C3 How does human digestion break down fats? – Saponification of edible oil

These simple experiments for the emulsification and saponification of fats are well suited as an introduction to the topic of digestion and metabolism in the human body. Starting from the experiment that merely demonstrates that fats can be split into fatty acids, the topic must then be related to human digestion. These teacher instructions provide information and suggestions for this. The materials and apparatus supplied are sufficient to allow eight groups of students to conduct the experiments simultaneously.

1 Main question

This series of experiments is intended as an introduction to the topic of fat digestion in the human body at the organ and cellular level. For this purpose, the properties and reactions of fats and oils as constituents of food will be made accessible experimentally and their chemical structure formulated. The health aspects of fat digestion should also be discussed during class.

2 Integrating the experiment into the teaching context

2.1 Basic principles

Students are frequently already familiar with the subject of nutrition from elementary school. They already have prior knowledge of what we have to eat in order to be active: Food provides the energy for life. Students are mostly familiar with the main constituents of food – carbohydrates, fats, and proteins. The digestive process is also known to them from their own experience. This prior knowledge can be utilized as the basis for a subsequent detailed analysis of nutrient components (in this case fats), and their processing, conversion, and utilization in the human body.

2.1.1 Qualitative access at age levels 10 to 16

Different nutrients have different functions in the body. Fats are used to store energy and as a vehicle for transporting fat-soluble vitamins, and they are important building materials for the cells (for example, for biomembranes). Fats that are not needed for providing energy are stored in the form of fat deposits and as structural fat.
During physical activity, the carbohydrates are used first for supplying energy to the muscles (see experiment C1 [We burn sugar – Cellular respiration and respiratory chain] and C2 [Carbohydrates as providers of energy for metabolism – Starch and sugar]). The energy from fat deposits is tapped only when the energy from burning carbohydrates is depleted. This does not occur until after about 30 minutes of continuous physical exertion. Fat metabolism is activated in particular by light endurance exercise. The more intensive the sporting activity, the greater the proportion of energy needs that are covered by more quickly mobilized carbohydrates. The body also falls back on its fat reserves during long periods of hunger or nutritional deficiency.
Fats also have a heat-insulating function; this is especially noticeable in animals living in polar regions (for example, whales and seals). Humans, too, have an insulating layer of fat under the skin.
The absorption of fat in the human body occurs in several stages. Because of their poor water solubility, fats require special treatment in the gastrointestinal tract to break them down for conversion to energy. Fat digestion begins in the **stomach**. Here the fats are mechanically emulsified by muscle movement and reduced to droplets with a diameter of 0.5 – 2 µm.

Fat-splitting enzymes, the lipases, can now act on these droplets and break down the fat into its constituent parts, the long-chain fatty acids and glycerol. About 15% to 30% of fats are digested in the stomach in this way.
As soon as the bolus reaches the duodenum, the pancreas performs a key function in the further digestion of fats. Lipases and bile salts from the pancreas are activated to ensure that the fat droplets are even more finely divided and micelles form that are so small (max. 50 nm) that they penetrate between the microvilli of the brush border of the intestine and can attach themselves to the cell membranes. The micelles consist of the initial products of degradation (fatty acids, mono- and diglycerides), bile salts, and other lipids, with the water-soluble parts of the substances oriented outward into the aqueous phase (see Fig. 1). Absorption of the fatty acids and other products of degradation occurs in the **small intestine**. The degradation products and other lipids pass through the membrane into the cells where they are reassembled into triglycerides. These newly composed fats are packed together with transport proteins and reach the bloodstream via the lymph. Short- and medium-chain fatty acids are absorbed directly into the brush border membrane and from there pass into the portal blood.

