5. PHYSIO-CHEMICAL PROPERTIES OF GROUNDNUT OIL

5.1 INTRODUCTION

Groundnut is an important food, feed and principal oil seed crop which is cultivated on a large scale throughout the world. It is an annual crop principally for its edible oil and protein rich kernel seeds, borne in pods which develop and mature below the soil surface (Ayoola and Adeyeye, 2010).

In India, 80% of the groundnut produce is crushed for extraction of oil and accounts for 36.10% of the total oil production. Although India has achieved great success in cereal production. Groundnut seed contains 44 – 56% oil and 22 – 30% protein on a dry seed and is a rich source of mineral (Phosphorous, calcium, magnesium and potassium) and vitamins E, K and B group (Savage and Keenan, 1994).

Edible oil from plant sources are of interest in various food and application industries. They provide characteristic flavours and textures to foods as integral diet components (Odoemelam, 2005) and can also serve as a source of oleochemicals (Morrison et al., 1995). Oleochemicals are completely biodegradable and so could replace a number of petrochemicals (Ayoola and Adeyeye, 2010). Vegetable oils has made an important contribution to the diet in many countries, serving as a good source of protein, lipid and fatty acids for human nutrition including the repair of worn-out tissues, new cells formation as well as a useful source of energy (Grosso et al., 1997).
Groundnut provides an inexpensive source of high quality dietary protein and oil. The vast food preparations incorporating groundnut to improve the protein level have helped in no small way in reducing malnutrition in the developing countries. The special taste and flavours of foods containing groundnut is important in the acceptance of these food preparation (Asibu et al., 2008). The quality of the oil and groundnut products depends to a large extent on quality, the relative proportion of fatty acids, geographical location, seasons and growing conditions (Adeyeye and Ajewole, 1992).

Beneficial effect of *Rhizobium* inoculation has been observed by several workers who reported an increase in yield and oil content of groundnut with such inoculation. Anandham and Sirdar (2004) concluded that double inoculation of *Thiobacillus* and *Rhizobium* increased the oil content of groundnut seed. Poultry manure application significantly increased the herbage, essential oil content and dry matter yield in *Java Citronella* plants (Adholeya and Prakash, 2004).

Groundnut oil (arachis oil) is an organic material oil derived from groundnuts, noted to have the aroma and taste of its parent legume. In U.K., it is marketed as groundnut oil. Its major component fatty acids are oleic acid (56.6%) and linoleic acid (26.7%). The oil also contains some palmitic acid, arachidic acid, arachidonic acid, behenic acid, lignoic acid and other fatty acids.

Refractive index plays an important role in many branches of physics, biology and chemistry. Knowledge of refractive index of aqueous solutions and oil is one of the crucial importance in applications of adulteration of oil and purity (Yunus et al., 2009).
A small quantity of free fatty acids is usually present in oils along with the triglycerides. The free fatty acids content is known as acid number or acid value. The keeping quality of oil therefore replies upon the free fatty acid content (Sadasivam and Manickam, 2008). The fatty acid composition of the oil in seed crops plays an important role in determining the functional properties, self-life, nutritional value and flavours of the food products derived from them (Lea, 1962).

The saponification value is a measure of the alkali reactive groups in fats and oils and is expressed as the number of milligrams of potassium hydroxide which react with one gram of sample.

The iodine value is a measure of the degrees of unsaturation in an oil. It is constant for a particular oil or fat. Iodine value is useful parameter in studying oxidative rancidity of oils since higher the unsaturation the greater the possibility of the oils to go rancid (Sadasivam and Manickam, 2008).

Fats are oxidized at the sites of unsaturated bonds in fatty acid chains. Oxidation of unsaturated bonds results in a variety of compounds being formed including free radicals and hydroperoxides. The peroxide value Ip is the number that expresses in milliequivalents of active oxygen the quantity of peroxide contained in 1000 g of the substance.

