

CLS 281

Basic Biochemistry and Biomolecules

جامعة
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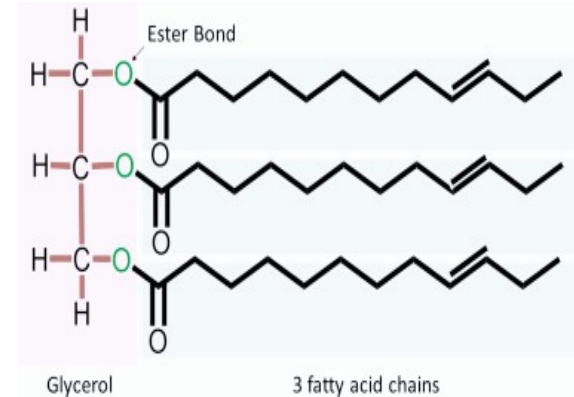
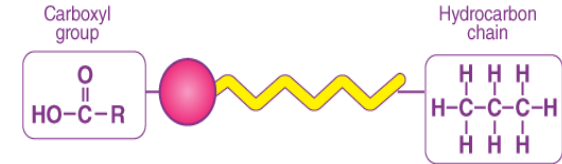


Experiment 8

Rancidity Testing Methods of Fat

Introduction

- Fats (lipids) are one of the main macronutrient groups in the human diet.
- Fat is a fuel source. It is a major storage form of energy in the body.
 - Fatty Acids are the building units of fat.
 - A triglyceride:
 - It is the major type of lipid used for energy storage.
 - It consists of glycerol and three fatty acids.

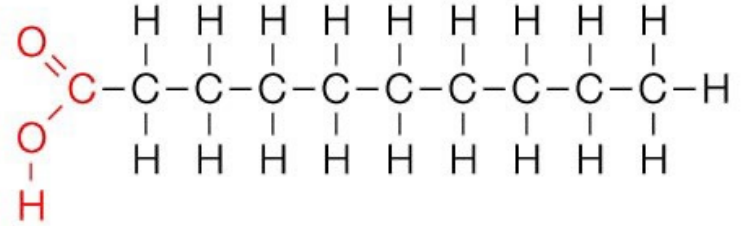


Types of fatty acid

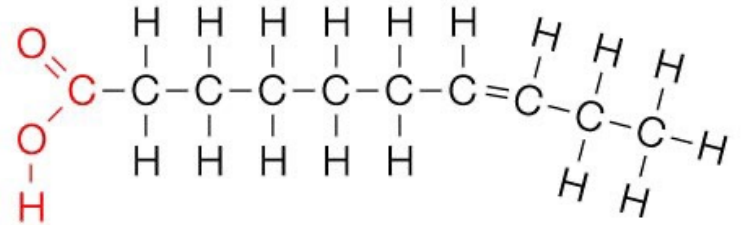
- **Saturated fatty acids**
- Have hydrocarbon chains connected by single bonds only.
- Form: Solid at room temperature.

- **Unsaturated fatty acids**
- Have one or more double bonds.
- Form: Liquid.

Saturated



Unsaturated



Rancidification of Fat

- **Rancidity** happens when fat is exposed to **heat, light, or oxygen** over a period of time.
 - The atmospheric **oxygen** reacts with the double bond in fatty acids to form peroxide.
 - The microorganisms **hydrolyze** the fat with the liberation of free fatty acids and glycerol.
 - The more free fatty acids in the oil, the more acidity and age the oil is.
 - Thus, the amount of free fatty acids present gives an indication of the age and quality of the fat.
- The result of this change: the fat and oil become rancid, creating **a bad smell, sour taste, and color changes**.

