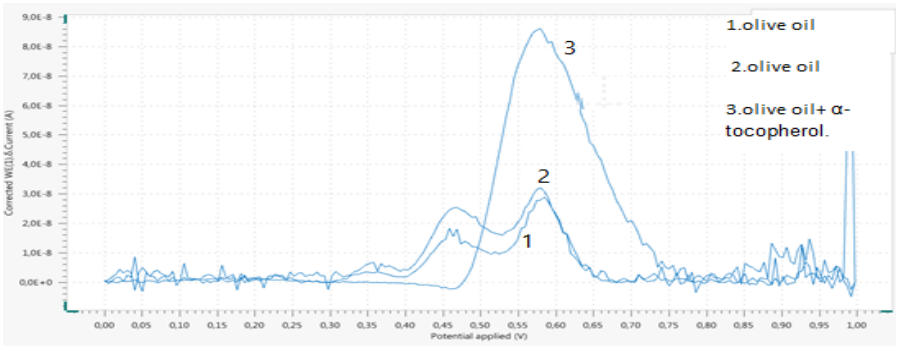

Figure 5.4: DPV voltammograms recorded for olive oil and with added standard: 1.olive oil; 2.olive oil 3.olive oil + α-tocopherol. In 0.5 M (ABS), pH is 4.8 and E vs Ag/Agcl 0 – 1.2 (V).

5.3 Electrochemical Behavior of Tocopherols for Each Olive Oil Sample

5.3.1 Sample A -EVOO

Figure 5.5 shows the voltammograms of A brand. As can be observed analytes were simultaneously oxidized giving rise to oxidation peak at +0.32V. At the higher potential (+0.44V) a second peak was shown for tocopherol oxidation.

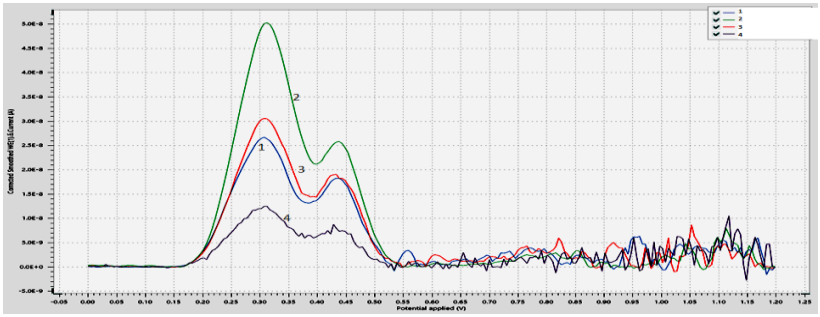

Figure 5.5: The voltammograms of A brand EVOO which are coded as (1,2,3,4). In 0.5 M (ABS), pH is 4.8 and E vs Ag/Agcl 0 – 1.2 (V).

5.3.2 Sample B - EVOO

Between +0.25V and +0.5 V two peaks were observed in DPV voltammograms of B brand in Figure 5.6. As can be observed analytes were simultaneously oxidized giving rise to oxidation peak at +0.32V. At the higher potential (+0.42V) a second peak was observed in B brand corresponding to tocopherol oxidation.

