International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.7, No.4, pp 1938-1942, 2014-2015

Square wave voltammetry peak separation of long chain polyunsaturated fatty acids of oil rich with tocopherols

Assia Djouadi[1, 2], Touhami Lanez[2]*

[1]University of Ouargla, department of material Sciences, PO Box 511, 30000, Ouargla, Algeria
[2]University of El Oued, VTRS Laboratory, PO Box 789, 39000, El Oued, Algeria

Abstract: This present study aims to separate the electrochemical response of the components of long chain polyunsaturated fatty acids (PUFAs) using electrochemical technics based on square wave voltammetry. The proposed method is based on the saponification of PUFAs followed by double liquid-liquid extraction using omega-3 dietary supplement as a source of PUFAs. Results showed the detection of two well-defined response peaks for omega-3 components respectively, at 1.1 and 1.42 V versus saturated calomel. The method can successfully applied to electrochemical determination of PUFAs in natural oil and dietary supplement.
Keywords: Omega-3; PUFAs; Tocopherol; saponification; SWV.

Introduction

The oil extracted from a natural material is a mixture of different lipid compounds as free fatty acids, glycerides, phospholipids, sterols, pigments or tocopherols, and, sometimes, toxics such as heavy metals[1]. From a nutritional point of view, the polyunsaturated fatty acids, especially omega-3, are essential in human nutrition since they play an important role in the organism and prevent several diseases[2]. The various health benefits of consuming omega-3 (n-3, PUFAs), particularly eicosapentaenoic acid (20:5 n-3; EPA) and docosahexaenoic acid (22:6 n-3; DHA), have been widely reported[3-8]. The evidence that they have a positive effect on the cardiovascular system by lowering cholesterol level[9-13], but these FA are not synthesized by humans and must be provided in the diet[14]. The nature provides a different source rich in n-3 PUFA acids for the human nutrition. Unfortunately, this PUFA are highly susceptible to lipid oxidation. Lipid oxidation of fish oil and other PUFA-rich foods is a serious problem that often leads to loss of shelf-life, consumer acceptability, functionality, nutritional value, and safety[15]. Protection against oxidation of omega-3 PUFA concentrates is always necessary, one of the most common methods to prevent oil oxidation and improve oil stability is stabilization with antioxidants[16]. The tocopherols represent one of the largest groups of natural antioxidants[17]. Several methods reported for characterization of lipid compound as tocopherols, sterols, and fatty acids of some oil samples are based on the chromatography analysis because it allows their easy separation and quantification[18-22]. The study of fatty acids is considered essential for research, clinical and quality control applications. Gas chromatography (GC) stands out among other methods of analysis of fatty acids, but this methodology involve long derivation step and need the time for fatty acids analysis[23-24]. Consequently, the availability of rapid and effective analytical methods in this specific field is upmost importance for both scientific and industrial communities. Recently, an electrochemical method has been considered as an alternative methods used to characterize a range of bioactives substance. A few researchers[25-26] employed electrochemical techniques for studying the components of oils. Reviewing the literature revealed that there is no study concerning the characterization of fatty acids from oils samples at simple electrode e.g., glassy carbon. Using electrochemical analysis implies several advantages (short times, not expensive), but also some drawbacks, especially, masking phenomena,

which is a serious problem that often leads to disappears of peaks. Since it cannot obtain any results with tocopherols-rich oils, a prior step of hydrolysation or saponification, as developed in the present study, is necessary. The saponification, removing the tocopherol and other non-saponifiable lipids present in the samples was developed for total fatty acids analysis[27]. Furthermore, the combination of analysis with a previous saponification, would lead to a highly accurate method for fatty acids analysis. Thus, the present investigation was aimed to evaluate the effectiveness of the saponification step method for the characterization of polyunsaturated fatty acids obtained from sample rich in omega-3 PUFA and vitamin E as food ingredient capsule of fish oil by square wave voltammetry.

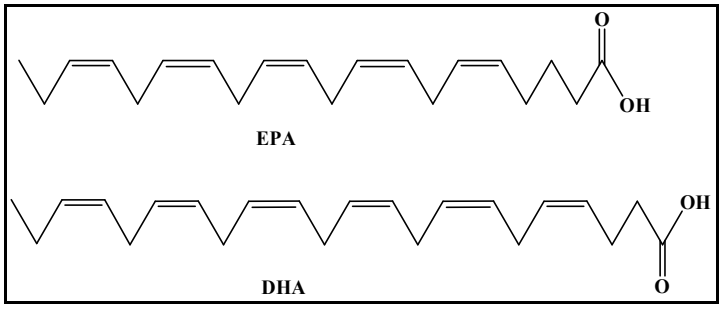

Figure 1. Molecular Structure of Omega-3 PUFA components.