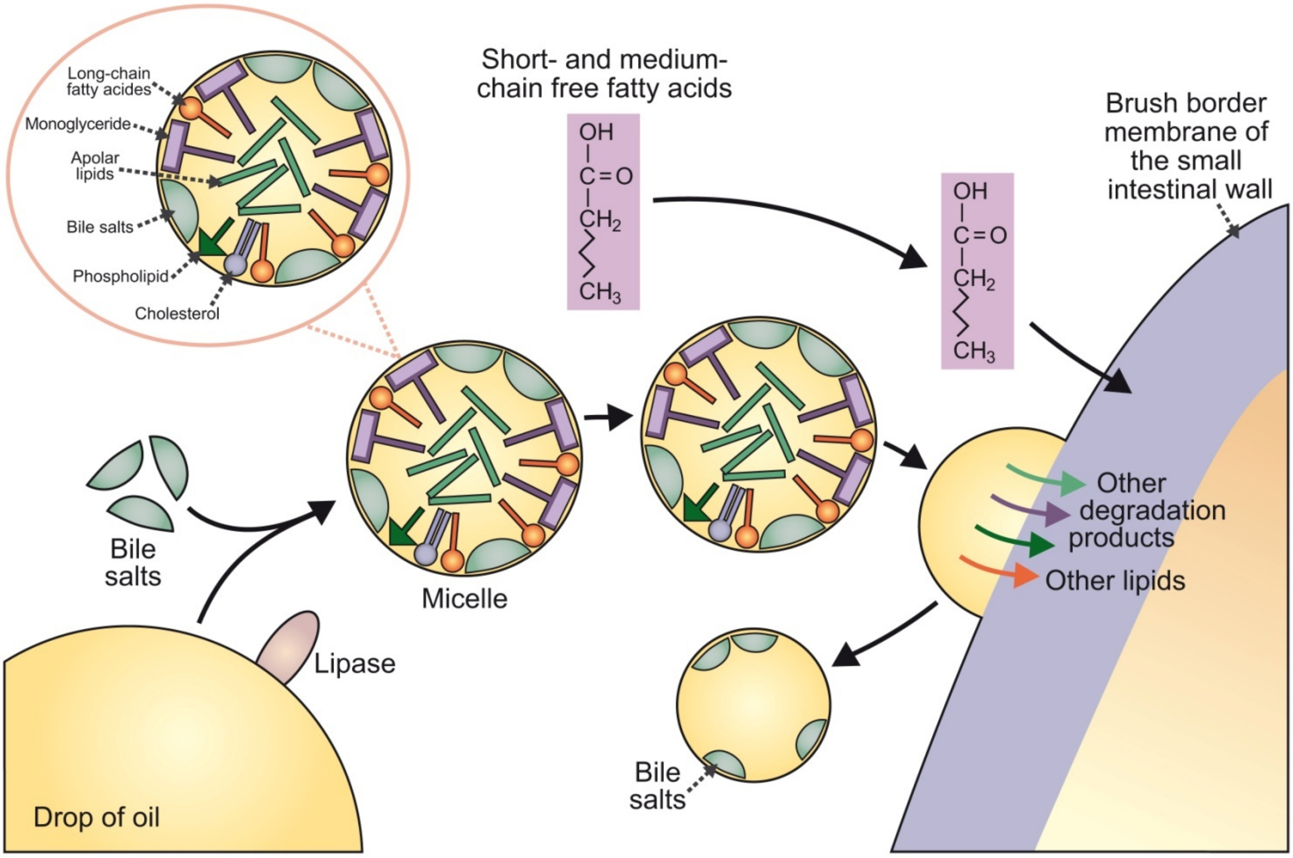

Fig. 1: Absorption of fat in the small intestine.

2.1.2 More detailed approach for the age group 16+

Properties and reactions of fats

Solid fats, liquid fats, and cholesterol belong to the lipids. Fats are the product of the esterification of the trivalent alcohol glycerol with fatty acids of different lengths (between 12 and 20 carbon atoms). A distinction is made between the fatty acids that the human body can produce from other substances through synthesis and conversion, on the one hand, and the essential fatty acids that have to be supplied with the food intake, on the other. These include those having one or more double bonds in their carbon skeleton (→ polyunsaturated fatty acids). The larger the proportion of polyunsaturated fatty acids, the more liquid the fat will be. Fats that consist only of saturated fatty acids are solid.