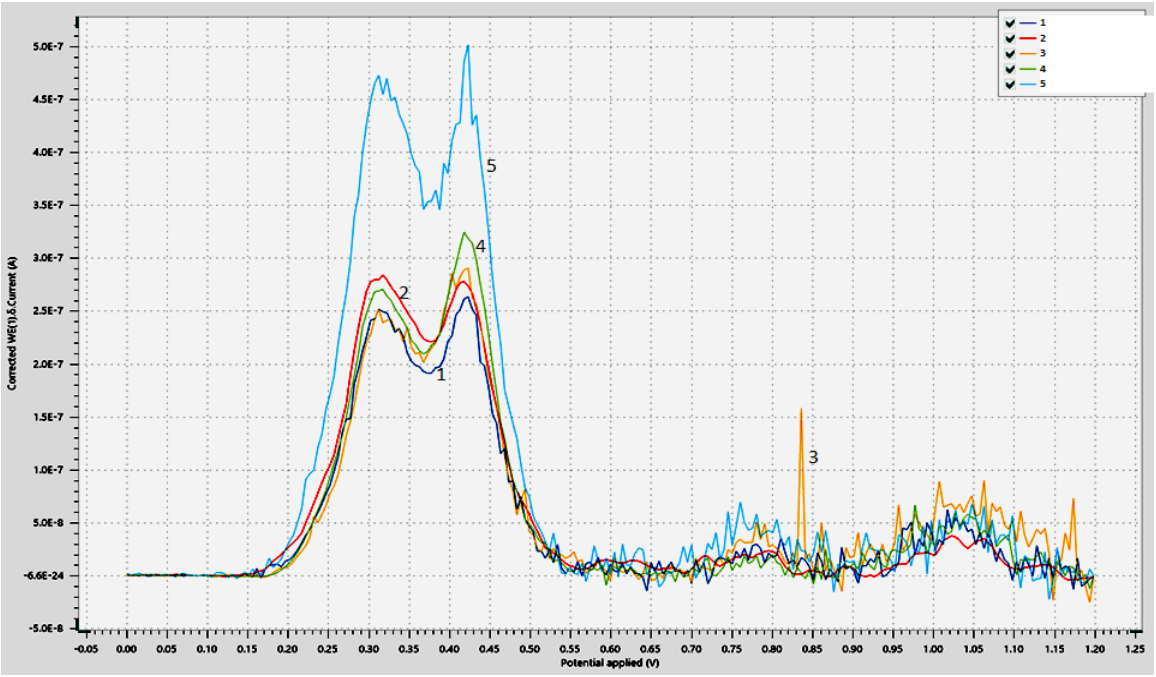

Figure 5.6: The voltammograms of B brand EVOO which are coded as (1,2,3,4,5). In 0.5 M (ABS), pH is 4.8 and E vs Ag/Agcl 0 – 1.2 (V).

5.3.3 Sample C- EVOO

Figure 5.7 shows signals obtained from C-EVOO sample. Tocopherol was oxidized, leading to an increase in an oxidation peak at +0.34V, at the higher potential +0.42V. A second peak was shown for tocopherol.

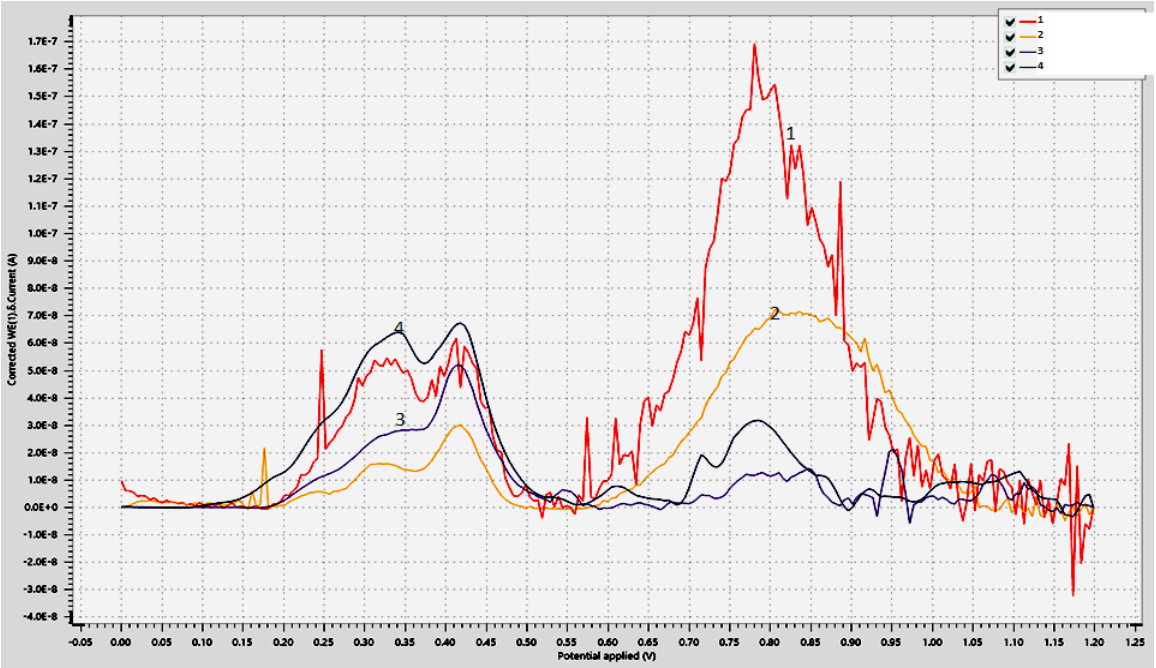

Figure 5.7: The voltammograms of C brand EVOO which are coded as (1,2,3,4). In 0.5 M (ABS), pH is 4.8 and E vs Ag/AgCl 0 – 1.2 (V).

