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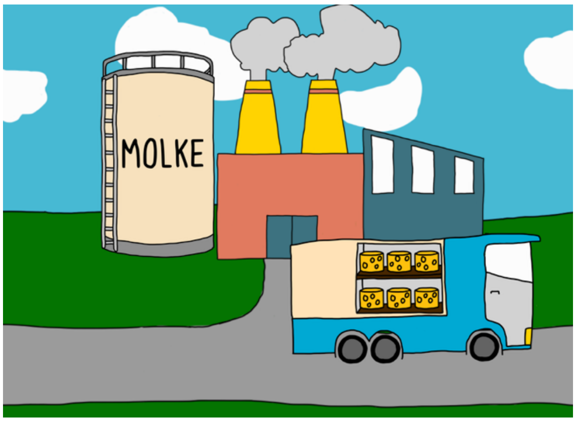
Whey: What‘s left over of milk



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The overall project is divided into three parts.

This document is **Part 2 of 3**.

Part 1 of 3: Fungi and Their Mycelium
Part 3 of 3: Mycelium and Whey for the Production of Sustainable Food

The content is being developed under the direction of Dr. Paul Bubenheim.

Table of contents accompanying documents

Bundesministerium für Landwirtschaft, Ernährung und Heimat
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Knowledgebox I: Cow's milk

Although cow's milk originally is meant to feed calves, many human cultures consume it lifelong, processing it into various food.

What is Cow's milk made of?

Indeed, Milk consists of 80% water, but the remaining 20% contain numerous essential nutrients for the human body. Those who want to know which nutrients are found in particular foods should look up the nutritional table on the package.

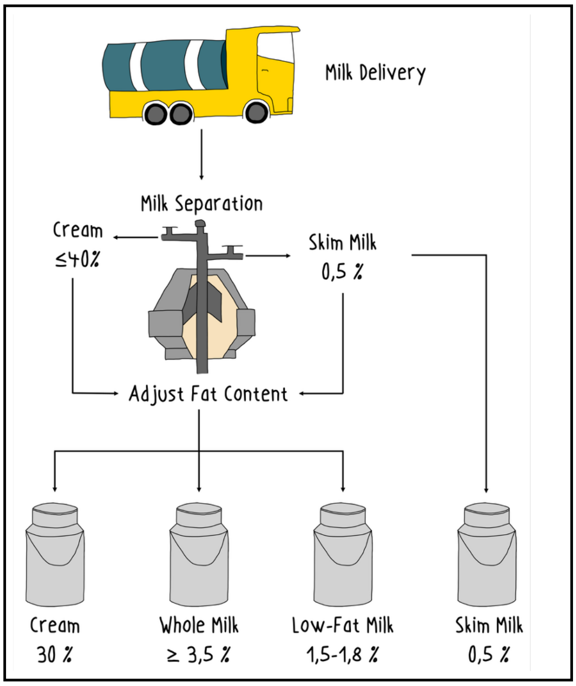
For most processed foods, the inclusion of a nutrient table is required by the EU. It shows the energy provided by particular foods and the nutrients it contains. These nutrients are **energy (calories), fat, carbohydrates, albumen (protein),** and **salt**. Generally, declarations are made per 100 ml or 100 g. 100 ml of whole milk contains approximately 68 calories, 4 g of fat, 4.8 g of carbohydrates, 3.3 g of protein, and 0.11 g of salt (see picture 1). Alongside these contents, milk has a lot of **minerals** and **vitamins**. Since our body is unable to produce some of these substances, we need to take them in through food. At first glance, these terms simply describe groups of nutrients — but what specific substances does milk actually contain? Carbohydrates in milk almost entirely consist of **lactose**, a sugar, the so-called milk sugar. Lactose is composed of two sugars, glucose and galactose, which are connected. Proteins play an important part in milk as well. The major part of proteins in milk is **casein**. This is particularly important for the manufacturing of cheese. Roundabout 80% of milk protein is casein, the remaining 20% are whey-protein. Another vital ingredient is fat. Milkfat is to be found in very small fat pellets. Altogether, milkfat contains more than 400 different fatty acids. Drinking milk is available with varying fat rates – from skimmed milk with 0,5 % fat up to whole milk with more than 3,5 % fat. But: how do all the different fat rates develop?