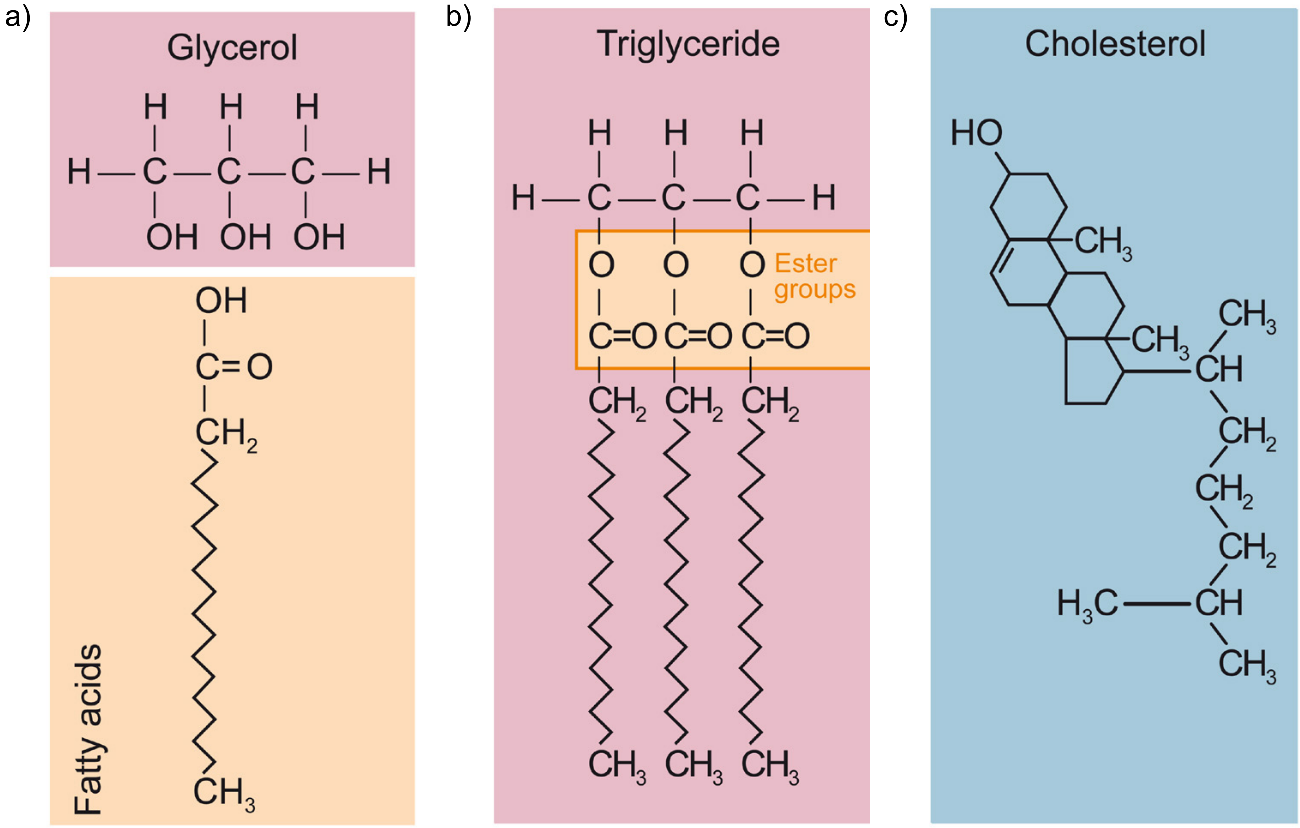

Fig. 2: a) Basic building blocks of fats, glycerol and fatty acids, as well as two important representatives of the lipids: b) Triglyceride (90% of fats) and c) Cholesterol.

The ester groups of fats can be easily split under acidic or alkaline conditions. This reaction is known as the saponification of a fat (see Fig. 3).

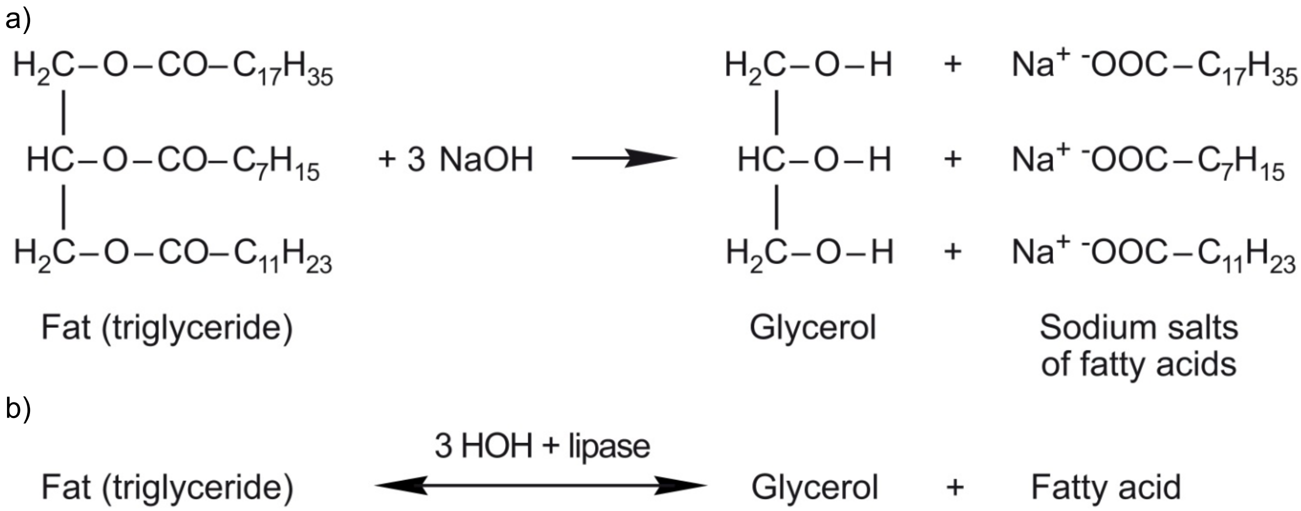

Fig. 3: a) Example of basic hydrolysis (alkaline saponification) of a fat. b) Breakdown of fat in human digestion.

