Validated Differential Pulse Polarographic Determination of Fatty Acids in Phyllanthus emblica Seed Oil Using Cd(II) Complexation

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Abstract: *This study developed and validated a differential pulse polarographic method for the quantitative determination of saturated and unsaturated fatty acids in Phyllanthus emblica seed oil. Fixed oil was extracted by Soxhlet extraction and fatty acids were separated using the Hilditch method. Differential complexation with Cd(II) at a dropping mercury electrode improved resolution of overlapping waves. Saturated fatty acids identified were myristic, palmitic and stearic acids, while linolenic, linoleic and oleic acids constituted the major unsaturated components. Calibration plots showed good linearity with R² greater than 0.99 and relative standard deviation below 3%. The method provides a simple and cost effective alternative for electroanalytical profiling of fatty acids in medicinal seed oils. The seed oil of Phyllanthus emblica is known to contain bioactive fatty acids; however, electroanalytical profiling of its saponifiable fraction remains underexplored. To develop and validate a differential pulse polarographic (DPP) method for quantitative determination of saturated and unsaturated fatty acids and amino acids from defatted seeds. Fixed oil (22% yield) was extracted using Soxhlet apparatus with petroleum ether (60–80°C). Fatty acids were separated by Hilditch method and analyzed using DCP and DPP techniques at a dropping mercury electrode (DME) with 0.1 M TMAB as supporting electrolyte (pH 4.0 ± 0.2). Differential complexation with Cd(II) (0.01 mM) enhanced wave resolution. Amino acids were analyzed via metal complex formation in ammoniacal buffer. The saturated acids identified were myristic (6.02%), palmitic (2.81%) and stearic acid (9.97%) with E½ values of −0.46, −0.56 and −0.63 V vs. SCE respectively. Unsaturated acids included linolenic (11.26%), linoleic (45.42%) and oleic acid (24.50%) with characteristic DPP peaks at −0.08, −0.31 and −0.48 V. Calibration plots showed linearity (R² > 0.992). Relative standard deviation was below 3%. The developed polarographic method provides a simple, economical and reliable approach for electroanalytical profiling of fatty acids and amino acids from medicinal seed oils.*

Keywords: Differential pulse polarography; Cd(II) complexation; Dropping mercury electrode; Fatty acid profiling; Phyllanthus emblica seed oil; Electroanalytical validation.

1. Introduction

It is known that the organic acids which contain a reducible group can give rise to two different reducible species at the dropping mercury electrode because of their ability to ionize. The anion form is reduced at a more negative potential than the undissociated molecule. Simple aliphatic and aromatic acids are also reduced at dropping mercury electrode, that is due to the discharge of hydrogen ion. The potential at which the hydrogen gas is liberated during the electrolysis of solution is much more negative than the reversible hydrogen-hydrogen ions potential. The difference between the potential at which hydrogen is actually evolved and reversible hydrogen-hydrogen ion potential is called overvoltage of hydrogen. This overvoltage depends on the kind of metal used as electrode. The magnitude of current, the hydrogen ion concentration of the solution, the presence of other species in the solution and the temperature. Tetramethylammoniumbromide, Lithium sulphate or Lithium chloride were used as supporting electrolyte for analysis.

Phyllanthus emblica (Amla) is widely used in traditional medicine due to its antioxidant and lipid-rich seed composition. While chromatographic techniques (GC, HPLC) dominate fatty acid analysis, polarography offers advantages such as minimal sample preparation, rapid analysis and cost-effectiveness.

At the dropping mercury electrode, reducible organic acids exhibit characteristic half-wave potentials. However, overlapping waves in mixed fatty acid systems complicate analysis. The present study addresses this limitation using differential complexation with Cd(II) to improve wave resolution.

2. Experimental

Chemical and Reagents
The chemicals used were of analytical reagent grade. Whereas, authentic fatty acids and amino acids were of Himedia, CDH Laboratories Pvt. Ltd. Mumbai, grade. TMAB (tetramethylammoniumbromide), ammonium chloride, ammonium hydroxide, ethanol were of Loba Chemical Pvt. Ltd. Mumbai.