Groundnut may be one of the most cardioprotective foods readily consumed according to the groundnut institute. The health benefits of groundnut come from its monosaturated fatty acid content. Diets higher in monosaturated fatty acids from groundnut butter improve blood lipid profiles (Kris-Etherton, 1999). The regular consumption of groundnuts and groundnut products help to lower the blood
cholesterol level (Lokko, 2007). Kris-Etherton (2007) reviewed the scientific data concerning groundnut consumption and coronary heart disease and concluded regular consumptions of groundnuts significantly reduces risk. The monosaturated fats and antioxidant properties found in the groundnuts protect against the oxidation of low density lipoprotein cholesterol.

The present investigation aims the effect of mono, dual and combined biofertilizers such as *Azotobacter*, *Mycorrhizae* and *Rhizobium* on the physiochemical properties of oil in *Arachis hypogaea* L. var TMV-7.
5.2 MATERIALS AND METHODS

5.2.1 Estimation of oil in groundnut seed (Sadasivam and Manickam, 2008)

Principle

Oil from a known quantity of the seed is extracted with petroleum ether. It is then distilled off completely, dried, the oil weighed and the percentage oil is calculated.

Materials

- Petroleum Ether (40 – 160ºC)
- Whatman No. 2 Filter Paper
- Absorbant Cotton
- Soxhlet Apparatus

Procedure

1. Fold a piece of filter paper in such a way to hold the seed meal. Wrap around a second filter paper which is left open at the top like a thimble. A piece of cotton wool is placed at the top to evenly distribute the solvent as it drops on the sample during extraction.

2. Place the sample packet in the butt tubes of the Soxhlet extraction apparatus.

3. Extract with petroleum ether (150 drops/min) for 6 hours without interruption (For caster beans use hexane) by gentle heating.
4. Allow to cool and dismantle the extraction flask. Evaporate the ether on a steam or water-bath until no odour of ether remains. Cool at room temperature.

5. Carefully remove the dirt of moisture outside the flask and weigh the flask. Repeat heating until constant weight is recorded.

**Calculation**

\[
\text{Oil in ground sample} \% = \frac{\text{Weight of oil (g)}}{\text{Weight of sample (g)}} \times 100
\]

\[
\text{Oil to dry weight basis} = \frac{\% \text{ oil in ground sample}}{100\% \text{ moisture in whole seed}}
\]

**5.2.2 Physical property of oil**

**5.2.2.1 Refractive index** (Minimum deviation method)

The prism table is released and rotated so as to have the edge of the prism turned away from the collimator. The refracted image is seen through the telescope. The prism table is then rotated so that the image moves towards the axis. At a particular position the image turns back and moves in the opposite direction. The position where it turns back is the position of minimum deviation. The prism table and telescope are fixed in this position. The main scale and vernier scale readings are taken for both verniers. Let the total reading be \( R_3 \) for both the verniers. The prism is then removed. The telescope is brought in line with the
collimator and the direct ray reading is noted as $R_4$. The difference between the readings $R_3$ and $R_4$ gives the angle of minimum deviation $D$. $D = R_3 - R_4$.

The refractive index of the material of the prism is calculated using the formula

$$\mu = \frac{\sin \frac{A + D}{2}}{2 \sin \frac{A}{2}}$$

5.2.3 Chemical properties of oil

5.2.3.1 Estimation of free fatty acid (Cox and Pearson, 1962)

Principle

The free fatty acid in all oil is estimated by titrating it against KOH in the presence of phenolphthalein indicator. The acid number is defined as the mg KOH required to neutralize the free fatty acids present in 1 g of sample. However, the free fatty acid content is expressed as oleic acid equivalents.

Materials

- 1% phenolphthalein in 95% ethanol
- Potassium hydroxide
- Neutral solvent: Mix 25 ml ether, 25 ml 95% alcohol and 1 ml of 1% phenolphthalein solution and neutralize with M/10 alkali.

Procedure

1. Dissolve 1 – 10 g of oil or melted fat in 50 ml of the neutral solvent in a 250 ml conical flask.
2. Add a new drops of phenolphthalein.

3. Titrate the contents against 0.1 N potassium hydroxide.

4. Shake constantly until a pink colour which persists for fifteen seconds is obtained.

**Calculation**

\[
\text{Acid value (mg KOH/g)} = \frac{\text{Titre value x Normality of KOH x 56.1}}{\text{Weight of the sample (g)}}
\]

The free fatty acid is calculated as oleic acid using the equation 1 ml N/10 KOH = 0.028 g oleic acid.

