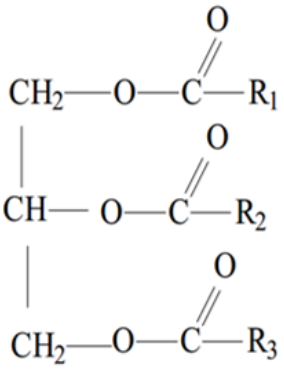
LABORATORY WORK №5
Determination of Lipids in Plant Raw Materials and Food Products

Lipids are a complex mixture of organic substances extracted from plant and animal sources. They share similar physicochemical properties, primarily **insolubility in water** and **good solubility in a number of organic solvents** (such as diethyl ether, benzene, chloroform, and alcohols).

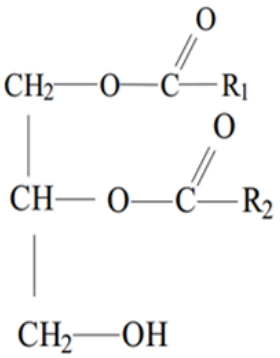
The main bulk of these substances consists of **complex esters of the trihydric alcohol glycerol and high-molecular-weight fatty acids**—called **glycerides**. In addition to glycerides, lipids include **free fatty acids, waxes, phospho- and glycolipids, fat-soluble pigments, sterols, fat-soluble vitamins**, and various **products of their transformations**.

The majority of lipids are **glycerides**, which are essentially **fats**.

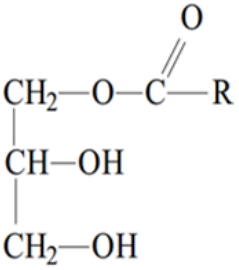
Fats mainly consist of **triglycerides**, but **di- and monoglycerides** are also present.



Triglyceride



diglyceride



monoglyceride

Lipids are important components of food and largely determine its **nutritional value and taste qualities**. Lipids play an **extremely important role in various food processing technologies**.
For example, the **spoilage of grain and its processed products during storage (such as rancidity)** is primarily associated with **changes in their lipid complex**.

Since fat contains **unsaturated fatty acids**, it can easily undergo **oxidation**. The process of fat oxidation — the oxidation of unsaturated fatty acids — can occur **spontaneously** through the attachment of **oxygen from the air** at the sites of **double bonds**.
However, this process can be **significantly accelerated** under the influence of a specific **enzyme** found in **grain, flour, and groats — lipoxygenase**. This enzyme is especially active in **soybeans** and **soy flour**.

As a result of lipoxygenase activity, **unsaturated fatty acids** form **peroxides and hydroperoxides**.

1. **Unsaturated fatty acid reacts with oxygen in the presence of lipoxygenase:**

$$\mathrm{R—CH_2—}CH=CH\mathrm{—}CH_2\ldots\mathrm{COOH}+O_2\xrightarrow{\text{lipoxygenase}}\mathrm{R—CH_2—}CH\mathrm{—}CH\mathrm{—}CH_2\ldots\mathrm{COOH}$$

This forms a **peroxide of unsaturated fatty acid**, where an oxygen bridge (–O–O–) is added between carbon atoms.

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2. **Further reaction of the unsaturated fatty acid with oxygen:**

$$\mathrm{R—CH_2—}CH=CH\mathrm{—}CH_2\mathrm{—}CH=CH\mathrm{—}CH_2\ldots\mathrm{COOH}+O_2\rightarrow\ldots$$

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3. **Formation of hydroperoxide of the unsaturated fatty acid:**

$$\mathrm{R—CH_2—}CH=CH\mathrm{—}CH\mathrm{—}CH=CH\mathrm{—}CH_2\ldots\mathrm{COOH}$$

with a hydroperoxide group (–OOH) attached:

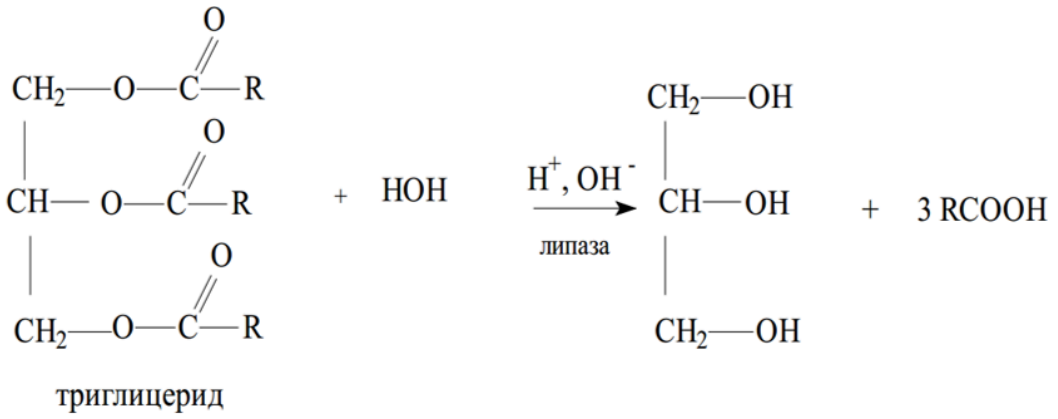
$$\begin{array}{c}\\|\\OOH\end{array}$$

Hydroperoxides and peroxides are highly active oxidizing agents. They easily oxidize **fatty acids**, resulting in the formation of **unpleasant-tasting and foul-smelling substances**, which causes the **fat to become rancid**. Therefore, the presence of **lipoxygenase** in grain contributes to the **rancidity of flour and groats during storage**.

Peroxides and hydroperoxides can also easily oxidize the **yellow pigments in flour—carotenoids**, which leads to the **lightening of flour and dough**.

This factor is particularly important in the **production and drying of pasta**. As a result, in recent years, there has been increased research into the **lipoxygenase activity in different varieties of durum wheat**, which is used to produce the flour for the pasta industry.

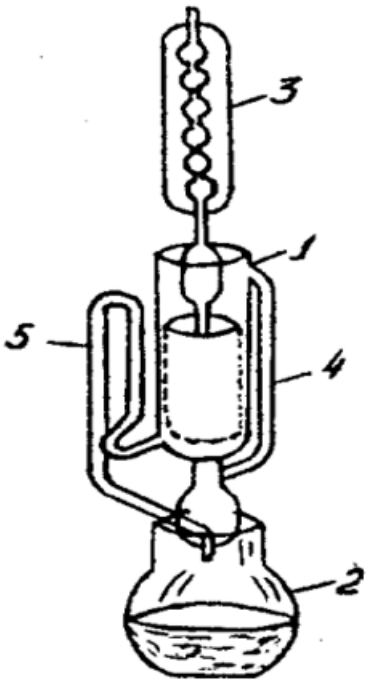
Under the influence of the enzyme **lipase**, as well as **acids, alkalis, or special mixtures**, **fats (triglycerides)** undergo **hydrolysis**, first forming **diglycerides**, then **monoglycerides**, and ultimately **fatty acids and glycerol**.



Determination of Crude Fat Using the Soxhlet Apparatus

Test material: Flour, ground grain
Reagents: Organic solvents – diethyl ether *(or petroleum ether, gasoline, hexane, ethanol, propanol)*.

The essence of the method lies in **extracting fat from the product using an organic solvent** (according to GOST – **diethyl ether**).
The extracted fat is called **crude fat** because it includes not only the actual fat (**glycerides**) but also **all other substances soluble in organic solvents** (i.e., **lipids**).
Crude fat is extracted using a **Soxhlet apparatus**. *(Figure 1)*



A sample of flour or finely ground grain weighing **8–10 g**, which passes completely through a sieve with **1 mm diameter holes**, is placed into a bag made of filter paper. The sample is

weighed **in the bag**, and the mass of the sample is determined by the difference between the weight of the bag with the sample and the empty bag.

The bag with the sample is placed into the extractor, which is then connected to a condenser (3) and a flask (2). Beforehand, the flask is filled with solvent to **2/3 of its capacity**. The amount of solvent should be **1.5 to 2 times the volume required to fill the extractor**.

Water is turned on in the condenser, and the flask with the solvent is heated to **40–50°C** by immersing it shallowly in an electric water bath.

The **solvent vapors** pass through the wide tube of the extractor, condense in the condenser, and drip back into the extractor as droplets. To prevent the solvent vapors from escaping through the condenser, the solvent should not boil too vigorously.

The apparatus should be adjusted so that the solvent siphons back **8–15 times per hour**. Under normal operation, extraction is carried out for **6 hours**. For more precise determinations and depending on the fat content in the sample, extraction can continue for **10 to 12 hours**.

At the end of the extraction, the solvent is allowed to drain from the extractor one last time, heating is stopped, and the apparatus is disassembled.

The bag with the defatted sample is air-dried (under a draft) and then dried to a constant weight in a drying oven at **60°C**.

The fat content on a dry matter basis is calculated using the formula:

$$X = \frac{(M_1 - M_2) \times 100}{P(100 - W)} \times 100$$

where:

- M_1 = mass of the bag with the sample before extraction, g
- M_2 = mass of the bag with the sample after extraction, g
- P = mass of the sample, g
- W = moisture content of the product, %