5.3.4 Sample D - EVOO

DPV voltammograms of D brand are shown in Figure 5.8. As can be observed from Figure 5.8 analytes were simultaneously oxidized giving rise to oxidation peak at +0.32V. A second peak in the sample D – EVOOs corresponding to tocopherol oxidation was observed at the higher potential of + 0.43V.

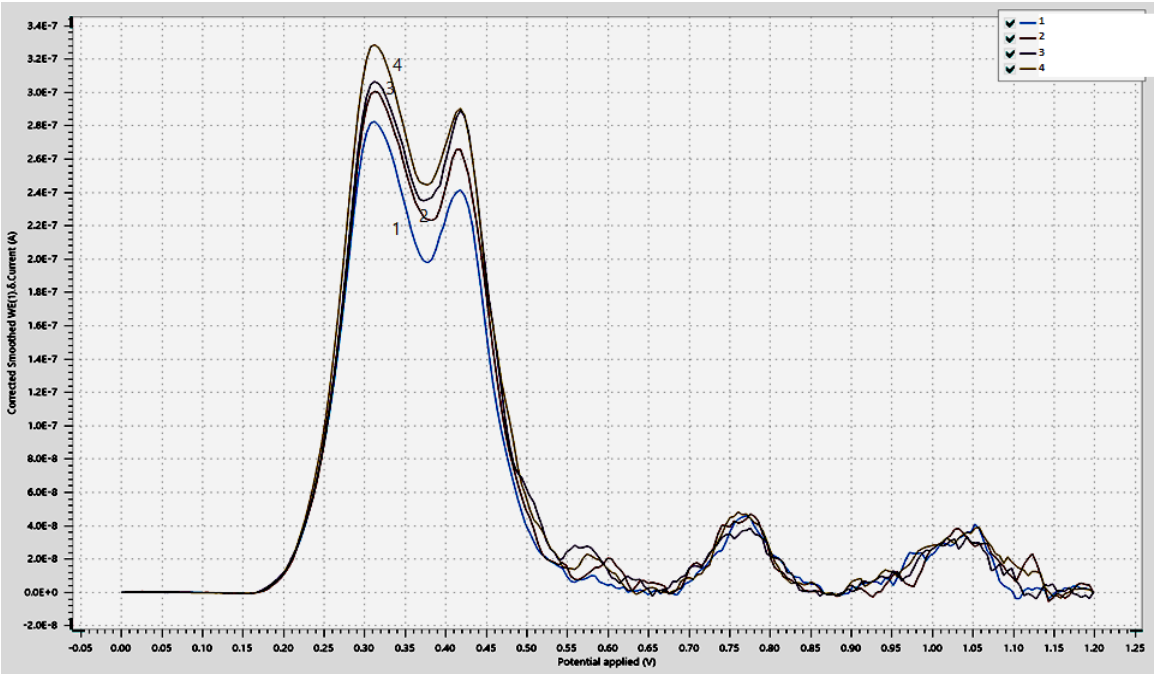

Figure 5.8: The voltammograms of D brand EVOO which are coded as (1,2,3,4). In 0.5 M (ABS), pH is 4.8 and E vs Ag/AgCl 0 – 1.2 (V).

5.3.5 Sample E -EVOO

Two peaks at +0.31V and +0.42V were observed in DPV voltammograms of E brand EVOO as can be seen in Figure 5.9.