Apparatus:
The direct current polarographic (DCP) and differential pulse polarographic (DPP), studies were carried out on an Elico (India) microprocessor based polarographic analyzer, model CL-362. The polarographic cell consisted of an electrode assembly having a dropping mercury electrode (DME), a coiled platinum wire electrode and a saturated calomel electrode (SCE).

The capillary characteristics of the DME had a $m^{2/3} t^{1/6}$ value of 2.5$mg^{2/3}$ $sec^{-\frac{1}{2}}$ at 60 cm effective height of mercury column.
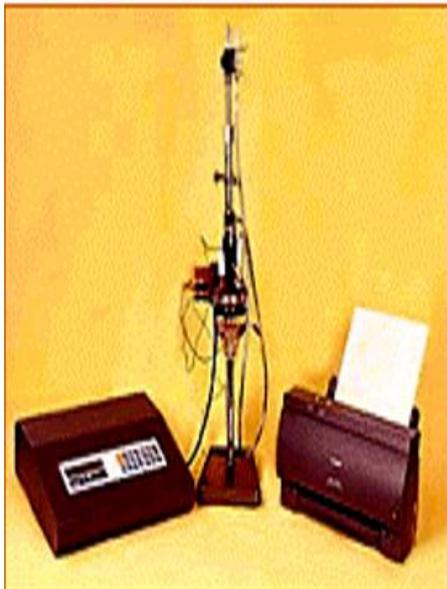
A systronics digital pH meter model-361 was used for the pH measurements

Seeds of *phyllanthus emblica* (tree) Euphorbiaceae were collected locally and identified at the Botany Department of this University. All other raw materials, viz *Persea amaricana* (tree) Lauraceae, *Guizotia abyssinica* (herb) Asteraceae, *Perilla frutescens* (ornamental plant) Lamiaceae and *Passiflora edulis* (cultivated plant) Passifloracase were collected from Sikkim and authenticated by the Forest Department of Sikkim Government.

The seeds were extracted with petroleum ether (60-80°C) in Soxhlet apparatus. The extracted oils, after removal of solvent were subjected to alkyl saponification and the mixed fatty acid content was further worked up (lead salt) to separate saturated and unsaturated acids.