Experimental

Reagents

Dietary complement of fish oil rich in omega-3 was supplied by Vitarmonyl laboratory, France. Each capsule contained 400 mg of omega-3 (218 mg EPA and 145 mg DHA) and 10 mg natural vitamin E. Petroleum ether, toluene, concentrated sulfuric acid (96-98%), sodium sulfate anhydrous (Na_2SO_4) were purchased from Biochem Chemopharma Co (Canada). Potassium hydroxide (KOH) was purchased from Biochem Chemopharma Co (United Kingdom). Absolute ethanol (≥ 99.8%) was purchased from Sigma–Aldrich GmbH (Sternheim, Germany). All solvents and reagents were of analytical grade.

Fatty acids extraction method

Oil sample (2 g) was saponified in 25 mL of 2 M KOH in Ethanol. The mixture was agitated under reflux and kept in a water bath at 80°C for 1 h. After reaction is performed, the mixture was poured into a separating funnel and 25 mL of distillated water and 50 mL of petroleum ether were added. The final biphasic system is allowed to separate into two layers and the petroleum ether phase (upper phase), which contain the non-saponifiable matter, was drained off into an Erlenmeyer flask. For complete unsaponifiable components extraction, the procedure was repeated until the organic layer becomes colorless after which the aqueous soap solution phase obtained above was acidified with concentrated sulfuric acid until obtaining an amorphous product of the fatty acid. The free fatty acids was extracted with petroleum ether, then the petroleum ether fractions were washed with distilled water and dried over anhydrous sodium sulfate, shaken thoroughly to remove the residual water and decanted into a rounded-bottom flask through a filter paper. The petroleum ether was evaporated at 40°C, using a rotary evaporator to acquire the lipid and then weighed. All samples were protected from direct light and stored in glass vials until analysis.

Instrumentation and methods

Measurements were performed in electrochemical cell containing a glassy-carbon working electrode, a Pt wire counter electrode and an Hg/Hg_2Cl_2 reference electrode. SWV experiments were carried out using a PGZ402 potentiostat, Voltalab 80 (radiometer analytical SAS) with VoltaMaster4 software. All square-wave voltammograms were obtained by using a frequency of 1 Hz, a pulse height (Esw) of 50 mV, and a potential step increment (Δ Esc) of 15 mV. The potential was swept in direct scanning mode starting from 0 to +1500 mV.

Preparation of solutions:

A. Supporting electrolyte:

The background electrolyte consisted of 0.1 M sulfuric acid in EtOH/toluene (1:1, v/v) solvent mixture.

B. Saponifiable solution

Omega-3 solution was prepared by adding 1 mL of EtOH/toluene (1:1, v/v) solvent mixture to oil obtained after saponification and double extraction.

C. Unsaponifiable solution

Vitamin E solution was prepared by adding 1 mL of EtOH/toluene (1:1, v/v) solvent mixture to oil obtained after saponification and one extraction.

Experimental procedure:

The general procedure used for electrochemical measurements was as follows: a 25 ml aliquot of the supporting electrolyte was transferred into a clean dry cell. After obtaining a voltammogram of the background electrolyte, the required volume of the prepared solution of the analyte was added by a micropipette. After a stirring period of 30 s, square wave voltammograms were recorded at a scan rate of 15 mV s^{-1}.

Results and Discussion

Typical cyclic voltammogram for omega-3 PUFA solution in EtOH/toluene solvent mixture containing 0.1 M sulfuric acid are shown in Figure 2. Before elimination of vitamin E the voltamogram shows only one well-defined peak at 0.54 V versus Hg/Hg_2Cl_2. After removal of vitamin E, the obtained voltammogram shows two well-defined peaks which characterize two electrode steps of omega-3 PUFA electro-oxidation at 1.1 V and 1.42 V respectively. These two picks can be attributed to EPA and DHA respectively.

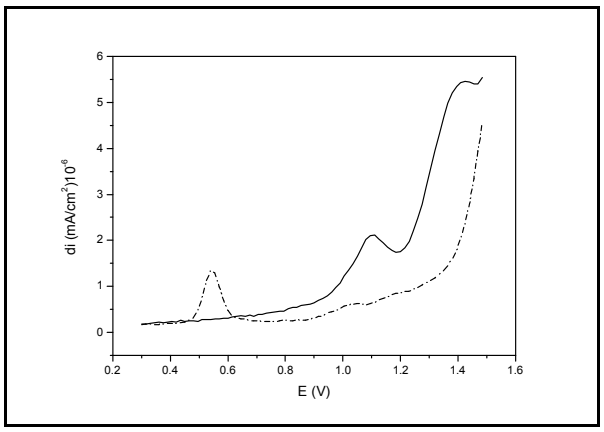

Figure 2. Square wave voltammograms of omega-3 PUFA before removal of vitamin E (dotted line) and after removal of vitamin E (full line).

