CHAPTER IV

Studies on Triglyceride Profile of Acacia arabica Seed Oil
Acacia arabica is also known as A. nilotica and A. indica belonging to subfamily Mimosaceae of family Leguminosae. The seed oil is antifungal. Composition of separate lipid classes has been reported. Although Physicochemical properties and sum of the fatty acids of the oil were determined by Maity et al., natural fats and oil are complex mixtures of triacylglycerols and a complete determination of the profile would require combination of procedures. This has been attempted in the present study. Chromatography has resulted in improved resolution in shorter time. Column, mobile phase selectivities and choice of detectors have been studied by several workers.

Experimental:

Coarse seeds were extracted with petroleum ether (40-60°C) in a Soxhlet apparatus. C18 reverse phase column was used for separation of triacylglycerol fractions. The fractions were separated using a Shimadzu (LC-10A) HPLC unit equipped with LC-10AD pump and Rheodyne injector with loop and a UV detector at 210 nm. 10 μl oil sample dissolved in chloroform (30%) was injected on the column using acetonitrile/alcohol (80:20) as mobile phase. Each separated peak was collected as it eluted. Repeated injections and collections were necessary to get enough material for analysis. Flow rate of 2ml/min was maintained.

74
Fig. IV/1: HPLC Profile – *Acacia arabica* seed oil triacylglycerols
CHAPTER-IV: STUDIES ON TRIGLYCERIDE PROFILE OF ACACIA ARABICA SEED OIL

Solvents were reversed by rotatory evaporation (at 40°C). The fractions were then subjected to GLC for determination of fatty acid composition and carbon number of the triacylglycerol in the fraction. Fatty acid composition was determined by saponification (KOH/MeOH) followed by esterification (MeOH/H₂SO₄) and separating the fatty acid methyl ester (FAME) on EGSS column using a Nucon. GC equipped with FID. Carbon number of the triacylglycerol fractions were determined by GLC using a Dексsil column at 290°C. Peaks were identified by comparison with standard triacylglycerol of known composition (Sigma). Further identification of the triacylglycerol fractions was made by Mass Spectral analysis of each fraction using Hewlett- Packard 5985 electron impact mass spectrometer. The MS equipment has computer attachment for data collection. The fragmentation pattern of each fraction was noted.

Results and Discussion:

The oil was extracted in an yield of 15%.

Fig. IV/1 shows the HPLC chromatogram of Acacia arabica seed oil triacylglycerols on reserve phase column. 10 peaks could be identified. The fatty acid composition (as FAME) and carbon number of each peak was determined by GLC (Table). Each concentrated fraction was subjected to mass spectrometry.

Fig. IV/2-11 shows the Mass scans. The findings have been summarized in the Table.
Fig. IV/2 : MS of Fraction 1 (HPLC) – *Acacia arabica* seed oil
Fig. IV/3: MS of Fraction 2 (HPLC) - *Acacia arabica* seed oil
Fig. IV/4 : MS of Fraction 3 (HPLC) – Acacia arabica seed oil
Fig. IV/5: MS of Fraction 4 (HPLC) – Acacia arabica seed oil
Fig. IV/6 : MS of Fraction 5 (HPLC) – *Acacia arabica* seed oil
Fig. IV/7: MS of Fraction 6 (HPLC) – *Acacia arabica* seed oil
Fig. IV/8: MS of Fraction 7 (HPLC) – *Acacia arabica* seed oil
Fig. IV/9: MS of Fraction 8 (HPLC) – Acacia arabica seed oil
Fig. IV/10 : MS of Fraction 9 (HPLC) – Acacia arabica seed oil
Fig. IV/11: MS of Fraction 10 (HPLC) – Acacia arabica seed oil
<table>
<thead>
<tr>
<th>Peak no (HPLC)</th>
<th>Fatty acid composition (GLC)</th>
<th>Carbon number</th>
<th>Mol. wt. (MS)</th>
<th>Possible composition of oil triglyceride</th>
<th>Effective carbon no. (carbon no – 2 X No. of double bonds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>18:2 (81%)</td>
<td>54</td>
<td>879</td>
<td>18:2 18:2 18:2</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>18:1 (31%) 18:2 (64%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>18:0 (13%) 18:1 (24%) 18:2 (62%)</td>
<td>52</td>
<td>856</td>
<td>16:0 18:1 18:2</td>
<td>46</td>
</tr>
<tr>
<td>3.</td>
<td>18:1 (61%) 18:2 (36%)</td>
<td>54</td>
<td>883</td>
<td>18:1 18:1 18:2</td>
<td>46</td>
</tr>
<tr>
<td>4.</td>
<td>16:0 (21%) 18:1 (41%) 18:2 (38%)</td>
<td>52</td>
<td>857</td>
<td>16:0 18:1 18:2</td>
<td>46</td>
</tr>
<tr>
<td>5.</td>
<td>18:0 (11%) 18:1 (68%) 18:2 (16%)</td>
<td>54</td>
<td>884</td>
<td>18:0 18:1 18:2</td>
<td>48</td>
</tr>
<tr>
<td>6.</td>
<td>18:0 (5%) 18:1 (75%)</td>
<td>54</td>
<td>886</td>
<td>18:0 18:1 18:1</td>
<td>50</td>
</tr>
<tr>
<td>7.</td>
<td>18:1 (45%) 18:2 (15%) 20:1 (12%)</td>
<td>56</td>
<td>911</td>
<td>18:1 18:2 20:1</td>
<td>48</td>
</tr>
<tr>
<td>8.</td>
<td>18:1 (48%) 18:2 (12%) 20:0 (6%)</td>
<td>56</td>
<td>912</td>
<td>18:1 18:2 20:0</td>
<td>50</td>
</tr>
<tr>
<td>9.</td>
<td>16:0 (21%) 18:2 (18%) 22:0 (9%)</td>
<td>56</td>
<td>913</td>
<td>16:0 18:2 22:0</td>
<td>52</td>
</tr>
</tbody>
</table>
CHAPTER-IV: STUDIES ON TRIGLYCERIDE PROFILE OF ACACIA ARABICA SEED OIL

The UV detector (210nm) takes advantages of the molar absorptivity of the double bond, resulting in enhanced detection of triacylglycerols with unsaturation. The presence of double bonds in a triacylglycerol decreases its retention time on reverse phase columns.

The above data gives the complete characterization of major triacylglycerols in Acacia arabica seed oil. It has been observed in the present study that liquid chromatography in combination with fraction identification has resulted in improved resolution in shorter time.
CHAPTER-IV: STUDIES ON TRIGLYCERIDE PROFILE OF ACACIA ARABICA SEED OIL

REFERENCES

1. Glossary of Indian Medicinal Plants (Eds. Asolkar, L.V., Kakkar, K.K., and Chakre, O.J.), National Institute of Science Communication (CSIR), New Delhi, 1, 10 (2000).