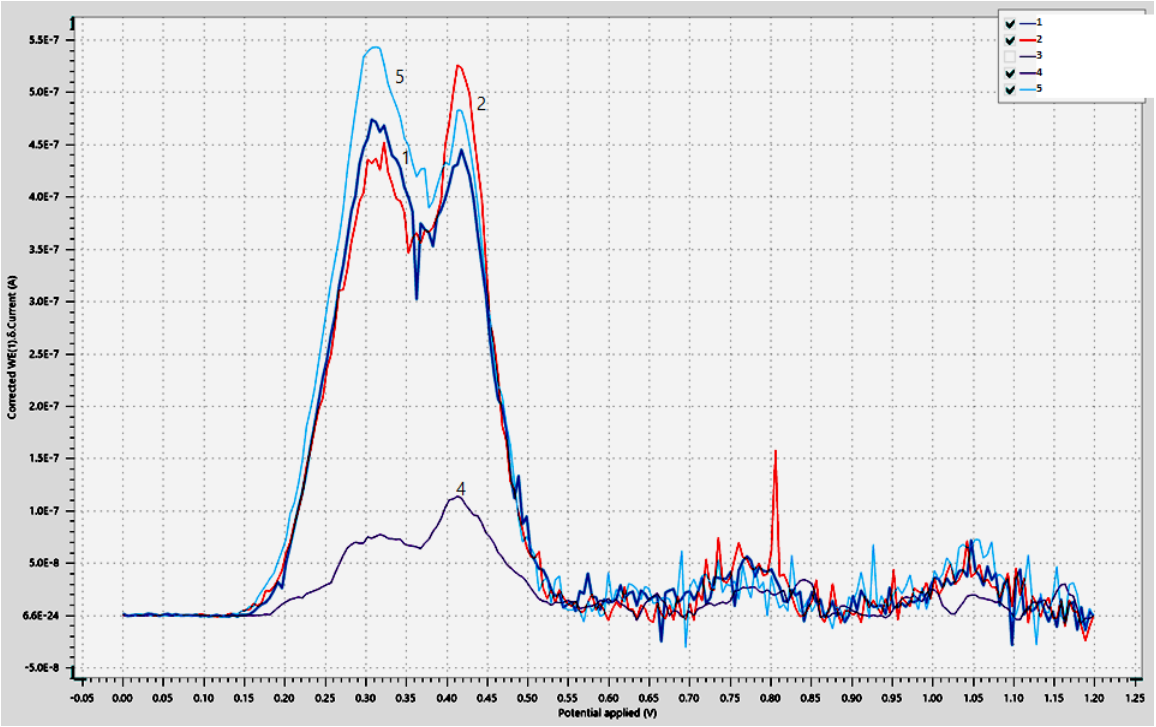

Figure 5.9: The voltammograms of E brand EVOO which are coded as (1,2,3,4). In 0.5 M (ABS), pH is 4.8 and E vs Ag/AgCl 0 – 1.2 (V).

5.3.6 Sample F -VOO

DPV voltammograms of F brand are shown in Figure 5.10 tocopherol were oxidized, resulting in oxidation peak at + 0.44V, as can be observed. A second peak in the sample F – VOOs was observed at the higher potential + 0.94V.