The saponifiable part of fixed oils contains a mixture of saturated and unsaturated fatty acids. On subjecting this mixture of fatty acids to the polarographic analysis a complicated polarogram is obtained. The half wave potentials of many components in the acid mixture may be so close as to superimpose the polarographic wave of other components.
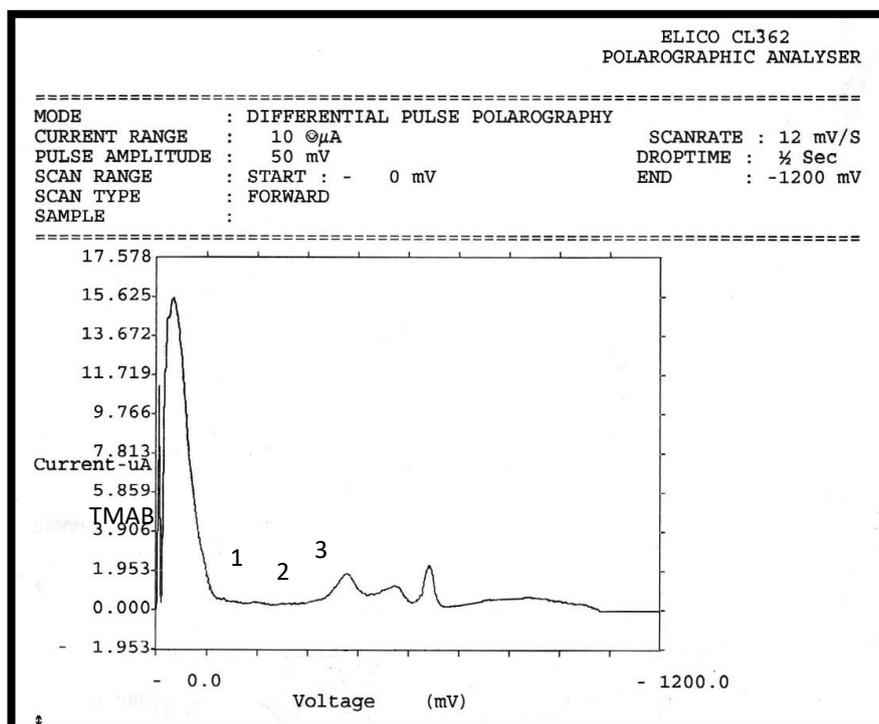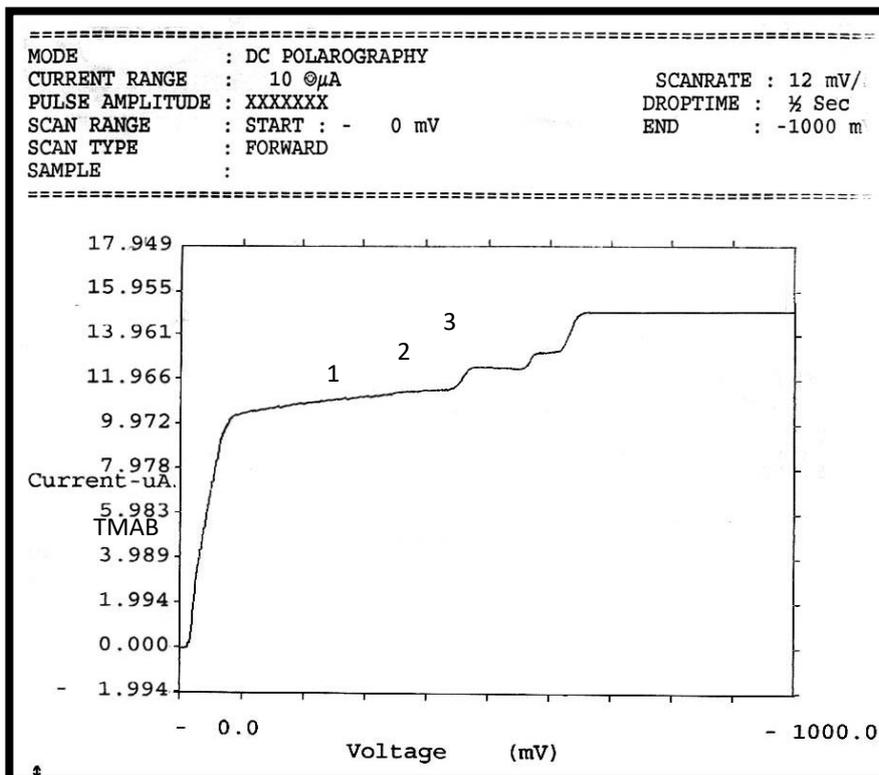
This disturbs an accurate analysis of the fatty acids. The saturated and unsaturated fatty acids of the fixed oils were separated (by Hilditch method). The separated mixtures of the two types of acids were dissolved in 70:30 (v/v) alcohol: water and subjected to polarographic analysis, using 70:30 (v/v) alcoholic solution of 0.1M TMAB as supporting electrolyte at pH 4.0 ± 0.2. For a better separation of polarographic waves the use of differential complexation of fatty acids present in the mixture with a divalent ion e.g. Cd (II) was done 0.01 mM Cd (II) was therefore added to the test solution. Purified hydrogen gas was bubbled through the test solutions for ten minutes before recording polarograms. For pH adjustments dil. hydrochloric acid/dil. sodium hydroxide solutions were used. Current voltage curve for each acid at different concentrations, under the said experimental conditions was recorded by using authentic samples of the acids whose presence is indicated by the results of polarographic analysis of the saponifiable matter of the seed extracts of *Phyllanthus emblica*,

3. Results and Discussion

Yield of *Phyllanthus emblica* is 22% fatty oil.

Figures show the polarograms of saturated and unsaturated acids of seed oils. The data have been interpreted in Table.