**5.2.3.2 Saponification value (Horowitz, 1975)**

**Principle**

A known quality of oil is refluxed with an excess amount of alcoholic KOH. After saponification, the remaining KOH is estimated by titrating it against standard acid.

**Materials**

- Hydrochloric acid 0.5 N, accurately standardized.
- Alcoholic KOH: Dissolve 40 g KOH in one litre of distilled alcohol keeping the temperature below 15.5°C while the alkali is being dissolved. This solution should remain clear.
- Phenolphthalein indicator: 1% in 95% alcohol.
- Air Condenser
Procedure

1. Melt the sample if it is not already liquid and filter through paper to remove any impurities and the last traces of moisture. The sample must be completely moisture free.

2. Weigh 4 – 5 sample into a flask. Add 50 ml of alcoholic KOH from burette by allowing it to drain for a definite period of time.

3. Prepare a blank also by taking only 50 ml of alcoholic KOH allowing it to drain at the same duration of time.

4. Connect air condenser to the flasks and boil them gently for about 1 hour.

5. After the flask and condenser get cooled, rinse down the inside of the condenser with a little distilled H₂O and then remove the condenser.

6. Add about 1 ml of indicator and titrate against 0.5 N HCl until the pink colour just disappears.

Calculation

\[
\text{Saponification value} = \frac{28.05 \times (\text{titre value of blank} - \text{titre value of sample})}{\text{Weight of sample (g)}}
\]

5.2.3.3 Determination of Iodine value (Horowitz, 1975)

Principle

The oils contain both saturated and unsaturated fatty acids. Iodine gets incorporated into the fatty acid chain wherever the double bonds exist. Hence, the
measure of iodine absorbed by an oil, gives the degree of unsaturation. Iodine value/number is defined as the ‘g’ of iodine absorbed by 100 g of the oil.

Materials

- Hanus Iodine Solution

Weigh 13.6 g of iodine and dissolve in 825 ml glacial acetic acid by heating, and cool. Titrate 25 ml of this solution against 0.1 N sodium thiosulphate. Measure another portion of 200 ml of glacial acetic acid and add 3 ml of bromine to it. To 1 ml of this solution add 10 ml of 15% potassium iodide solution and titrate against 0.1 N sodium thiosulphate. Calculate the value of bromine solution, to double halogen content of the remaining 800 ml of the above iodine solution as follows:

\[ X = \frac{B}{C} \]

where \( X = \) ml of bromine solution required to double the halogen content, \( B = 800 \times \) thiosulphate equivalent of 1 ml of iodine solution and \( C = \) thiosulphate equivalent of 1 ml of bromine solution.

- 15% Potassium Iodide Solution

- % Sodium Thiosulphate

- 1% Starch

Procedure

1. Weigh 0.5 or 0.25 g of oil into an iodine flask and dissolve iodine 10 ml of chloroform.
2. Add 25 ml of Hanus iodine solution using a pipette, draining it in a definite
time. Mix well and allow to stand in dark for exactly 30 min with
occasional shaking.

3. All 10 ml of 15% KI, shake thoroughly and add 100 ml of freshly boiled
and cooled water, washing down any free iodine on the stopper.

4. Titrate against 0.1N sodium thiosulphate until yellow solution turns almost
colourless.

5. Add a few drops of starch as indicator and titrate until the blue colour
completely disappears.

6. Towards the end of titration, stopper the flask and shake vigorously so that
any iodine remaining in solution in CHCl₃ is taken up by potassium iodide
solution.

7. Run a blank without the sample.

**Calculation**

\[
\text{Iodine number} = \frac{(B - S) \times N \times 12.69}{(g) \text{ sample}}
\]

where \( B = \text{ml thiosulphate for blank} \)

\( S = \text{ml thiosulphate for sample.} \)

\( N = \text{normality of thiosulphate solution.} \)

Amount of fat/oil taken should be adjusted such that the excess iodine in the
added 25 ml of Hanus iodine solution has about 60% of excess iodine of the
amount added, i.e. if \((B - S)\) is greater than \(B/2\), repeat with smaller amount of sample.

