

Polarographic Analysis of Fatty Acids Obtained from the Seed of *Persea americana*

NEELAM VAIDYA*, RAKESH CHOURE, A.K. BANERJEE and K.S. PITRE
Department of Chemistry, Dr. H. S. Gour University, Sagar-470 003, India
E-mail: neelam.vaidya15@rediffmail.com

Persea americana is grown as an oil seed crop in Sikkim. The saponifiable portion of fixed oil from the seeds of the tree has been analyzed using polarographic technique, which has revealed the presence of saturated fatty acids with $E_{1/2}/E_p$ values equal to (1) -0.46/-0.48 V, (2) -0.56/-0.57 V, (3) -0.63/-0.69 V and (4) -0.80/-0.80 V versus SCE, corresponding to myristic acid (1.8 %), palmitic acid (10.9 %), stearic acid (0.85 %) and arachidic acid (0.56 %), respectively. Whereas the unsaturated acid mixture produced four waves/peaks with $E_{1/2}/E_p$ values equal to (1) -0.30/-0.32 V, (2) -0.48/-0.48 V, (3) -0.56/-0.567 V and (4) -0.64/-0.66 V versus SCE, indicating the presence of linoleic acid (10.2 %), oleic acid (70.6 %), gadoleic acid (0.67 %) and palmitoleic acid (4.37 %), respectively. The method has been successfully applied for the qualitative as well as quantitative analysis of fatty acids. Each of fatty acid present in the oil produced is well defined polarographic DCP and DPP signals in 0.1 M TMAB and 0.1 mM Cd^{2+} used as a supporting electrolyte as pH 4.0 ± 0.2 .

Key Words: Fatty acid, Polarographic analysis, *Persea americana*.

INTRODUCTION

The organic chemists very much depended on the use of chromatographic methods viz., thin layer chromatography, paper chromatography, gas liquid chromatography, HPLC etc., for the identification and separation of fatty acid present in the fixed oils¹⁻⁴ *Persea americana* (tree) Lauracea was collected from Sikkim⁵ and authenticated by the Forest Department of Sikkim Government. Yield of *Persea americana* is 40 %. Seed oil content ranged from with 70.6 % oleic acid being the major part of fatty acid of the oil. The present communication reports the fatty acid composition of the fixed oil from the tree using a comparatively new electrochemical technique of polarography in the field of plant product. The present method is quick, accurate and economic. The technique applied in present studies as reported earlier⁶⁻⁸.

EXPERIMENTAL

Crued seeds of *Persea americana* were extracted by petroleum ether in soxhlet apparatus for 3 days. The extract, on removal of solvent, gave a yellow coloured oil. Mixed fatty acids obtained by saponification of fixed oil. 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 method⁹. 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 0.1 M TMAB as supporting electrolyte at pH 4.0 ± 0.2 . For a better separation of polarographic waves the use of differential complexation¹⁰ of fatty acids present in the mixture with a divalent ion *e.g.*, Cd(II) was done by adding 0.01 mM Cd(II) to the test solution. Purified hydrogen gas was bubbled through the test solutions for 10 min 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 *Persea americana*.

The polarograms were recorded on an Elico (India) micro processor 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 $2.5 \text{ mg}^{2/3} \text{ s}^{-1/2}$ at 60 cm effective height of mercury column. A systronics digital μ pH meter model-361 was used for the pH measurements.

RESULTS AND DISCUSSION

Figs. 1a and 1b clearly reveal the presence of four waves/peaks related to saturated fatty acids with $E_{1/2}/E_p$ values equal to (1) -0.46/-0.48 V, (2) -0.56/-0.57 V, (3) -0.63/-0.69 V and (4) -0.80/-0.80 V, *versus* SCE, corresponding to myristic acid (1.8 %), palmitic acid (10.9 %), stearic acid (0.85 %) and arachidic acid (0.56 %), respectively. Whereas the unsaturated acid mixture produced four waves/peaks in Figs. 2a and 2b with $E_{1/2}/E_p$ values equal to (1) -0.30/-0.32 V, (2) -0.48/-0.48 V, (3) -0.56/-0.567 V and (4) -0.64/-0.66 V *versus* SCE, indicating the presence of linoleic acid (10.2 %), oleic acid (70.6 %), gadoleic acid (0.67 %) and palmitoleic acid (4.37 %), respectively. The identity of acids was also confirmed by recording the polarogram of authentic samples under the mentioned 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.