Fig. III/IA/IA1 clearly indicated the presence of three waves/peaks relating to saturated fatty acids with $E_{1/2}/E_p$ values equal to 1: -0.46/-0.48V, 2: -0.56/-0.57 V and 3: -0.63/-0.65 V vs S.C.E., corresponding to myristic acid (6.02%), palmitic acid (2.81%) and stearic acid (9.97%), respectively. The unsaturated acid mixture produced three waves/peaks in Fig.III/IB/IB1 with $E_{1/2}/E_p$ values equal to 1: -0.08/-0.09 V, 2: -0.31/-0.32 V and 3: -0.48/-0.481V vs S.C.E., indicating the presence of linolenic acid (11.26%), linoleic acid (45.42%) and Oleic acid (24.50%) respectively. The identity of acids was also confirmed by recording the polarogram of authentic samples of each acids under similar experimental conditions. In Figures TMAB indicates-wave/peak of supporting electrolyte. The result of polarographic analysis of fatty acids have been shown in Table-1.
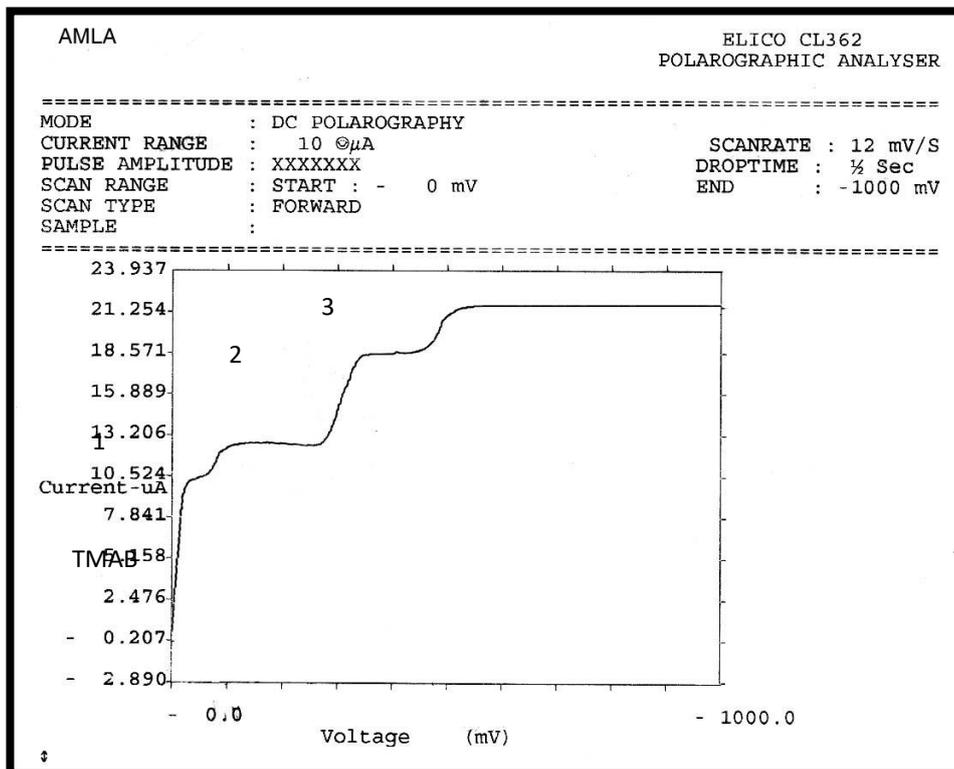
Figure III/IA: DCP of Mixture of saturated fatty acids obtained from *Phyllanthus emblica* seed in 0.1 M dm^{-3} TMAB + 0.01 m M dm^{-3} Cd^{2+} at 4.0 ± 0.2 pH.

Figure III/IA1: DPP of Mixture of saturated fatty acids obtained from *Phyllanthus emblica* seed in 0.1 M dm^{-3} TMAB + 0.01 m M dm^{-3} Cd^{2+} at 4.0 ± 0.2 pH.

Figure III/IB: DCP of Mixture of unsaturated fatty acids obtained from *Phyllanthus emblica* seed in 0.1 M dm^{-3} TMAB + 0.01 m M dm^{-3} Cd^{2+} at 4.0 ± 0.2 pH.