Release of energy from fatty tissue in animal cells – β-oxidation
The stored fat deposits (trialkylglycerides) are hydrolyzed into free fatty acids and glycerol in the fatty tissue by a lipase. The free fatty acids are discharged into the blood, transported bonded to proteins, and converted to ATP in the mitochondria by ß-oxidation. For this, the fatty acids are first activated by coupling to coenzyme A, whereby ATP is consumed. Acetyl-CoA molecules are then split in several steps. One acetyl-CoA molecule is created in each cycle and then introduced into the citric acid cycle. The complete conversion of a long-chain fatty acid delivers three to four times as much ATP as the decomposition of a glucose molecule (see experiment C1). The glycerol resulting from the hydrolysis of the fat is similarly introduced into the citric acid cycle after conversion to pyruvate.
Whereas equilibrium is established between educts and products in the case of enzymatic ester hydrolysis in the body, alkaline saponification with addition of sodium chloride (table salt) is an irreversible process. The resulting sodium salts of the fatty acids are precipitated in the form of poorly soluble soaps (curd soap) and are thus removed from the equilibrium reaction. If you carry out saponification with potassium hydroxide solution, it produces soft soap. (See also experiment C6 [Skin and hygiene – Why do we wash our hands?] with respect to the washing effect of soaps and tensides.)

2.2 Relevance to the curriculum

In the age group from 10 to 16 years, the main focus of attention is on the human biology aspects of nutrition and digestion. In the first subexperiment, the principle of fat digestion in the stomach and intestine can be presented qualitatively and the effect of tensides/bile salts discussed. It is also advisable to brush up on prior knowledge with respect to the digestive organs and in relation to the miscibility of polar and nonpolar substances.
The chemistry of fats with their properties and reactions should not be dealt with in detail until the age group 16+. Basic knowledge of organic chemistry is essential for this (alkanes, alkenes, alcohols, esters, carboxylic acids: structure and reactions; forms of isomerism). The same applies to covering the topic of metabolic physiology of fat degradation at the cellular level. There is also an interdisciplinary component provided through the significance of the chemical concepts mentioned for application within the biological context of fat digestion.

Topics and terms: alkyl radical, bile salt, carboxyl group, digestion, diglyceride, emulsion, enzymes, ester hydrolysis, esterification, fat splitting, fats (lipids), fatty acid, glycerol, hydrophilic, hydrophobic, lipase, metabolism, micelle, monoglyceride, nonpolar, nutrition, polar, saponification, tenside, triglyceride

2.3 Skills

The students will …

- know the path taken by food through the human body.
- know the stages of fat digestion and be able to explain the processes taking place there.
- reflect upon their eating habits and discuss the health consequences of eating too much or too little food.
- understand the construction and basic functioning of a tenside and be able to apply it to fat digestion.
- be able to explain the chemical structure of fats.
- be able to formulate the reaction equation for fat saponification.

2.4 Explaining the experiment in the teaching context

2.4.1 Subexperiment 1: We emulsify oil

This subexperiment relates to the students' everyday experience. The fact that fats do not readily mix with water is something they will have observed, for example, when washing a greasy pan. They are also familiar with the phenomenon that fats can be removed by using dishwashing detergents. The experimental approach to this phenomenon therefore offers the possibility of brushing up on the chemical principles of the miscibility of substances and dealing explicitly with the way in which tensides function.