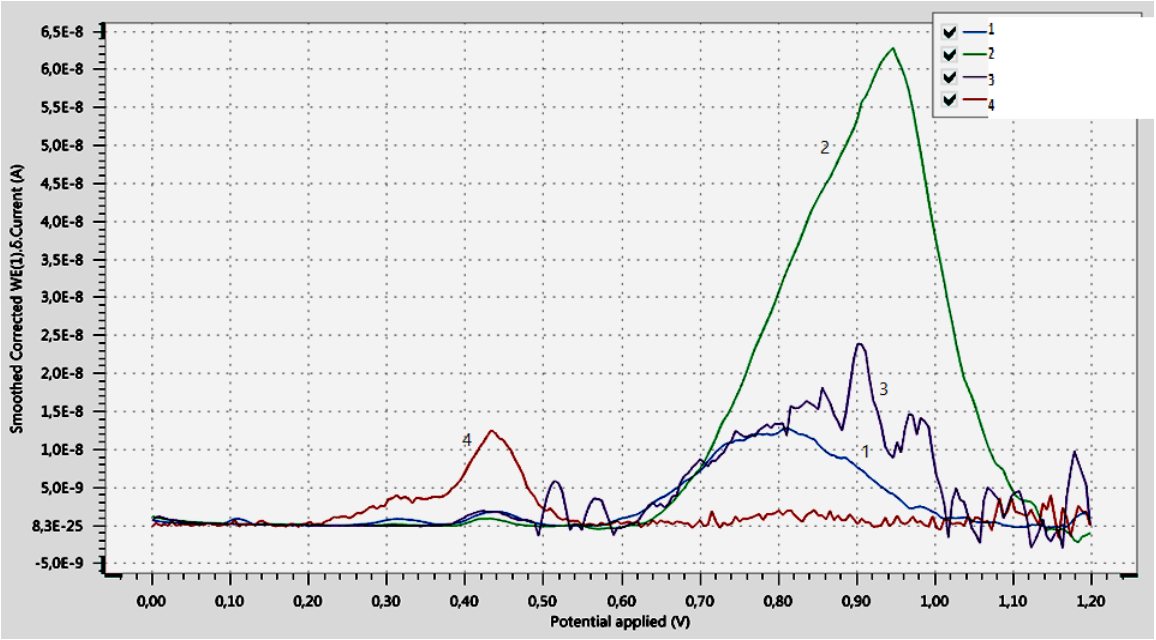

Figure 5.10: The voltammograms of F brand VOO which are coded as (1,2,3,4). In 0.5 M (ABS), pH is 4.8 and E vs Ag/AgCl 0 – 1.2 (V).

5.3.7 Sample G –VO6O

Figure 5.11 shows signals obtained from sample G-VOO sample. Tocopherol were oxidized, leading to an increase in an anodic peak at +0.43V. A second peak in the sample G - VOOs was observed at the higher potential + 0.84V.

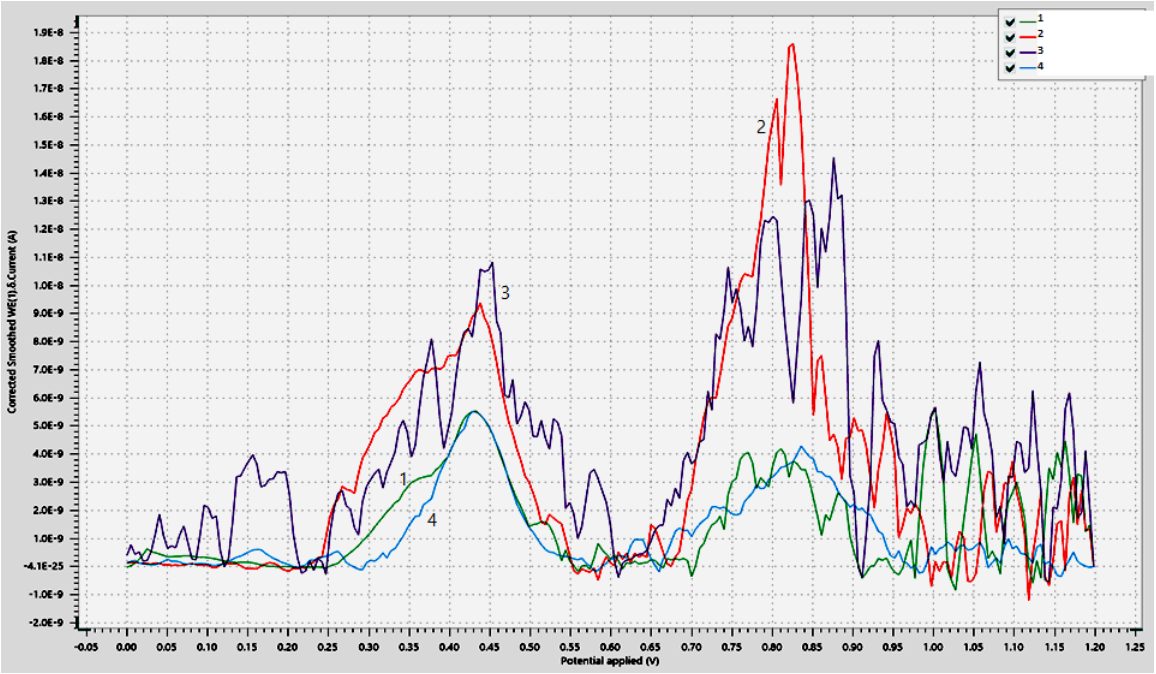

Figure 5.11: The voltammograms of G brand VOO which are coded as (1,2,3,4). In 0.5 M (ABS), pH is 4.8 and E vs Ag/AgCl 0 – 1.2 (V).