The presence of acids in the respective acid mixtures was confirmed by adding authentic samples of the acids to the analyte under similar experimental conditions and recording the polarogram. The resulting polarograms showed an increase of wave/peak height for the polarographic signal of each acid, without much difference in its $E_{1/2}/E_p$ value. Thus confirming the usefulness of the DCP and DPP methods

TABLE-1
POLAROGRAPHIC DATA OF SATURATED AND UNSATURATED FATTY ACIDS
PRESENT IN FIXED OIL OF *Persea americana* SEEDS

Name of fatty acids	$E_{1/2}$ (V)	E_p (V)	i_d (μ A)	Percentage in the group	Total percentage in the oil
Saturated acids					
Myristic acid	-0.46	-0.48	0.40	12.75	1.80
Palmitic acid	-0.56	-0.57	1.00	77.00	10.90
Stearic acid	-0.63	-0.69	0.20	6.07	0.85
Arachidic acid	-0.80	-0.80	0.15	4.00	0.56
Unsaturated acids					
Linoleic acid	-0.30	-0.32	0.50	11.80	10.20
Oleic acid	-0.48	-0.48	3.1	81.70	70.60
Gadoleic acid	-0.56	-0.56	0.2	0.77	0.67
Palmitoleic acid	-0.64	-0.66	0.5	5.05	4.37

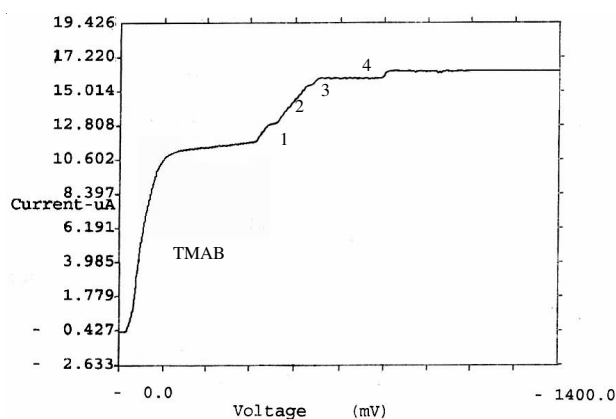


Fig. 1a. DCP of mixture of saturated fatty acids obtained from *Persea americana* seed in 0.1 M dm^{-3} TMAB + 0.01 mM dm^{-3} Cd^{2+} at 4.0 ± 0.2 pH

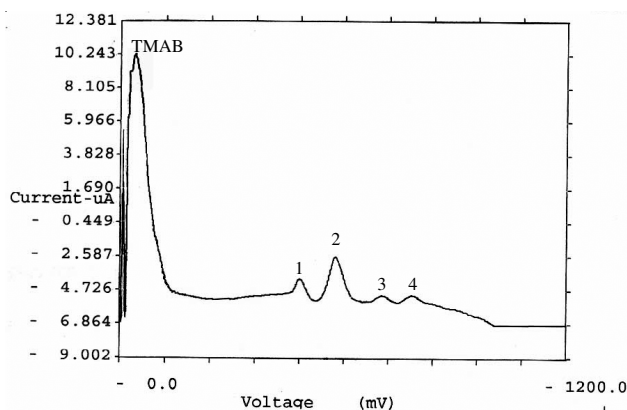


Fig. 1b. DPP of mixture of saturated fatty acids obtained from *Persea americana* seed in 0.1 M dm^{-3} , TMAB + 0.01 mM dm^{-3} , Cd^{2+} at 4.0 ± 0.2 pH