2.4.2 Subexperiment 2: Saponification of edible oil

This subexperiment shows the alkaline saponification of an edible oil. This takes place in several steps.
Sodium carbonate dissolves in water with the development of heat. It reacts in an acid-base reaction with the solvent, forming hydrogen carbonate and hydroxide ions:

$$CO_3^{2-} + H_2O \rightarrow HCO_3^{-} + OH^{-} \text{ and}$$

$$HCO_3^{-} + H_2O \rightarrow CO_2 + H_2O + OH$$

This results in a strongly alkaline solution. The hydroxide ions in this solution act on the ester bonds in the oil and hydrolyze them, producing glycerol and carboxylations as shown in Fig. 3. Sodium chloride added to the solution increases the sodium ion concentration to such a level that the solubility product of the sodium soap is exceeded. (To express this more simply: Since there are already so many sodium ions due to the table salt in the aqueous solution, the sodium salt of the fatty acid can no longer dissolve.)
Note: In this experiment it is certainly possible to recognize the formation of the curd soap from the highly visible change in consistency from clear, light oil to an opaque, viscous soap. Since curd soap remains liquid for a relatively long time when hot, it may be necessary to wait for a fairly long time until solid soap precipitates.

2.5 Experimental variations

The experiments for emulsification and saponification of edible oil do not require much material or time, and can be integrated into lessons as student experiments that can be conducted individually or in pairs. Because of the complexity of the overall topic of nutrition, digestion, and cell metabolism, there are teaching methods available to spur students to action. These methods provide additional material for background information in addition to the experimental approach. Typical examples of such approaches could be learning at workstations or a group puzzle on the overall topic. These methods are likewise well suited to allow for the different pace of learning and learning progress of individual students.

3 Additional information on the experiment

You will find additional media for preparing or for further study of this experiment on the Siemens Stiftung Media Portal:
https://medienportal.siemens-stiftung.org

4 Notes on conducting the subexperiments

4.1 Facilities

The experiments can be conducted by the students on their own under the supervision of a teacher in any well-ventilated classroom.

4.2 Time required

	Preparation	Execution	Analysis
Subexperiment 1	5 min.	3 min.	5 min.
Subexperiment 2	5 min.	25 min.	7 min.

4.3 Safety aspects

The students may conduct the experiments only in the presence and under the supervision of the teacher. The teacher is to point out to the students that the provided materials may be used only according to the respective instructions.
For these experiments, watch out for the following potential dangers and make your students aware of them:

- Working with a flame and hot alkaline solution can result in burns or fires. Before a lighter is used for the first time, the teacher must check that it is working properly and, above all, adjust the flame height.
- Make sure that no damage can occur to water-sensitive materials and apparatus.
- Do not allow students to play with fire. The aluminum bowl can be used as a fireproof base.
- Students must wear safety goggles for this experiment. Point out to the students the first aid measures they must follow if sodium carbonate splashes into their eyes or onto their skin.

According to the international hazardous substance labeling (GHS): “Caution”

Hazard Statements: H319
Precautionary Statements: P260, P305, P351, P338

4.4 Apparatus and materials

Required materials that are not supplied:

- Tap water
- One lighter per student group (if possible, a gas igniter) or matches

Supplied:

The apparatus and materials supplied are sufficient to allow **eight** groups of students to conduct the experiments simultaneously. Safety-relevant materials and apparatus must be tested for proper functioning before being handed out to the students.
The following materials included in the kit are needed for **one** group of students:

Material	Quantity
Boiling chip, bottle	1x
Bowl, aluminum	1x
Cooking oil (“vegetable oil”), bottle	1x
Dishwashing detergent, bottle	1x
pH test strip, package	1x for entire class
Plant clip (as test tube holder)	1x
Plastic cup (clear), 500 ml	1x
Safety goggles	1x*
Table salt, box	1x for entire class
Tea light	1x
Teaspoon	1x
Test tube, glass, 13 cm	2x
Test tube clamp, wooden	1x
Test tube stopper	1x
Washing soda (sodium carbonate), package	1x for entire class

*A total of 16 pairs of safety goggles are supplied for all students in all groups. If more than 16 students participate in the experiments, additional pairs may need to be provided by the school.

Fig. 4: Supplied apparatus and materials for one group of students.

4.5 Cleanup, disposal, and recycling

All apparatus and nearly all materials from the kit can be reused. Therefore, after the students have completed the respective experiment, they should put the apparatus and materials back in the appropriate boxes and return them to the kit. This practice will ensure that you and your colleagues will find everything again quickly the next time the kit is used.
Apparatus that become dirty during the experiment, such as cups, bowls, spoons, and test tubes, should be cleaned before being returned to the kit. We recommend that you have the students do this immediately after they have completed the experiment.
Also make sure that the apparatus are in working order for the next time. For example, recharge used accumulators immediately (It makes sense to charge the accumulators even if they will not be used for an extended period.).
Materials that cannot be reused, such as used pH test strips and filter paper, should be disposed of properly.
The waste that accumulates during this experiment can be disposed of in the regular trash or poured down the sink.