5.3.8 Sample H –VOO

Figure 5.12 shows voltammograms obtained from sample H -VOOs α-tocopherol were oxidized, leading to an increase in oxidation peak at +0.43V.

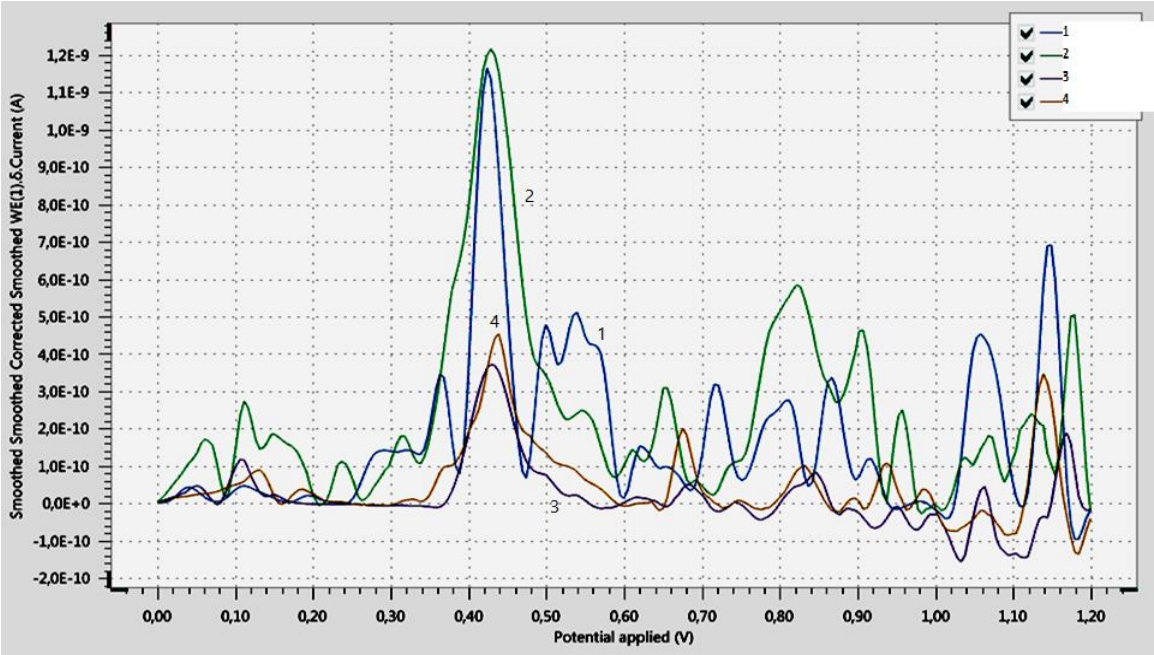

Figure 5.12: The voltammograms of H brand VOO which are coded as (1,2,3,4). In 0.5 M (ABS), pH is 4.8 and E vs Ag/AgCl 0 – 1.2 (V).

5.3.9 Sample I –VOO

Figure 5.13 shows signals obtained from sample I -VOOs. α-tocopherol were oxidized, leading to an increase in an oxidation peak at +0.42V.