Two tests to study the quality of fat (oil):

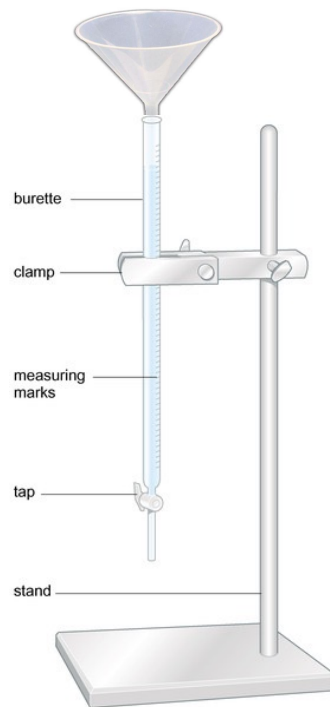
1. Acid value of fat.
2. Iodine number.

01 Acid value of fat

- **Aim**
 - To determine the age of the fat.
 - To determine the quality of the fat.
- **Method**
 - Titration with a **base**.
 - The most common base is **KOH**.
 - The result of the titration is the acid value.
- **The acid value** is the number of milligrams of basic compound required to neutralize the free fatty acid present in 1 g of fat.

01 Materials

1. Balance
2. Funnel
3. Burette clamp and stand
4. Burette
5. Cylinder
6. Flask



01

Procedure

1. Add 1.2 ml of **oil** (equivalent to 1 gm of fat) to a flask.
2. Add 25 ml of **fat solvent** to the flask.
3. Add 1 ml of **phenolphthalein solution** (pH indicator) and mix well.
4. Fill the Burette with 0.01 N KOH till 0.
5. Start the titration with 0.01N KOH.

Note: The endpoint is reached when the **faint pink color** persists for 30 seconds.

5. Once you reach the endpoint, measure the used volume (V_2) of KOH in the titration process.
6. Calculate the acid value.