5.2.3.4 Determination of peroxide value (Cox and Pearson, 1962)

Principle

Peroxide value is a measure of the peroxide in the oil. The peroxide are determined by titration against thiosulphate in the presence of KI. Starch is used as indicator.

Materials

- Solvent mixture: Mix two volumes of glacial acetic acid with one volume of chloroform.
- 5% Potassium Iodide Solution.
- 1% Starch Solution.
- N/500 Sodium Thiosulphate Solution. Prepare N/10 solution and dilute to N/500 on the day of use.

Procedure

1. Weigh 1 g of oil or fat into a clean dry boiling tube and add 1 g of powdered potassium iodine and 20 ml of solvent mixture.

2. Place the tube in boiling water so that the liquid boils within 30 seconds and allow to boil vigorously for not more than 30 seconds.

3. Transfer the contents quickly to a conical flask containing 20 ml of 5% potassium iodide solution.
4. Wash the tube twice with 25 ml water each time and collect into the conical flask.

5. Titrate against N/500 sodium thiosulphate solution until yellow colour is almost disappeared.

6. Add 0.5 ml of starch, shake vigorously and titrate carefully till the blue colour just disappears.

7. A blank should also be set at the same time.

Calculation

\[
\text{Peroxide value (milliequivalent peroxide/kg sample) = } \frac{S \times N \times 100}{(g) \text{ sample}}
\]

where \( S = \text{ml Na}_2\text{S}_2\text{O}_3 \) (Test-Blank) and \( N = \text{normality of Na}_2\text{S}_2\text{O}_3 \).
5.3 RESULTS

5.3.1 Estimation of oil

The results on percentage yield of oil was presented in the table 28 and figure 28.

The control showed the minimum amount of oil 32.6 ± 0.76%. In mono inoculations *Azotobacter* showed the value of 36.3 ± 1.18%, *Mycorrhizae* it was 38.4 ± 1.09%, and *Rhizobium* showed the value of 39.6 ± 0.58%. In dual inoculations, *Azotobacter* and *Mycorrhizae* showed the value of 40.4 ± 0.97%, *Azotobacter* and *Rhizobium* showed the value of 42.1 ± 1.28% and *Mycorrhizae* and *Rhizobium* showed the value of 43.5 ± 0.69%. The maximum amount of soil showed the combined inoculation of biofertilizers. The amount of oil was 45.8 ± 0.34%.

The statistical analysis of one way ANOVA test conducted between percentage yield of oil as a function of different biofertilizers were statistically significant (F = 731.9584; P < 0.005).
Table 28. Response of biofertilizers on the percentage yield of oil in *Arachis hypogaea* L. Var TMV-7

<table>
<thead>
<tr>
<th>Inoculations</th>
<th>Percentage of oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.6 ± 0.76</td>
</tr>
<tr>
<td><em>Azotobacter</em></td>
<td>36.3 ± 1.18</td>
</tr>
<tr>
<td><em>Mycorrhizae</em></td>
<td>38.4 ± 1.09</td>
</tr>
<tr>
<td><em>Rhizobium</em></td>
<td>39.6 ± 0.58</td>
</tr>
<tr>
<td><em>Azotobacter + Mycorrhizae</em></td>
<td>40.4 ± 0.97</td>
</tr>
<tr>
<td><em>Azotobacter + Rhizobium</em></td>
<td>42.1 ± 1.28</td>
</tr>
<tr>
<td><em>Mycorrhizae + Rhizobium</em></td>
<td>43.5 ± 0.69</td>
</tr>
<tr>
<td><em>Azotobacter + Mycorrhizae + Rhizobium</em></td>
<td>45.8 ± 0.34</td>
</tr>
</tbody>
</table>

P < 0.05 is statistically significant

Figure - 28. Response of different biofertilizers on the percentage yield of oil in *Arachis hypogaea* L. Var TMV-7
5.3.2 Physical property of oil (Refractive index)

The results on refractive index of groundnut oil was presented in the table 29 and figure 29.