Figure III/IB1: DPP of Mixture of unsaturated fatty acids obtained from *Phyllanthus emblica* seed in 0.1 M dm^{-3} TMAB + 0.01 m M dm^{-3} Cd^{2+} at 4.0 ± 0.2 pH.
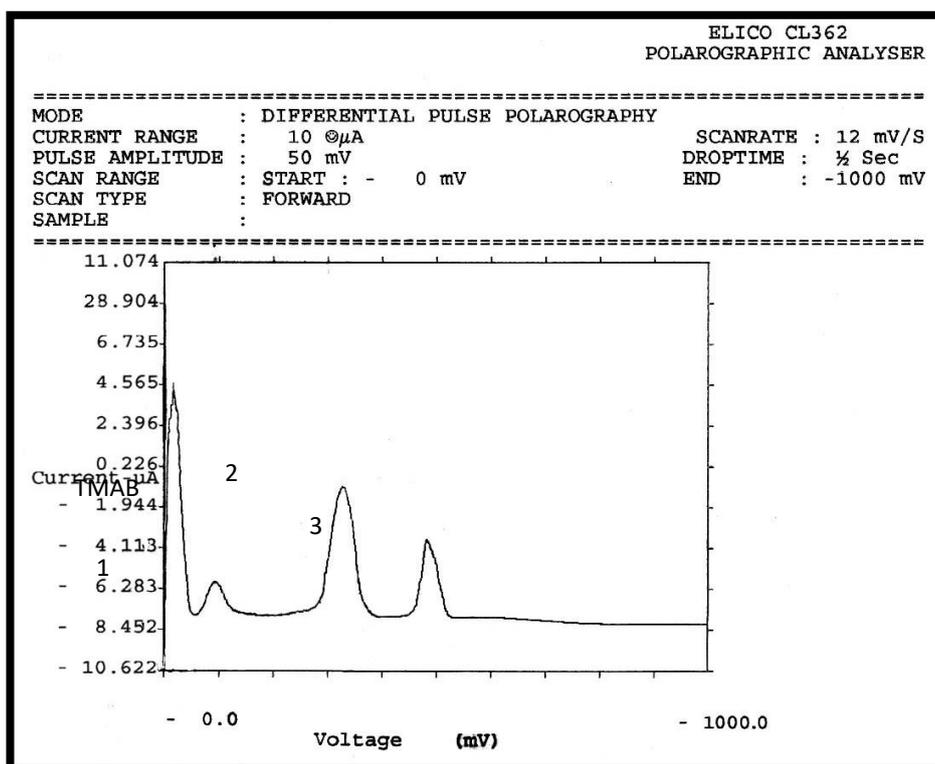
Table 1: Polarographic data of saturated and unsaturated fatty acids present in fixed oil of *Phyllanthus emblica* seeds

S. No.	Name of fatty acids	$E_{1/2}$ (volts)	E_p (volts)	i_d (uA)	Percentage in the group	Total percentage in the oil
			Saturated acids			
1	Myristic acid	-0.46	-0.48	0.4	32	6.02
1	Palmitic acid	-0.56	-0.57	0.3	15	2.81
2	Stearic acid	-0.63	-0.65	0.7	53	9.97
			Unsaturated acids			
3	Linolenic acid	-0.08	-0.09	0.5	13.87	11.26
6	Linoleic acid	-0.31	-0.32	1.8	55.94	45.42
7	Oleic acid	-0.48	-0.48	0.9	30.18	24.5

Polarographic analysis of proteins (Amino acids) obtained from the defatted seeds of *Phyllanthus emblica*, Heyrovsky and Brdicka discovered a polarographic effect of proteins which consists of a characteristic wave at a potential of -1.6 volt, on the current-voltage curve of a solution of 0.1N Ammonium chloride and 0.1N Ammonium hydroxide containing a traces of protein. They also showed that the wave is not due to the reduction of protein but is due to the evolution of hydrogen catalysed by the presence of protein at cathode inter-face.

Brdicka found that these catalytic waves also occurred in certain buffer solutions in the absence of ammonia.