Phenolphthalein Indicator

pH=0-3



Orange

pH=4-7



Colourless

pH=8-10



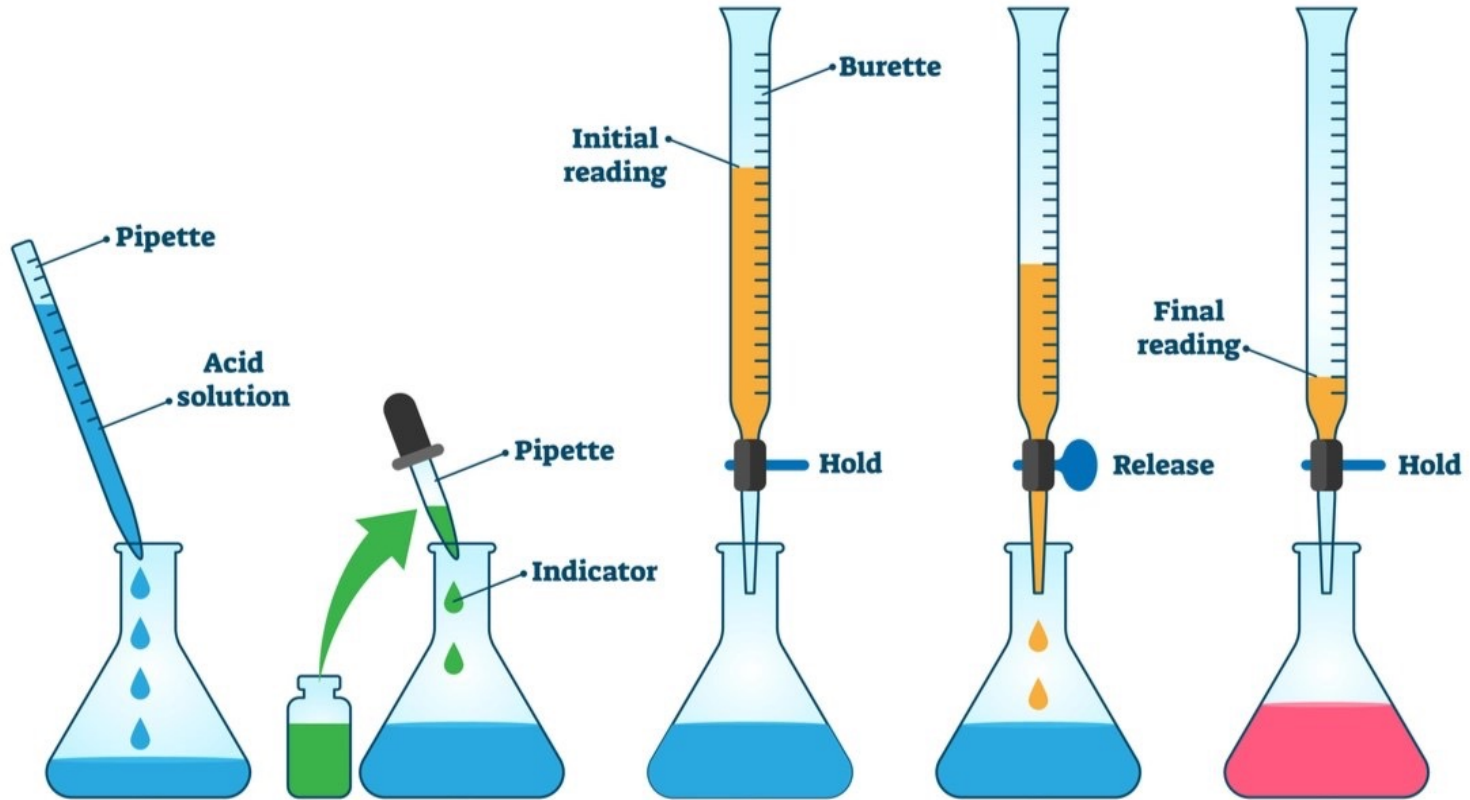
Pink

pH=11-14



Colourless

Acid-Base Titration



The endpoint = faint pink color persists for 30 seconds.

01

Calculation

To calculate the Acid value of fat, use one of the following formulae:

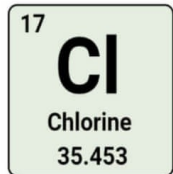
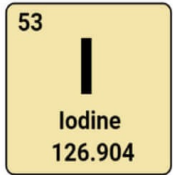
- **Acid value = (V of KOH X 0.56 mg) / Weight of fat**
- **Or: $M_w(\text{KOH}) \times N \times V / W(\text{fat})$**

- **Range of Acid value for olive oil = 0.3-1.0**

- **V of KOH** = Volume of KOH used to reach the endpoint.
- **W of fat** = weight of fat used in the sample
- **The molecular weight of KOH is 56**
- 1 liter N KOH contains 56g.
- 1 ml of N KOH contains 56 mg.
- 1 ml of 0.01N KOH contains **0.56 mg**

02 The Iodine Number of Fat

- Double bonds in unsaturated fatty acids are capable of breaking down and binding to **halogens** (iodine, bromine, and chloride).
- **Aim** to measure the degree of unsaturation of fat.
- The greater the degree of unsaturation in a fatty acid (i.e., the more double bonds in the fatty acid), the more vulnerable it is to **lipid peroxidation** (rancidity).
- **What is the Iodine Number of Fat?**
 - The number of grams of iodine absorbed by 100 g of fat called the "iodine number."