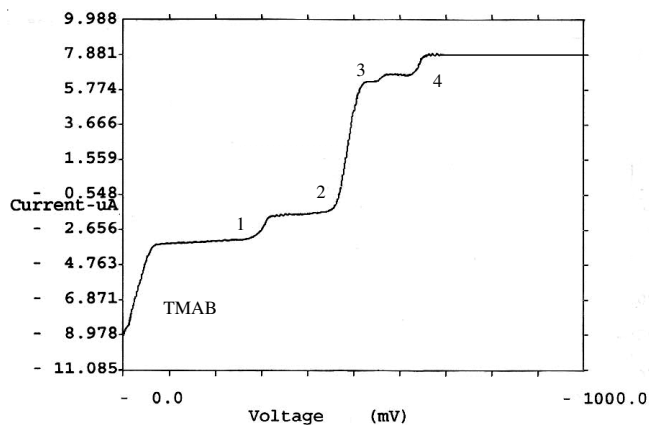


Fig. 2a. DCP of mixture of unsaturated fatty acids obtained from *Persea americana* seed in 0.1 M dm^{-3} , TMAB + 0.01 mM dm^{-3} , Cd^{2+} at $4.0 \pm 0.2 \text{ pH}$

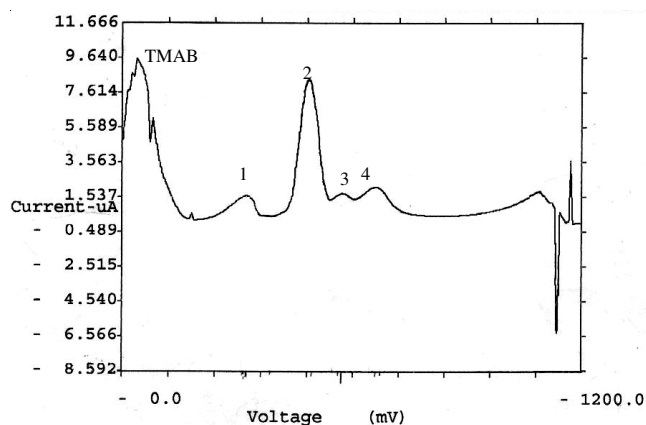


Fig. 2b. DPP of mixture of unsaturated fatty acids obtained from *Persea americana* seed in 0.1 M dm^{-3} , TMAB + 0.01 mM dm^{-3} , Cd^{2+} at $4.0 \pm 0.2 \text{ pH}$

for qualitative and quantitative analysis of fatty acids present in the two mixtures. Besides, it also helps in avoiding the problem due to matrix effects.

The saturated and unsaturated fatty acids produce polarographic waves¹¹ at the dropping mercury electrode. In case of the saturated fatty acids the polarographic waves may be produced due to the discharge of H^+ where as unsaturated fatty acids produce polarographic waves due to the reduction of double bond. An analysis of the wave indicates that the potential determining step involves one electron, which was calculated from the log plot slopes and $(E_{3/4} - E_{1/4})$ values¹¹. From the observed polarographic data of the fatty acids, it is clear that with the increase of carbon chain of the fatty acid, the discharge of H^+ starts at a relatively lower potential and hence the $E_{1/2}$ value is also low. A similar explanation regarding the relation between the unsaturation and $E_{1/2}$ value may be given. It may be said that more the unsaturation

in a compound, its reduction at the DME would take place at a relatively lower potential and hence the $E_{1/2}$ value would be accordingly low.

The present method is significant as a complimentary tools for qualitative as well as quantitative analysis of fatty acids present in fixed oil obtain from the seed of *Persea americana* with great accuracy and precision of determination.

Conclusion

The present method is significant as a complimentary tools for qualitative as well as quantitative analysis of fatty acids present in fixed oil obtain from the seed of *Persea americana* with great accuracy and precision of determination. Besides the observed results are in good agreement with those reported in the literature.

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