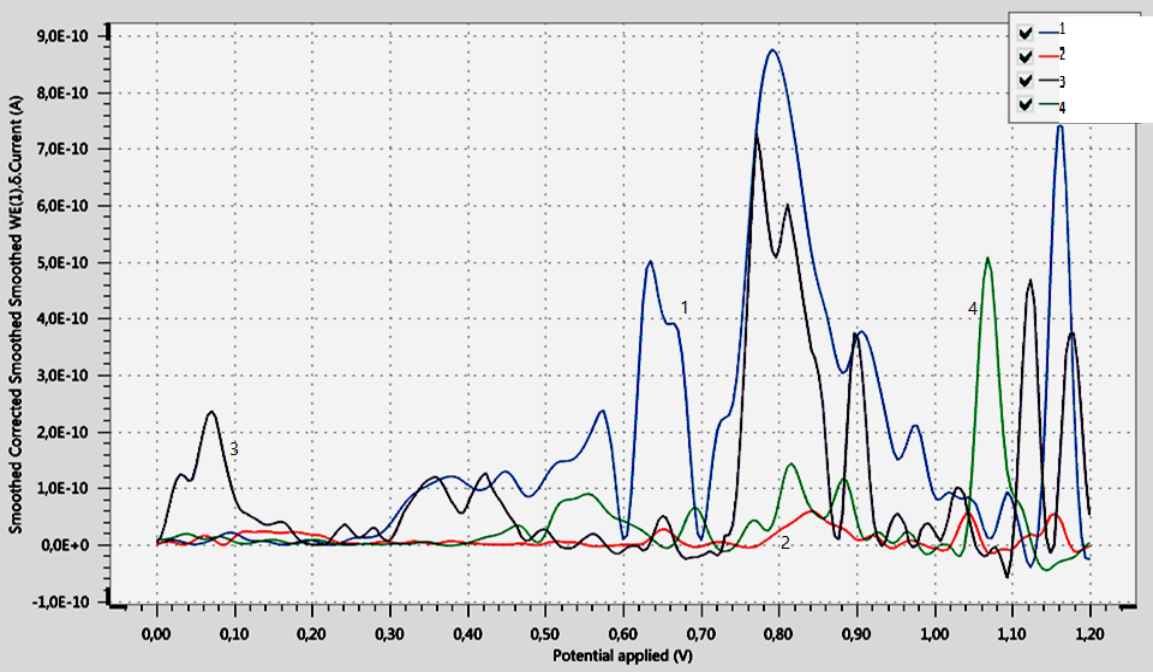

Figure 5.13: The voltammograms of I brand VOO which are coded as (1,2,3,4). In 0.5 M (ABS), pH is 4.8 and E vs Ag/AgCl 0 – 1.2 (V).

5.3.10 Sample J –VOO

Figure 5.14 shows signals obtained from sample J -VOO. α-tocopherol was oxidized, leading to an increase in an oxidation peak at +0.43V.

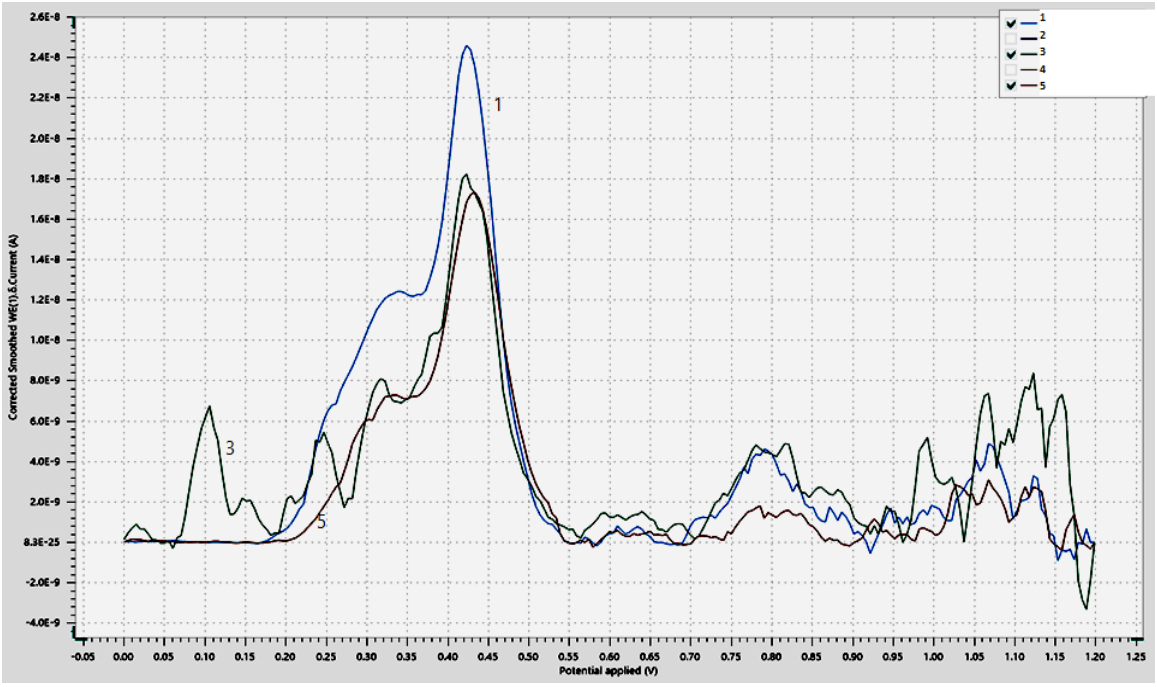

Figure 5.14: The voltammograms of J brand VOO which are coded as (1,2,3,4). In 0.5 M (ABS), pH is 4.8 and E vs Ag/AgCl 0 – 1.2 (V).