In order to study the influence of vitamin E on the peaks separation of omega-3 PUFA components, a series of voltammograms were obtained in the absence and in the presence of gradually concentration addition of vitamin E. Figure 3 shows the gradually appearance of the pick at 0.64 V. this peak can be attributed to vitamin E which initially appear at 0.54 V, this anodic shift can be explain by the increase in the resistance of the solution due to addition of vitamin E.

In this paper, a sensitive, reproducible, and faster determination of long chain polyunsaturated fatty acids components present in oil rich with vitamin E using square wave voltammetry technics was presented. The separation of the electrochemical response was achieved by a saponification procedure of the sample before electrochemical analysis.

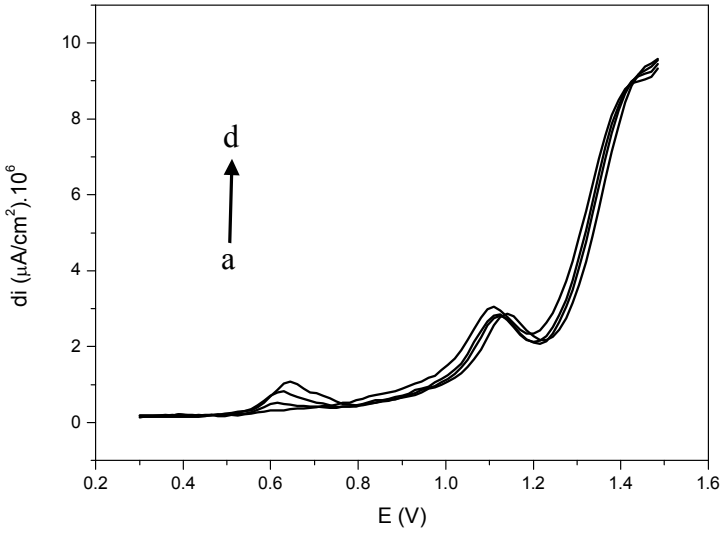

Figure 3. Square wave voltammograms of 350 µL omega-3 PUFA in the absence of tocopherol (a) and presence of 100 µL (b), 200 µL (c), 300 µL tocopherol (d).

References

1. Cheryan M., Ultrafiltration and microfiltration handbook, 2nd ed., Technomic Lacaster, USA, 1998.
2. Rubio-Rodríguez N., Beltrán S., Jaime I., De Diego S. M., Sanz M. T.,Carballido J. R., Production of omega-3 polyunsaturated fatty acid concentrates: A review, Innov. Food Sci. Emerg. Tech., 2010, 11, 1-12.
3. Connor W.E., Importance of n-3 fatty acids in health and disease. Am. J. Clin. Nutr., 2000, 71, 171-175.
4. De Lorgeril M., Salen P., Martin J.L., Monjaud I., Delaye J., Mamelle N., Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. Circulation, 1999, 99, 779-785.
5. Ollis T.E., Meyer B.J., Howe P.R.C., Australian food sources and intakes of omega-6 and omega-3 polyunsaturated fatty acids. Ann. Nutr. Metab., 1999, 43, 346-355.
6. Simopoulos A.P., Essential fatty acids in health and chronic disease, Am. J. Clin. Nutr.,1999, 70, 560-569.
7. Siscovick D.S., Raghunathan T.E., King I., Weinmann S., Wicklund K.G., Albright J., Bovbjerg V., Arbogast P., Smith H., Kushi L.H., Cobb L.A., Copass M.K., Psaty B.M., Lemaitre R., Retzlaff B., Childs M., Knopp R.H., Dietary intake and cell membrane levels of long-chain n 3 polyunsaturated fatty acids and the risk of primary cardiac arrest, J.A.M.A., 1995, 274, 1363-1367.
8. Von Schacky C., Angerer P., Kothny W., Theisen K., Mudra H., The effect of dietary omega-3 fatty acids on coronary atherosclerosis- A randomized, double-blind, placebo-controlled trial, Ann. Intern. Med., 1999, 130, 554-562.
9. Bigger J. T., El-Sherif T., Polyunsaturated fatty acids and cardiovascular events. A fish tale. Circulation, 2001, 103, 623-625.
10. Oh S. Y., Ryue J., Hsieh C. H., Bell D. E., Eggs enriched in omega-3-fatty acids and alterations in lipid concentrations in plasma and lipoproteins and in blood-pressure, Am. J. Clin. Nutr., 1991, 54, 689-695.
11. Kris-Etherton P.M., Harris W.S., Appel L.J., Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Arterioscler. Thromb. Vasc. Biol., 2003. 23, 20-30.
12. Ruxton C.H.S., Reed S.C., Simpson M.J.A., Millington K.J., The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence, J. Hum. Nutr. Diet., 2004, 17, 449–459.
13. Wang C., Harris W.S., Chung M., Lichtenstein A.H., Balk E.M., Kupelnick B., Jordan H.S., Lau J., n-3 Fatty acids from fish or fish-oil supplements, but not{alpha}-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review, Am. J. Clin. Nutr., 2006, 84, 5-17.
14. Neuringer M., Anderson G.J., Connor W.E., The essentiality of n-3 fatty acids for the development and function of the retina and brain, Annu. Rev. Nutr., 1988, 8, 517–541.