The control showed the refractive index of 1.4651. In mono inoculation the *Azotobacter* showed the value of 1.4653, *Mycorrhizae* it was 1.4656 and the *Rhizobium* showed the value of 1.4659. In dual inoculations *Azotobacter* and *Mycorrhizae* showed the value of 1.4668, *Azotobacter* and *Rhizobium* showed the value of 1.4667 and *Mycorrhizae* and *Rhizobium* showed the value of 1.4673. The combined inoculation showed the refractive index of 1.4678.

The statistical analysis of one way ANOVA test conducted between refractive index of oil as a function of different biofertilizers were statistically significant (F = 17840941; P < 0.05).
Table 29. Response of biofertilizers on the refractive index of oil in *Arachis hypogaea* L. var TMV-7

<table>
<thead>
<tr>
<th>Inoculations</th>
<th>Refractive index of oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.4651</td>
</tr>
<tr>
<td>Azotobacter</td>
<td>1.4653</td>
</tr>
<tr>
<td>Mycorrhizae</td>
<td>1.4656</td>
</tr>
<tr>
<td>Rhizobium</td>
<td>1.4659</td>
</tr>
<tr>
<td>Azotobacter + Mycorrhizae</td>
<td>1.4668</td>
</tr>
<tr>
<td>Azotobacter + Rhizobium</td>
<td>1.4667</td>
</tr>
<tr>
<td>Mycorrhizae + Rhizobium</td>
<td>1.4673</td>
</tr>
<tr>
<td>Azotobacter + Mycorrhizae + Rhizobium</td>
<td>1.4678</td>
</tr>
</tbody>
</table>

P < 0.05 is statistically significant

Figure - 29. Response of different biofertilizers on the refractive index of oil in *Arachis hypogaea* L. var TMV-7
5.3.3 Chemical properties of oil

5.3.3.1 Free fatty acid

The results on free fatty acids of different biofertilizers treated oil was presented in the table 30 and figure 30.

The control showed the highest amount of free fatty acid, the value was 1.55 ± 0.01 g oleic acid. In mono inoculation the *Azotobacter* showed the value of 0.96 ± 0.03 g oleic acid, *Mycorrhizae* it was 0.93 ± 0.02 g oleic acid and *Rhizobium* showed the value of 0.89 ± 0.003 g oleic acid. In dual inoculations *Azotobacter* and *Mycorrhizae* showed the value of 0.81 ± 0.02 g oleic acid. *Azotobacter* and *Rhizobium* showed the value of 0.69 ± 0.01 g oleic acid and *Mycorrhizae* and *Rhizobium* showed the value of 0.45 ± 0.01 g oleic acid. The combined inoculation showed the lowest amount of free fatty acid was 0.33 ± 0.03 g oleic acid.

The statistical analysis of one way ANOVA test conducted between free fatty acid value of oil as a function of different biofertilizers were statistically significant (F = 39.69174; P < 0.05).
Table 30. Response of biofertilizers on the free fatty acid content in *Arachis hypogaea* L. var TMV-7

<table>
<thead>
<tr>
<th>Inoculations</th>
<th>Free fatty acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.55 ± 0.01</td>
</tr>
<tr>
<td><em>Azotobacter</em></td>
<td>0.96 ± 0.03</td>
</tr>
<tr>
<td><em>Mycorrhizae</em></td>
<td>0.93 ± 0.02</td>
</tr>
<tr>
<td><em>Rhizobium</em></td>
<td>0.89 ± 0.03</td>
</tr>
<tr>
<td><em>Azotobacter</em> + <em>Mycorrhizae</em></td>
<td>0.81 ± 0.02</td>
</tr>
<tr>
<td><em>Azotobacter</em> + <em>Rhizobium</em></td>
<td>0.69 ± 0.01</td>
</tr>
<tr>
<td><em>Mycorrhizae</em> + <em>Rhizobium</em></td>
<td>0.45 ± 0.01</td>
</tr>
<tr>
<td><em>Azotobacter</em> + <em>Mycorrhizae</em> + <em>Rhizobium</em></td>
<td>0.33 ± 0.03</td>
</tr>
</tbody>
</table>

P < 0.05 is statistically significant

Figure - 30. Response of biofertilizers on the free fatty acid content in *Arachis hypogaea* L. var TMV-7
5.3.3.2 Saponification value of oil

The results on the saponification value was presented in the table 31 and figure 31.

