Medical Laboratory Techniques Department

Practical Biochemistry

SECOND STAGE \ SECOND COURSE

Lab 3-4

Oil and Fat analysis

Assist Lecturer

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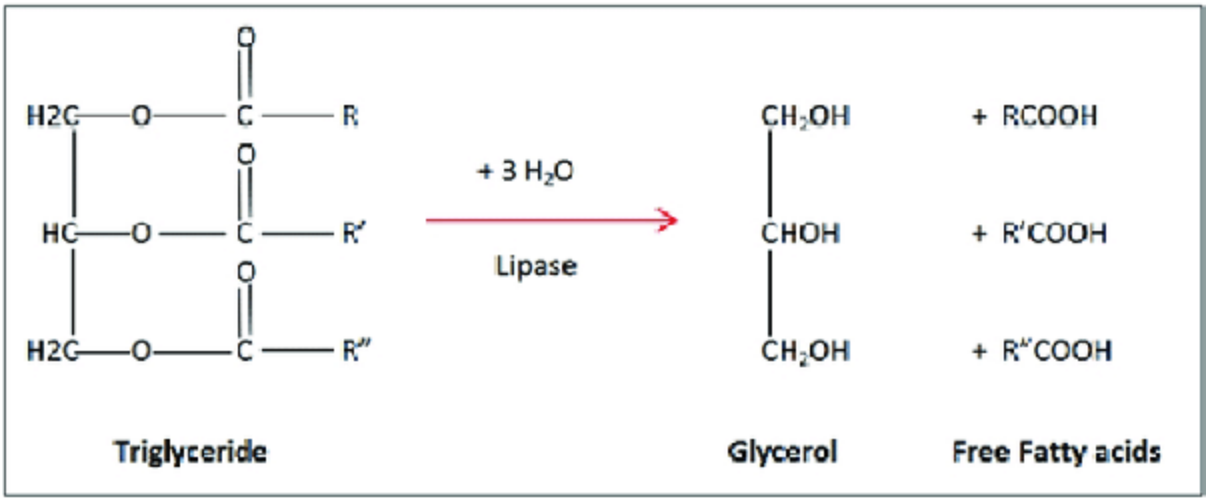
Lipid constants

They are called constants because they do not change for one type of fatty substance, because any change in their value indicates The impurity of this fatty substance.

1- acid value (free fatty acid value)

The acid value is defined as the number of milligrams of KOH required to neutralize the amount of free fatty acids present in 1 gm of the fat or oil.

*Fat or oil is hydrolyzed by different microorganisms with the formation of free fatty acids. The acid value is often a good measure of the breakdown of the triglycerides into free fatty acids, which has an adverse effect on the quality and the age of many lipids.



When fat or oil is dissolved in an ethanol solution containing an indicator and this solution is then titrated with alkali (KOH) until a pinkish color appears due to the formation of free fatty acid. Thus the high acid number indicates a stale oil or fat and stored under improper conditions.

Note:

✓ The oils and fats contain more or less free fatty acids according to the condition of manufacture, age and storage

✓ Acid value is a measure of the extent to which the glycerides in the oil have been decomposed.

✓ As rancidity is usually accompanied by free fatty acid formation, the determination of acid value is often used as a general indication of the condition and edibility of the oil.

✓ Oils intended for edible purposes should not contain more than 1% free fatty acids

Reagents:

1- Oil or Fat.
2- Hydrochloric acid (0.5 N).
3- KOH solution (0.1 N).
4- Fat solvent (a 1:1 mixture of ethanol (95%) and ether)
5- Phenolphthalein indicator 1% (in alcohol).

Procedure:

1-Weigh 5 gm of fat or 5 ml of oil and transfer it into 250 ml conical flask.

2- Add 10 ml fat solvent to the oil solution.

3- Add 1 ml of phenolphthalein indicator and mix well.

4- Titrate this against the KOH solution until a faint pink color appears and persists.

Calculation:

Calculate the acid number (mg KOH/g) of the fats:

$$\text{Acid number} = \frac{\text{Volume of KOH}}{\text{Weight of sample}} \times N_{KOH} \times \text{M.wt (56.1)}_{KOH}$$

2- peroxide value

The peroxide value is defined as the number of milliliters of sodium thiosulfate required to neutralize the peroxides contained in 1 gm of fat or oil.

Lipid oxidation (also called auto-oxidation) at fatty acid occurs by reaction of double bonds in fatty acids with oxygen present in the air, causing the formation of labile peroxides.

*The peroxides (ROOH) formed during auto-oxidation are unstable and decompose into free radicals .

*These initiate chain reactions which lead to eventually to decomposition of the fatty acid into various low molecular weight e.g., aldehydes, ketones, organic acids, and hydrocarbons.

