Laboratory Work No. 6

Determination of the Acid Number

The **acid number** characterizes the presence of **free fatty acids in fat** and is expressed as the **amount of potassium hydroxide (mg)** required to neutralize the free fatty acids and other substances associated with triacylglycerols that can be neutralized by alkali, contained in **1 gram of fat** (**mg KOH/g fat**).

The acid number value reflects the **degree of hydrolytic decomposition of fats** and determines the **commercial grade and quality** of edible fats. It is regulated by **GOST standards** and **technical specifications**.

For example, **oil obtained from mature seeds** has a **low content of free fatty acids**, while the **oil from immature seeds** contains a **high level of free fatty acids**.

The acid number varies depending on the degree of oil purification. For unrefined oils, the acid number is higher than for refined oils.

Acid Numbers of Sunflower Oil	mg KOH/g fat:
refined deodorized	0.40
refined non-deodorized	0.40
refined hydrated, highest grade	1.50
hydrated, 1st grade	2.25
hydrated, 2nd grade	6.00
unrefined, highest grade	1.50
unrefined, 1st grade	2.25
unrefined, 2nd grade	6.00

The acid number for refined vegetable oils should not exceed **0.4 mg KOH/g fat** (GOST R 50457–92 (ISO 660–83). "Vegetable oils. Determination of acid number and acidity").

To characterize the freshness of milk fat, unlike other animal fats, the concept of acidity is used, which is expressed in **Kettstoffer degrees** and should not exceed **4.0 °K** (GOST 52971–2008. "Clarified butter and milk fat. Technical specifications").

Depending on the value of the acid number, edible rendered fat (pork, beef, lamb, horse), obtained from slaughtered livestock, is classified into higher and first grades. The acid number for rendered fat of the higher grade should not exceed **1.1 mg KOH/g fat**; for the first grade, it should not exceed **2.2 mg KOH/g fat**. (GOST 25292–82. "Edible rendered animal fats").

Fat from chilled and frozen carcasses of all types of poultry with an acid number up to **1 mg KOH/g fat** is considered fresh; chicken fat from chilled carcasses with an acid number of **1.0–2.5 mg KOH/g**, goose fat **1.0–2.0 mg KOH/g**, and duck and turkey fat **1.0–3.0 mg KOH/g** is considered not fresh.

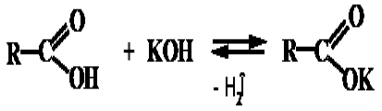
Fat from frozen carcasses of all types of poultry with an acid number of **1.0–1.6 mg KOH/g** is considered of doubtful freshness.

For fats from marine mammals and fish intended for food production, the acid number should not exceed **4.0 mg KOH/g** (GOST 8714–72 "Edible fat from fish and marine mammals").

If the conditions and storage periods of fats are not observed, the acid number increases, which is mainly caused by the hydrolysis of triacylglycerides. Hydrolysis occurs under the influence of enzymes from microorganisms, high temperatures, humidity, light, and other factors. As a result of hydrolysis, free fatty acids are released, the content of which is determined by the acid number.

Since free fatty acids oxidize faster than bound ones, an increase in the acid number accelerates both chemical and enzymatic oxidative rancidity processes of unsaturated fatty acids. On the other hand, the oxidation of free unsaturated fatty acids by lipoxygenases contributes to an increase in the acid number. However, a high acid number is not always an indication of fat spoilage. Often, fats with a high acid number are not rancid, while rancid fats may have a low acid number.

The method for determining the acid number is based on acid-base titration of the oil with potassium hydroxide in the presence of phenolphthalein.



A sample of the oil is dissolved in a neutralized mixture of ethanol and diethyl ether (1:2). Ethyl alcohol is used not only to dissolve the soap formed during the titration process but also to prevent the reverse reaction — the hydrolysis of the soap. Titration can be carried out using either an alcoholic alkali solution or an aqueous one. In the latter case, the volume of the titrant (aqueous potassium hydroxide solution) should not exceed the volume of ethyl alcohol used to dissolve the oil by more than 20%, in order to prevent the hydrolysis of the soap formed during the analysis.

Analysis Procedure

1. In a 100 ml flask, accurately weigh about **3 g of vegetable oil** or **1 g of animal fat** using an analytical balance.
2. Add **50 ml of an alcohol-ether mixture (1:2)** to the flask.
3. Carefully dissolve the fat with gentle heating.
4. After the fat is completely dissolved, cool the flask with the sample to **room temperature**.
5. Add **1–2 drops of phenolphthalein alcoholic solution** to the cooled solution.
6. Slowly titrate the solution **dropwise** with **0.01 N aqueous potassium hydroxide solution** until a **faint pink color** appears.
7. The volume of titrant used should **not exceed 4 ml** (which is 20% of the alcohol volume).
8. If an alcoholic solution of alkali is used for titration, the fat sample can be dissolved in **ether or benzene without adding alcohol**.
9. Calculate the acid number (AN, mg KOH/g) using the formula:

$$AN = \frac{V_{KOH} \times 0.5611}{m}$$

where:

- V_{KOH} — volume of 0.01 N potassium hydroxide solution used for titration, in ml
- 0.5611 — titer of 0.01 N potassium hydroxide solution, mg/ml
- m — mass of the fat sample, in grams

To determine the acid number of unrefined vegetable oil, a **0.1 N potassium hydroxide solution** should be used as the titrant. The acid number (AN, mg KOH/g) is calculated using the formula:

$$AN = \frac{V_{KOH} \times 5.611}{m}$$

where:

- V_{KOH} — volume of 0.1 N potassium hydroxide solution used for titration of the fat sample, ml;
- 5.611 — titer of 0.1 N potassium hydroxide solution, mg/ml;
- m — mass of the fat sample, g.

The amount of titrant used in determining the acid number of unrefined vegetable oils neutralizes not only the free fatty acids but also other titratable substances present in the oils. For example, phosphatides or gossypol in cottonseed oil. Therefore, the true acid number of such oils, which is caused by the presence of free fatty acids themselves, cannot be determined by direct titration.

The true acid number (AN', mg KOH/g) of unrefined oils can be calculated using the formula, knowing the content of phosphatides and gossypol:

$$AN' = AN - (0.32 \times PF + 2.17 \times PG)$$

where:
AN – acid number of the oil determined by titration, mg KOH/g;
PF – mass fraction of phosphatides in the oil, %;
PG – mass fraction of gossypol in the oil, %;
0.32 and 2.17 – average neutralization numbers of phosphatides and gossypol respectively, divided by 100, mg KOH/g.

Calculation of free fatty acids (FFA, %) from the true acid number:

$$\mathrm{FFA} = \frac{AN' \times M \times 100}{56.11 \times 1000}$$

Where:

- AN' — true acid number (mg KOH/g fat)
- M — average molecular weight of fatty acids (g/mol)
- 56.11 — molecular weight of potassium hydroxide (KOH) (g/mol)
- 1000 — conversion factor from mg to g
- 100 — conversion factor to percentage (%)

Determination of the mass fraction of free fatty acids in sunflower oil, soybean oil, and confectionery fats is carried out recalculated as oleic acid (FFA_OLE, %) as the most common free fatty acid in these fats using the formula:

$$\mathrm{FFA}_{OLE} = AN' \times 0.5034$$

Where:

- AN' — true acid number (mg KOH/g fat)
- 282.47 — molecular weight of oleic acid (g/mol)
- 0.5034 — conversion factor to oleic acid