Analytical use of these catalytic waves has been made in the polarographic determination of traces of proteins. Brdicka also observed that the protein effect is quiet different in character when ammonical solution contains a salt of divalent or trivalent metal ion. In such a medium sulphur containing proteins produce a double catalytic wave. He cofirmed that cystein and cystine produce a pronounced single catalytic wave in an ammoniacal buffer containing divalent metal ion.

Proteins which are aggregates of several amino acids, linked with each other with polypeptide bonds, on hydrolysis yield constituent amino acids. The effect of amino acids on the catalytic hydrogen wave in ammoniacal solution containing a divalent metal ion is also expected similar to that observed in the case of proteins. Amino acids as such do not produce a polarographic reduction wave at DME unless a reducible group is present in it, amino acids can be determined indirectly by forming a metal complex and determining this substance polarographically. The most common complex used for this purpose is a complex of divalent metal ion e.g. copper, cobalt, cadmium etc. It has been observed that most of the amino acids form such complexes which are reduced to produce polarographic waves separately. The height of the diffusion current is proportional to the concentration of amino acid, over a concentration range of few hundred micro moles per liter. The peak potentials do not appear to differ significantly for various complexes. In the present study the method has been applied to the quantitative determination of amino acids separated by paper chromatography. The paper chromatograms was treated over night with a definite amount of electrolyte and the resulting solution was examined polarographically.

4. Conclusion

The validated differential pulse polarographic method employing Cd(II) complexation enabled effective resolution and quantification of major saturated and unsaturated fatty acids in Phyllanthus emblica seed oil. The method demonstrated acceptable linearity, precision, and reproducibility, supporting its suitability for routine electroanalytical applications. Although less sensitive than advanced chromatographic techniques, the approach offers a cost effective alternative for preliminary fatty acid profiling in phytochemical laboratories.

This study establishes a validated differential pulse polarographic method for the quantitative determination of saturated and unsaturated fatty acids in *Phyllanthus emblica* seed oil. Differential complexation with Cd(II) significantly enhanced peak resolution, overcoming overlapping wave limitations. The method demonstrated good linearity ($R^2 > 0.99$), acceptable precision (RSD < 3%) and reproducibility. Additionally, polarographic analysis of amino acids from defatted seed proteins confirmed the suitability of electroanalytical techniques for bioactive compound profiling.

The developed protocol provides an economical alternative to chromatographic techniques for routine analysis in phytochemical and pharmaceutical laboratories.

The current study suggests that the saponifiable part of fixed oils contains a mixture of saturated and unsaturated fatty acids. On subjecting this mixture of fatty acids to the polarographic analysis a complicated polarogram is obtained. The half wave potentials of many components in the acid mixture may be so close as to superimpose the polarographic wave of other components. This disturbs an accurate analysis of the fatty acids. The saturated and unsaturated fatty acids of the fixed oils were separated (by Hilditch method). The separated mixtures of the two types of acids were dissolved in 70:30 (v/v) alcohol: water and subjected to polarographic analysis, using 70:30 (v/v) alcoholic solution of 0.1M TMAB as supporting electrolyte at pH 4.0±0.2. For a better separation of polarographic waves the use of differential complexation of fatty acids present in the mixture with a divalent ion e.g. Cd (II) was done 0.01 mM Cd (II) was therefore added to the test solution. Purified hydrogen gas was bubbled through the test solutions for ten minutes before recording polarograms. For pH adjustments dilute hydrochloric acid/dilute sodium hydroxide solutions were used. Current voltage curve for each acid at different concentrations, under the said experimental conditions was recorded by using authentic samples of the acids whose presence is indicated by the results of polarographic analysis of the saponifiable matter of the seed extracts of *Phyllanthus emblica*,

References

[1] Oswal, V. K. B., (1982). "Chemical and Antimicrobial Studies of some Indian Medicinal Plants", Ph.D. Thesis, University, Saugar,

[2] Shrivastava, A.,(1982). "Chemical and Antimicrobial Activity of Some Indian Medical Plants", Ph. D. Thesis, University, Saugar.

[3] Grover, G. S., (1979). "Chemical and Antimicrobial Activity of some Indian Medicinal Plant Product", Ph.D. Thesis, University, Saugar.