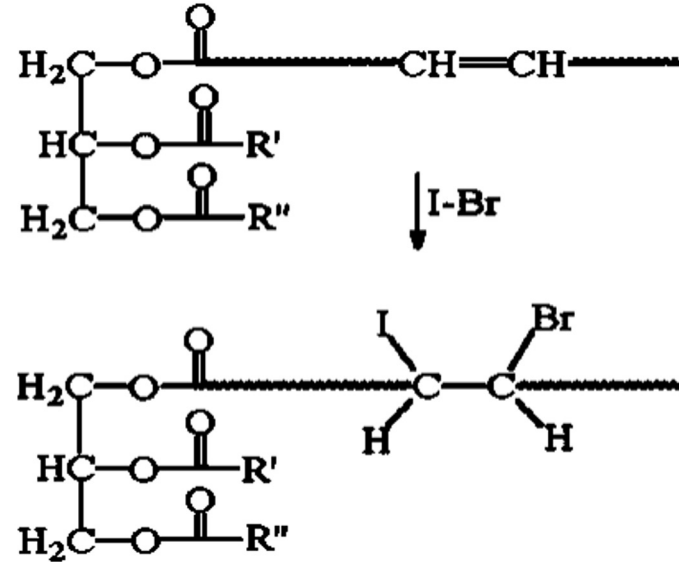


02 The Iodine Number of Fat

- Two methods are generally used to test the iodine number of fat:
 1. **The Wijs method** uses iodine chloride (ICL).
 - The Wijs method gives results 2 to 5 percent higher, and the iodine numbers are **closer to the theoretical values**.
 2. **The Hanus method** uses iodine bromide (IBr).
 - The Hanus reagent is **more stable**.

02 The Hanus method Principle

1. $I_2 + Br_2 \rightarrow 2 IBr$ (Iodine Bromide)
2. Fat + Excess $IBr \rightarrow$ halogenated fat + unreacted IBr
3. $KI + IBr \rightarrow I_2 + KBr$
4. $I_2 + 2 Na_2 S_2 O_3 \rightarrow 2 NaI + Na_2 S_4 O_6$ **pale yellow.**
5. $2 NaI + starch \rightarrow$ **blue color**



02 Procedure

1. Pipette 10 ml of **each fat sample** provided in separately labeled flasks.
2. Add exactly 25 ml of **Hanus iodine solution** from a burette to each flask.
3. **Set up a blank (separate flask) by adding 10 ml of chloroform to 25 ml of Hanus iodine solution.**
Note: Only one blank flask is enough for all the students.
4. Close the flasks with glass stoppers, and mix well by swirling.
5. Incubate the flasks at room temperature for 30 mins in a dark cabinet with occasional swirling.
6. Add 10 ml of 15% **potassium iodide** solution to each flask and mix.

02 Procedure

6. Add about 30 ml of **water**, washing down any iodine solution that may be found on the wall of the flask and the stopper.
7. Titrate the iodine with 0.1N **sodium thiosulphate** from a 50 ml burette until the color of the solution is **pale yellow**.
8. Add 2 ml of **1% starch solution** as an indicator. The solution in the flask **turns blue**.
9. Continue the titration until the blue color disappears, mixing well during the final stages of titration.
10. To ensure complete removal of the iodine, stopper the flask and shake vigorously. If the blue color returns, continue the titration.
11. Record the volume used for your sample and blank to reach the colorless endpoint.

02 Result

- Colorless is the endpoint



02

Calculation

- Iodine Number = $[(b-a) \times 12.69 \times N] / W$ of the sample
- b = the volume of titer (sodium thiosulphate) with the **blank**.
- a = the volume of titer (sodium thiosulphate) with the **sample**.
- The atomic mass of iodine = 126.90447

Summary of Rancidity Testing Methods of Fat

Test	Aim	Reagent	Principle	Result
Rancidity happens when fat is exposed to heat, light, or oxygen over a period of time.				
The acid value of fat.	To determine the age of the fat. And to determine the quality of the fat.	Titration with a base. The most common base is KOH.	Acid-Base Titration	Range of Acid value for olive oil = 0.3-1.0
Iodine number.	Aim to measure the degree of unsaturation of fat.	Iodine bromide (IBr).	The Hanus method	Iodine Number

Report Criteria

Total: 5 marks

- 1- Course # (CLS 281)
- 2- Experiment title
- 2- Date of the experiment
- 4- Student's names and university ID#
- 5- Section #
- 6- Experiment title
- 5- The aim of the experiment (objective, or what the test detects specifically) (1 mark)
- 6- Principle (chemical reaction) (1 mark)
- 7- Methodology
- 8- Result (2 mark)
- 9- Interpretation or Comment (1 mark)

Deadline: next lab

Submission: Handout or **email (docx. form only)**