The control showed the lowest saponification value of 184.10 ± 0.25 mg KOH/g. In mono inoculation Azotobacter showed the value of 186.60 ± 0.21 mg KOH/g, the Mycorrhizae it was 187.30 ± 0.15 mg KOH/g and the Rhizobium showed the value of 187.90 ± 0.20 mg KOH/g. In dual inoculations Azotobacter and Mycorrhizae showed the value of 188.50 ± 0.98 mgKOH/g, Azotobacter and Rhizobium showed the value of 189.20 ± 0.76 mg KOH/g and Mycorrhizae and Rhizobium showed the value of 190.70 ± 0.15 mg KOH/g. The combined inoculation showed the highest saponification value of 192.40 ± 0.20 mg KOH/g.

The statistical analysis of one way ANOVA test conducted between saponification value of oil was a function of different biofertilizers were statistically significant (F = 44045.05; P < 0.05).
Table 31. Saponification value of biofertilizers inoculated in *Arachis hypogaea* seed oil (mg KOH/g)

<table>
<thead>
<tr>
<th>Inoculations</th>
<th>Saponification value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>184.10 ± 0.25</td>
</tr>
<tr>
<td>Azotobacter</td>
<td>186.60 ± 0.21</td>
</tr>
<tr>
<td>Mycorrhizae</td>
<td>187.30 ± 0.15</td>
</tr>
<tr>
<td>Rhizobium</td>
<td>187.90 ± 0.20</td>
</tr>
<tr>
<td>Azotobacter + Mycorrhizae</td>
<td>188.50 ± 0.98</td>
</tr>
<tr>
<td>Azotobacter + Rhizobium</td>
<td>189.20 ± 0.76</td>
</tr>
<tr>
<td>Mycorrhizae + Rhizobium</td>
<td>190.70 ± 0.15</td>
</tr>
<tr>
<td>Azotobacter + Mycorrhizae + Rhizobium</td>
<td>192.40 ± 0.20</td>
</tr>
</tbody>
</table>

P < 0.05 is statistically significant

Figure - 31. Saponification value of biofertilizers inoculated in *Arachis hypogaea* seed oil (mg KOH/g)
5.3.3.3 Determination of Iodine value

The results on iodine value of different biofertilizers inoculated oil was presented in the table 32 and figure 32.

The control showed the lowest iodine value of 83.21 ± 0.97 g/100g. In mono inoculations Azotobacter showed the value of 86.73 ± 1.08 g/100g, Mycorrhizae it was 87.19 ± 0.73 g/100g and the Rhizobium showed the value of 88.80 ± 0.56 g/100g. In dual inoculation Azotobacter and Mycorrhizae showed the value of 89.36 ± 0.38 g/100g, Azotobacter and Rhizobium showed the value of 90.13 ± 0.44 g/100g and Mycorrhizae and Rhizobium showed the value of 93.25 ± 1.12 g/100g. The combined inoculation showed the highest iodine value of 95.87 ± 1.23 g/100g.

The statistical analysis of one way ANOVA test conducted between iodine value of oil as a function of different biofertilizers were statistically significant (F = 4142.197; P < 0.05).
Table 32. Iodine value of biofertilizers inoculated in *Arachis hypogaea* seed oil (g/100g)

<table>
<thead>
<tr>
<th>Inoculations</th>
<th>Iodine value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>83.21 ± 0.97</td>
</tr>
<tr>
<td><em>Azotobacter</em></td>
<td>86.73 ± 1.08</td>
</tr>
<tr>
<td><em>Mycorrhizae</em></td>
<td>87.19 ± 0.73</td>
</tr>
<tr>
<td><em>Rhizobium</em></td>
<td>88.80 ± 0.56</td>
</tr>
<tr>
<td><em>Azotobacter</em> + <em>Mycorrhizae</em></td>
<td>89.36 ± 0.38</td>
</tr>
<tr>
<td><em>Azotobacter</em> + <em>Rhizobium</em></td>
<td>90.13 ± 0.44</td>
</tr>
<tr>
<td><em>Mycorrhizae</em> + <em>Rhizobium</em></td>
<td>93.25 ± 1.12</td>
</tr>
<tr>
<td><em>Azotobacter</em> + <em>Mycorrhizae</em> + <em>Rhizobium</em></td>
<td>95.87 ± 1.23</td>
</tr>
</tbody>
</table>