5.4.1 Riviera olive oil

Figure 5.15 shows signals obtained from Riviera olive oil, tocopherol was leading to an oxidation peak at +0.4V, however its current was at back ground level.

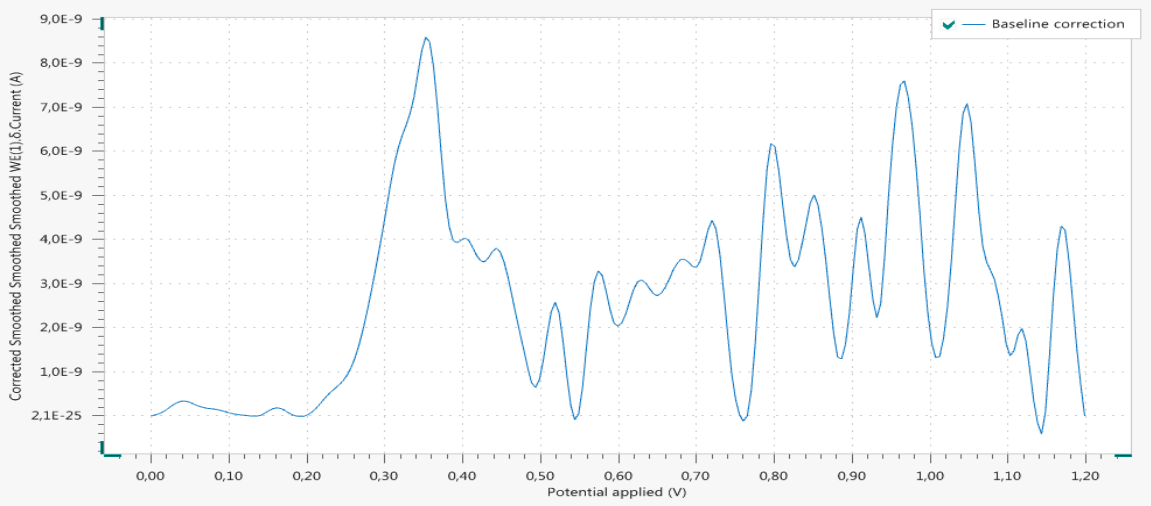

Figure 5.15: The voltammograms of Riviera oilve oil. In 0.5 M (ABS), pH is 4.8 and E vs Ag/AgCl 0 – 1.2 (V).

Table 5.4 : The peak position and peak height for Riviera olive oil.

Samples	Peak position (V)	Peak Height (A)
Riviera	0.4	5.6×10^{-9}

CHAPTER 6

CONCLUSION

This work presents and evaluates an electrochemical sensor based on a pencil graphite electrode. The sensor's electrochemical to olive oil has demonstrated exceptional benefits such as single use, easy sample preparation, high analytical performance, low cost and environmentally friendly. The PGE showed the good electrocatalytic activity towards the oxidation of tocopherols.

Alpha tocopherol peak current of extra virgin olive oil (EVOO) samples were changing in a range of 5.95×10^{-8} A to 1.04×10^{-7}A, while 1.33×10^{-8} A to 7.8×10^{-11}A in virgin olive oil (VOO) samples. Tocopherol content of Riviera oil was found just close to virgin olive oil Tocopherol content (5.6×10^{-9}A).

When we compare tocopherol levels of extra virgin olive oil (EVOO) samples and virgin olive oil (VOO) samples, α-tocopherol level (as peak height) might be considered distinctive parameter for screening purposes in adulteration.

All these options make this electrochemical approach very valuable as screening tools.

It would be useful to expand the study determining tocopherol contents of various olive oils, since geographical and climatic conditions, production and storage conditions can definitely effect tocopherol content.

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