15. Arab-Tehrany E., Jacquot M., Gaiani C., Imran M., Desobry S., Linder M., Beneficial effects and oxidative stability of omega-3 long-chain polyunsaturated fatty acids, Trends Food Sci. Tech., 2012, 25, 24-33.
16. Kamal-Eldin A., Yanishlieva N. V., N−3 fatty acids for human nutrition: Stability considerations, Eur. J. Lipid Sci. Technol., 2002,104 ,825−836.
17. Doelman C.J., in Antioxidant Therapy and Preventive Medicine (Eds: I. Emerit, C. Anclair), Plenum Press, New York, 1989, 9.
18. Canabate-Diaz B., Segura Carretero A., Fernandez-Gutierrez A., Belmonte Vega A., Garrido Frenich A., Martinez Vidal J.L., Duran Martos J., Separation and determination of sterols in olive oil by HPLC-MS, Food Chem., 2007,102, 593-598.
19. Samaniego-Sanchez C., Quesada-Granados J.J., Lopez-Garcia de la Serrana H., Lopez-Martinez M.C., β- Carotene, squalene and waxes determined by chromatographic method in picual extra virgin olive oil obtained by a new cold extraction system, J. Food Compos. Anal., 2010, 23, 671-676.
20. Mulinacci N., Ieri F., Ignesti G., Romani A., Michelozzi M., Creti D., Innocenti M., Calamai L., The freezing process helps to preserve the quality of extra virgin olive oil over time: A case study up to 18 months, Food Res. Int., 2013, 1-8.
21. Fadda C., Del Caro A., Sanguinetti A.M., Urgeghe P.P., Vacca V., Arca P.P., Piga A., Changes during storage of quality parameters and in vitro antioxidant activity of extra virgin monovarietal oils obtained with two extraction technologies, Food Chem., 2012, 134, 1542-1548.
22. Santos O.V., Corrêa N.C.F., Carvalho Jr. R.N., Costa C.E.F., França L.F.F., Lannes S.C.S., Comparative parameters of the nutritional contribution and functional claims of Brazil nut kernels, oil and defatted cake, Food Res. Int., 2013, 51, 841-847.
23. Ulberth F., Schrammel F., Accurate quantification of short, medium, and long chain fatty acid methyl esters by split injection capillary gas liquid chromatography, J. Chromatogr. A, 1995, 704(2), 455-463.
24. Seppanen Laakso T., Laakso I., Hiltunen R., Analysis of fatty acids by gas chromatography, and its relevance to research on health and nutrition, Anal. Chim. Acta, 2002, 465(1), 39-62.
25. Ceballos C., Fernández H., Synthetic Antioxidants in Edible Oils by Square-Wave Voltammetry on Ultramicroelectrodes, J. Am. Oil Chem. Soc., 2000,77(7), 731-735.
26. Clough A.E., The determination of tocopherols in vegetable oils by square-wave voltammetry, J. Am. Oil Chem. Soc., 1992, 69 (5) , 456-460.
27. Juarez M., Juarez A., Aldai N., Avilés C., Polvillo O.,Validation of a gas–liquid chromatographic method for analysing samples rich in long chain n-3 polyunsaturated fatty acids: Application to seafood, J. Food Compos. Anal., 2010, 23, 665-670.
28. Bard A.J., Faulkner L.R., Electrochemical Methods Fundamentals and Applications, 2nd ed., Wiley, New York, 2001, 494.

*****