P < 0.05 is statistically significant

Figure - 32. Iodine value of biofertilizers inoculated in *Arachis hypogaea* seed oil (g/100g)
5.3.3.4 Determination of peroxide value

The results on peroxide value of different biofertilizers inoculated oil was presented in the table 33 and figure 334.

The control showed the highest peroxide value of 1.82 ± 0.03 mEq/Kg. In mono inoculations *Azotobacter* showed the value of 1.79 ± 0.01 mEq/kg, *Mycorrhizae* it was 1.70 ± 0.04 mEq/Kg and *Rhizobium* showed the value of 1.66 ± 0.02 mEq/Kg. In dual inoculations *Azotobacter* and *Mycorrhizae* showed the value of 1.58 ± 0.01 mEq/Kg, *Azotobacter* and *Rhizobium* showed the value of 1.53 ± 0.02 mEq/Kg and *Mycorrhizae* and *Rhizobium* showed the value of 1.47 ± 0.02 mEq/Kg. The combined inoculation showed the lowest peroxide value of 1.33 ± 0.15 mEq/Kg.

The statistical analysis of one way ANOVA test conducted between peroxide value of oil as a function of different biofertilizers were statistically significant (*F* = 754.4574; *P* < 0.05).
Table 33. Peroxide value of biofertilizers inoculated in *Arachis hypogaea* seed oil (g/100g)

<table>
<thead>
<tr>
<th>Inoculations</th>
<th>Peroxide value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.82 ± 0.03</td>
</tr>
<tr>
<td><em>Azotobacter</em></td>
<td>1.79 ± 0.01</td>
</tr>
<tr>
<td><em>Mycorrhizae</em></td>
<td>1.70 ± 0.04</td>
</tr>
<tr>
<td><em>Rhizobium</em></td>
<td>1.66 ± 0.02</td>
</tr>
<tr>
<td><em>Azotobacter</em> + <em>Mycorrhizae</em></td>
<td>1.58 ± 0.01</td>
</tr>
<tr>
<td><em>Azotobacter</em> + <em>Rhizobium</em></td>
<td>1.53 ± 0.011</td>
</tr>
<tr>
<td><em>Mycorrhizae</em> + <em>Rhizobium</em></td>
<td>1.47 ± 0.02</td>
</tr>
<tr>
<td><em>Azotobacter</em> + <em>Mycorrhizae</em> + <em>Rhizobium</em></td>
<td>1.33 ± 0.15</td>
</tr>
</tbody>
</table>

P < 0.05 is statistically significant

Figure - 33. Peroxide value of biofertilizers inoculated in *Arachis hypogaea* seed oil (g/100g)
5.4 DISCUSSION

Biofertilizers are living microorganisms of bacterial, fungal and algae origin. Their mode of action differs and can be applied alone or in combination. Biofertilization is the most important factor affecting the yield, yield components and biochemical constituents. Mixed inoculation treatment increased oil content in Canola (Abd-El-Gawad et al., 2009).

In the present investigation, the percentage yield of oil and physio-chemical properties of oil showed the combined inoculation of biofertilizers are more significant when compared to control and other inoculations.

5.4.1 Percentage yield of oil

The application of *Azotobacter* and *Azospirillum* helped increase in the oil content of canola seeds (Yasari and Patwardhan, 2007). The fertilizing fennel plants with organic manure and biofertilizers showed maximum essential oil (Azzaz et al., 2009). The promoting effect of NPK, organic manure and biofertilizers treatments was emphasized (Hend et al., 2007) on peppermint plant.