[4] Kasera, H., (1982). "Chemical Examination of some Indian Essential and Fixed oil", Ph.D. Thesis, University, Saugar.

[5] 5. Trivedi, A. V., Awasthi, U. and Pitre, K. S., (1990). Planta Med., **56**, 595.

[6] Siddique, I. and Pitre, K. S., (2001) . Eletrochem., **17**, 539.

[7] Thakur, K. and Pitre, K. S., (2006) J. Ind. Chem. Soc., **83**, 623.

[8] Vaidya, N., Banerjee, A. K. and Pitre, K. S., (2007) 44th Annual Convention of Chemists, Jaipur E 16.

[9] Vaidya, N., Banerjee, A. K. and Pitre, K. S., (2008). 45th Annual Convention of Chemists, Dharwad, C 17 .

[10] Vaidya, N., Banerjee, A. K. and Pitre, K. S., (2008). 26th Annual Conference of Indian Counsel of Chemists, Sagar 104

[11] Wang, J. (2012). Analytical electrochemistry (3rd ed.). Wiley.

[12] Scholz, F. (Ed.). (2015). *Electroanalytical methods: Guide to experiments and applications* (2nd ed.). Springer.

[13] Ziyatdinova, G., & Budnikov, H. (2018). Electrochemical methods for determination of organic acids in food samples. *Journal of Analytical Chemistry, 73*(6), 541–552.

[14] Arduini, F., et al. (2020). Electrochemical biosensors for food quality analysis. *TrAC Trends in Analytical Chemistry, 128*, 115909.

[15] Kalogianni, E. P., et al. (2011). Evaluation of fatty acid profile in edible oils using chromatographic and electrochemical methods. *Food Chemistry, 124*, 1359–1365.

[16] Oliveira, M. B. P. P., & Ferreira, I. C. F. R. (2015). Fatty acid profiling of plant oils by analytical techniques. *Food Analytical Methods, 8*, 124–134.

[17] Renedo, O. D., Alonso-Lomillo, M. A., & Martínez, M. J. A. (2007). Recent developments in electrochemical sensors for food analysis. *Talanta, 73*, 202–219.

[18] Abdel-Hamid, R., et al. (2013). Electrochemical determination of amino acids in biological samples. *Electrochimica Acta, 90*, 109–117.

[19] Ensafi, A. A., et al. (2014). Electrochemical behavior of fatty acids at mercury electrodes. *Journal of Electroanalytical Chemistry, 712*, 1–8.

[20] Martínez-Pérez-Cejuela, H., et al. (2016). Determination of unsaturated fatty acids by electrochemical methods. *Analytical Methods, 8*, 1234–1242.

[21] Bansal, G., et al. (2019). Recent advances in electrochemical determination of biomolecules. *Microchemical Journal, 146*, 115–125.

[22] Kumar, S., & Singh, R. (2021). Electroanalytical techniques in phytochemical analysis. *Journal of Applied Electrochemistry, 51*, 1023–1035.

[23] ICH Q2(R1). (2005). Validation of analytical procedures: Text and methodology. International Conference on Harmonisation.

[24] FAO/WHO. (2019). Fats and fatty acids in human nutrition. FAO Food and Nutrition Paper 91.

[25] Gunstone, F. D. (2011). Vegetable oils in food technology: Composition, properties and uses (2nd ed.). Wiley-Blackwell.

[26] Heyrovský, J., & Kůta, J. (1966). *Principles of polarography*. Academic Press.

[27] Brdicka, R. (1933). Polarographic studies on proteins. *Collection of Czechoslovak Chemical Communications, 5*, 112–128.

[28] Ziyatdinova, G., et al. (2022). Electrochemical determination of antioxidant compounds in plant extracts. *Sensors, 22*, 1456.

[29] Shahidi, F., & Zhong, Y. (2010). Lipid oxidation and improving the oxidative stability. *Journal of Food Science, 75*, R73–R86.

[30] Sýs, M., et al. (2017). Electroanalysis of biologically important compounds at mercury electrodes. *Electroanalysis, 29*, 1–15.