Chapter 9

Isolation and Characterization of seed oil of *Basella rubra* Linn

9.1 Plant material

Plant samples of *Basella rubra* L growing in different parts of Barak Valley during the period of October to April were collected.

Mature seeds of the plant were collected from several growing areas in Barak Valley during early, mid and late cropping and air dried at ambient temperature (~25°C). These samples were analysed separately.

9.2 Extraction of seed oil

Dry seeds (100g) were ground in a mill and oil was extracted by shaking ground seeds in a shaker with 400ml hexane for 3 hr. at ambient temperature in a sealed container. The extract was filtered through Whatman No. 1 filter paper atop Buchner funnel applying suction. Residue was subjected to re-extraction, each time followed by filtration. The pooled filtered extracts were subjected to rotary vacuum evaporation at ~25°C. The resulting oil was transferred to amber bottle and last traces of solvent were removed by a nitrogen purge followed by continuous vacuum application overnight at ambient temperature.

Yield of oil (unrefined): 27 – 29% w/w of dry seeds.
9.3 Characterisation of the seed oil


9.3.1 Colour:

Lovibond tintometer is used to determine colour of the seed oil. Here colour is measured by comparison with Lovibond glasses. The cell of tintometer is cleaned with CCl₄ and dried. It is now filled with filtered oil. Lovibond yellow and red glasses are placed alongside to match the colour of the oil viewing through the eye piece. Colour of oil is reported in Lovibond units in 1cm length cell as the sum of the yellow (Y) and red (R) slides (*Sathe*, 1999).

9.3.2 Odour:

Odour of seed oil is determined by smelling (organoleptic evaluation) to evaluate unpleasant odour, if any.

9.3.3 Refractive index:

Abbe digital refractometer (Alsterdorfer, Germany), Model No. AR2008, is used for measuring the refractive index value. It is previously standardised with distilled water having R.I. value 1.330 at 20°C. The temperature of the refractometer is adjusted to 40°C. Now a few drops of the filtered oil is placed on the clean prism and wiped excess sample. The reading is noted.
9.3.4 Viscosity:

Viscosity of the oil is determined with Ostwald viscometer in centipoise and then expressed as Kinematic viscosity by dividing viscosity value with the specific gravity value to get the centistokes value at 25°C.

9.3.5 Iodine Value (I.V.):

Wijs’ method is applied for determination of iodine value of the oil.

In a 500ml iodine flask accurately weighed 0.25 – 0.30 g sample oil by difference method. 25ml CCl₄ added to dissolve the oil and 25ml Wijs’ solution (ICl in glacial acetic acid, 0.2M) added and mixed thoroughly. It is kept in dark for ½ hr. with occasional shaking. 10ml of 10% KI solution and 100ml of freshly boiled and cooler distilled water and mixed. The liberated iodine is titrated with 0.1M Na₂S₂O₃ solution using starch as indicator towards the end.

A blank is run similarly with the oil sample. I.V. = 12.69 (V_{blank} - V_{sample}) / weight of sample. The result gives the amount (g) of I₂ absorbed by 100g of oil sample.

9.3.6 Acid Value (FFA):

Free fatty acid content is measured by titration with 0.1N KOH solution and expressed as oleic acid equivalents. 5g of the oil sample is accurately weighed and dissolved in 50ml of neutral solvent (25ml ether + 25ml 95% alcohol + 1ml 1% alcoholic phenolphthalein solution neutralised with 0.1N KOH solution). The contents titrated with 0.1N KOH (std) solution until a pink colour persistent for 15 sec. is observed.

\[
\text{FFA (as oleic acid)} = 56.1 \times \text{Titre value} \times \text{Normality of KOH} / \text{weight of sample (g)}.
\]
9.3.7 Saponification Value (S.V.) :

Saponification value is determined by the AOCS method (AOCS, 1979). 5g oil sample is taken in a conical flask with precise measurement. 50ml alcoholic KOH solution (4%) added in a definite period of time. For a blank similar addition of KOH solution without oil sample performed separately. Both connected with air condenser and heated for 1 hr. (clear medium). Condenser washed down by distilled water, 1ml 1% phenolphthalein indicator added and titrated against 0.5N HCl until the pink colour just disappears (Sadasivam et. al, 1996).

\[
S.V. = \frac{28.05 \text{ (titre of blank – titre of sample)}}{\text{wt. of sample (g)}}.
\]

9.4 Results

The values of different Physico-chemical parameters of the seed oil are presented in Table 9.1 below:

**Table 9.1**: Physico-chemical parameters of the seed oil

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield</td>
<td>27 – 29% w/w</td>
</tr>
<tr>
<td>Colour (Lovibond)</td>
<td>10 Y + 4R</td>
</tr>
<tr>
<td>Odour (Organoleptic)</td>
<td>Pleasant.</td>
</tr>
<tr>
<td>Viscosity (Ostwald)</td>
<td>480 centipoise at 26.2°C</td>
</tr>
<tr>
<td>Refractive index (R.I.)</td>
<td>1.4696 at 26°C</td>
</tr>
<tr>
<td>Saponification value (S.V.)</td>
<td>198.905</td>
</tr>
<tr>
<td>Iodine value (I.V.) Wijs</td>
<td>94.505</td>
</tr>
<tr>
<td>Acid value</td>
<td>11.457</td>
</tr>
<tr>
<td>Non-saponifiable fraction (NSF)</td>
<td>1.5% w/w</td>
</tr>
</tbody>
</table>
9.5 Conclusion

Various physical parameters of the seed oil of Basilla rubra were studied. The parameters were found to be similar to any common edible oil e.g., mustered oil.