The associative action of biofertilizers and organic wastes led to a significant increase in the oil percentage in seeds as compared to chemical fertilizer treatment (Radwan and Awad, 2002). Compost with mixed *Azotobacter* and *Bacillus megaterlum* gave the highest essential oil production in cumin plants (Safwat and Badran, 2002). The highest seed contents of oil percentage was recorded by treatment of 75% NPK + 25% FYM + microbein (El-Kramany et al., 2007).
The oil content of seed yield significantly increased in response to biofertilizers application as being compared to the control (Shehata and El-Khawas, 2003). Inoculation with VAM fungi increased seed oil of soybean (Mostafavian et al., 2008) combined application of Rhizobium inoculation phosphobacterium inoculation and cobalt gave higher values of oil content than single application of either inoculation or cobalt (Basu et al., 2006). The percentage of oil content was highest in the Pseudomonas plants treated with 40 units of nitrogen and biofertilizers (Gomma et al., 2006).

In the present study, combined inoculated groundnut plants showed maximum amount of oil when compared to control and other mono and dual inoculations.

5.4.2 Physical property of oil (Refractive index)

The combined inoculated groundnut seed oil showed higher refractive index than other inoculations and control. This result was correlated with the result of El-Nakhlawy and Bakhashwain (2009) and Nkafamiya et al., (2010). The refractive index was no significance difference in the degrees of flow or thickness of all the oil at room temperature (Cocks and Von Rede, 1992).

5.4.3 Chemical properties of oil

5.4.3.1 Free fatty acid

The control showed maximum amount of free fatty acids. The minimum amount of free fatty acid was observed in the combined biofertilizers inoculated
plants seed oils. The free fatty acid of the oil is low. This indicates the stability of the products (Olaposi and Adunni, 2010). The presence of free fatty acid and other fatty materials in oil brings about the offensive odour and taste in oil on long storage (Aluyor et al., 2009).

5.4.3.2 Saponification value

The biofertilizers treated Pseudomonas plants, the saponification value was increased when compared to the non-fertilized ones (Gomaa et al., 2006). The biofertilizers and organic manure treated plants showed the maximum amount of saponification value in fennel plants (Azzaz et al., 2009).

The high saponification value indicated oxidation and its decrease suggest the unset of oxidation (Nkafamia et al., 2010). The high saponification value also indicated the presence of greater number of ester bonds, suggesting that the fat molecules were intact (Denniston et al., 2004). These properties make it useful in soap making industry, it is not attractive as a raw material because of its economic and nutritive implications.

The combined inoculated groundnut seed oil showed maximum amount of saponification value when compared to control and other inoculations.

5.4.3.3 Iodine value

In Pseudomonas biofertilized plants showed higher amount of iodine than the unfertilized plants (Gomma et al., 2006). The iodine value was higher for canola genotypes treated with mixed inoculation treatment than those of control (Abd-El-Gawad, 2009).
The high iodine value indicates dehydrogenation. It is a measure of unsaturation in lipid, which again determines the degree of flow. Decrease in iodine value indicates lipid oxidation and this might be due to metallic ions present among other factors, which enhances or promotes oxidation after the formation of hydroperoxide (Ruize et al., 1995).

The combined inoculated groundnut seed oil showed the maximum amount of iodine value when compared to the control and other inoculations.

**5.4.3.4 Peroxide value**

In the present investigation control showed a maximum amount of peroxide value. The minimum amount of peroxide value was observed in the combined biofertilizers inoculated plants seed oil. This result was correlated with the result of Abd-El-Gawad (2009). It was noticed that the peroxide value was generally lower for canola genotypes treated with mixed inoculation treatment than those of control.

The low peroxide value indicated slow oxidation of oils, according to Demian (1990). The peroxide formation is slow at first during an induction period that may vary from few weeks to several months according to the particular oil and temperature (Pearson, 1981). The peroxide value is an indicator of deterioration of fat (Olaposi and Adunni, 2010).

The present study revealed that the combined inoculation showed the maximum amount of oil content, refractive index, saponification value and iodine value. The minimum amount was found in control. But in the chemical parameters
such as free fatty acid and peroxidase value were negatively correlated with the above observations.