Reagents:

1- Solvent mixture (mix 2 ml of glacial acetic acid with 1 ml of chloroform).

2- Potassium iodide powder.

3- Potassium iodide KI (15%)

4- Sodium thiosulfate Na2S2O3 (0.02 N or 0.02 M)

5-Starch indicator solution (0.5 %)

Procedure:

1- Weight 1 gm oil (with precision of 0.001 g) into a 250 ml Erlenmeyer flask.

2- Add 1gm of powdered KI, and 20 ml of solvent mixture (glacial acetic acid with chloroform 2:1). Then place the Erlenmeyer flask in boiling water for 1 minute.

3- Cool and add 20 ml of KI solution. Then wash the flask by 20 ml distilled water and add 1 ml starch indicator.

4- Titrate the solution with sodium thiosulfate until blue color disappears.

5- Do the same procedure as in the test without using oil under similar conditions and record the volume of sodium thiosulfate.

$$\text{Peroxide number} = \frac{\left[\begin{array}{l}\text{V. of } Na_2S_2O_3 \\ \text{for sample}\end{array}\right] - \left[\begin{array}{l}\text{V. of } Na_2S_2O_3 \\ \text{for blank}\end{array}\right]}{\text{Weight of sample}}$$

3- Iodine Value:

This is a test to measure the amount of unsaturation in fat and oil. The iodine value (IV) gives a measure of the average degree of unsaturation of a lipid: the higher the iodine value, the greater the number of C=C double bonds.

$$-CH=CH-+I_2 \rightarrow -CHI-CHI-$$

By definition the iodine value is expressed as the grams of iodine absorbed per 100g of lipid.

Iodine number is a useful characteristic for assessment of both purity and nutritive value of the fat. The iodine numbers of some important fats are mentioned below:

Fats	Iodine numbers
Butter fat	26-28
Human fat	65-70
Peanut oil	80-90
Corn oil	110-125
Soybean oil	137-143
Linseed oil	170-200

Reagents:

1- Oil or Fat.

2- Hanus solution IBr (dissolves 13.2 gm of I2 in 1 litter of glacial acetic acid, then add 3 ml of Br2.

3- Sodium thiosulfate (0.1 M).

4- Potassium iodide KI (10%).

5- Chloroform or CCl4

6-Starch (1%).

Procedure:

1- In a 250 ml conical flask, dissolve 0.25 ml of oil or 0.5 gm of fat in 10 ml chloroform or CCl4. Then add 25 ml of Hanus solution. Mix well, cover the mouth of the flask with a paper and keep it in dark place for 30 minutes for reaction to take place.

2- After 30 minutes, add about 50 ml of warm distilled water to wash down all the IBr into the solution. The colors of the solution turn blue-black. Mix well, and then add 10 ml of KI solution into it.

3-Titrate the contents of the flask with standard sodium thiosulfate till the color changes from blue-black to a pale strew color, add 10 drops of starch solution as indicator (blue color) and titrate again till there is no free iodine to change the blue color to colorless. Note down the titre value which is X ml.

4- To prepare a blank, use only 10 ml of chloroform or CCl4 only instead of oil or fat sample and repeat the same procedure as in the test. Note down the titre value which is Y ml.

Calculation:

The volume obtained for test titration = x ml

The volume obtained for blank titration = y ml

The difference between the two (i.e. blank-test) indicates the volume of sodium thiosulfate (0.1M) required to react with an equivalent volume of iodine .

To convert this volume into grams of iodine, multiply (Blank- Test) by 12.7/1000, as 1litter of 0.1 M iodine contains 12.7 gm of iodine.

According to Normality equation

1 ml of N/10 $Na_2S_2O_3$ solution = 1 ml of N/10 I-Br solution

1 ml of n/10 $Na_2S_2O_3$ solution = I ml of N/10 I-Br solution

= 1 ml of N/10 iodine solution

Normality of sodium $Na_2S_20_3$ = 0.1

Equivalent weight of iodine =127

1 ml of N/10 iodine = (127/1000) x (1/10) = 0.0127

Volume of Sodium thiosulphate used = [Blank- Test] ml

(Two molecules of Na2S2O3 are equivalent to one molecules of iodine; thus one molecules of Na2S2O3 is equivalent to one atom of iodine

1 ml of N/10 Na2S2O3 solution = 0.0127 gm of iodine

Amount of iodine absorbed by given amount of oil or fat = (y-x) x 0.0127 gm of iodine

$$\text{Iodine number} = \frac{\left[\text{V. of } Na_2S_2O_3 \text{ for blank}\right] - \left[\text{V. of } Na_2S_2O_3 \text{ for sample}\right]}{\text{Weight of sample}} \times 0.0127 \times 100$$