Average nutritional values per 100 ml:	
Energy	288 kJ (68 kcal)
Fat	4.0 g
of which saturates	2.6 g
Carbohydrate	4.8 g
of which sugars	4.8 g
Protein	3.3 g
Salt	0.11 g

Picture 1: Nutrition table of whole milk.

How do different fat contents develop in drinking milk?

Milk that comes directly from the cow (raw milk) has a naturally varying fat content. In the supermarket, however, milk must always have a certain fat content. What to do? The milk is divided into a **fat-reduced** and a **high-fat part** (see picture 2). Normally, the fat would rise to the surface on its own if you let the milk sit for a while. To accelerate the process, a fast-rotating machine (centrifuge) is used, which separates milk effectively into **skimmed milk**, containing almost no fat, and high-fat **cream.** After this, skimmed milk and cream are mixed again, to produce milk



Picture 2: How milk's fat content is adjusted.

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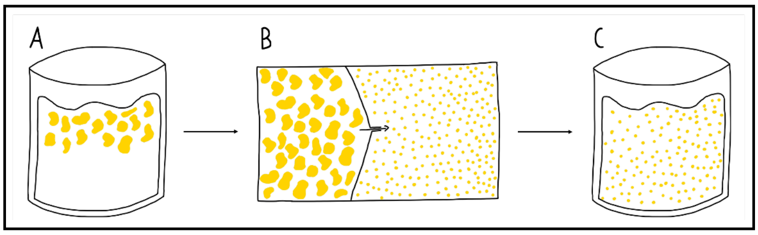
with different fat contents; for instance, whole milk, fat-reduced milk or cream. Without further processing, the fat would eventually rise to the surface again. But why doesn't that happen with milk from the supermarket?

How is fat separation on the surface of the milk prevented?

Among other things, milk consists of **fat and water**. Originally, the said substances don't mingle quite well. To avoid fat settling on the surface, milk is **homogenized**. The fat globules are forced through a small opening, which significantly reduces their size (picture 3). This ensures they are evenly distributed in the milk. You get a solid compound called an **emulsion**.

By homogenizing, fat stays evenly distributed, and milk will become more creamy. Milk is used for different products, such as yoghurt, cooking cream, or condensed milk. The milk used for cheese or butter is not going to be homogenized; for these products, you need the fat pellets to embed together. Apart from homogenizing, it is important for a dairy plant to make milk last longer.



Picture 3: Homogenization of milk. A: Fat settles on the surface of non-homogenized milk. B: Fat particles of milk are crushed. C: Crushed fat pellets are evenly distributed in milk.

How is milk preserved?

Raw milk can be kept for up to four days. To make it keepable, contained germs have to be reduced or killed (Table 1). One of the oldest methods is pasteurization. Hereby, milk is shortly heated. This process kills many germs while preserving most of the nutrients. Yet some heat-resistant germs may survive. Therefore, pasteurized milk has to be kept in the refrigerator, where it can be kept for up to ten days. In supermarkets, this kind of milk is called „fresh" milk. Alternatively, a procedure named **microfiltration** is used. Skimmed milk is led through a high-efficiency filter to extract bacteria. After this, it is mingled with heated cream to reach the requested fat content. Milk treated in this way is called **ESL-milk** (Extended Shelf Life) and is labeled as "longer-lasting" milk in supermarkets. It will stay fresh for up to 3 weeks if refrigerated. Milk can be made to last even longer using **ultra-high-temperature** (UHT) processing. In this process, the milk is heated to 135–150 °C for a few seconds. This allows it to be stored unrefrigerated for up to three months. It can now be labelled as **H-milk**. (the **h** for haltbar = durable).

Table 1: Methods of preserving milk.

Name of product	Method	Kind of treatment	Durability
Raw milk	-	-	4 days
Fresh milk	Pasteurization	72-75°C for 15-30 sec.	10 days
ESL-Milch	Skimmed milk: Microfiltration	Filtration through smallest voids	3 weeks
	Cream: Heating	104-108°C for 1-4 sec.	
H-Milch	ultra-high-temperature heating	135-150°C for 1-4 sec.	3 months

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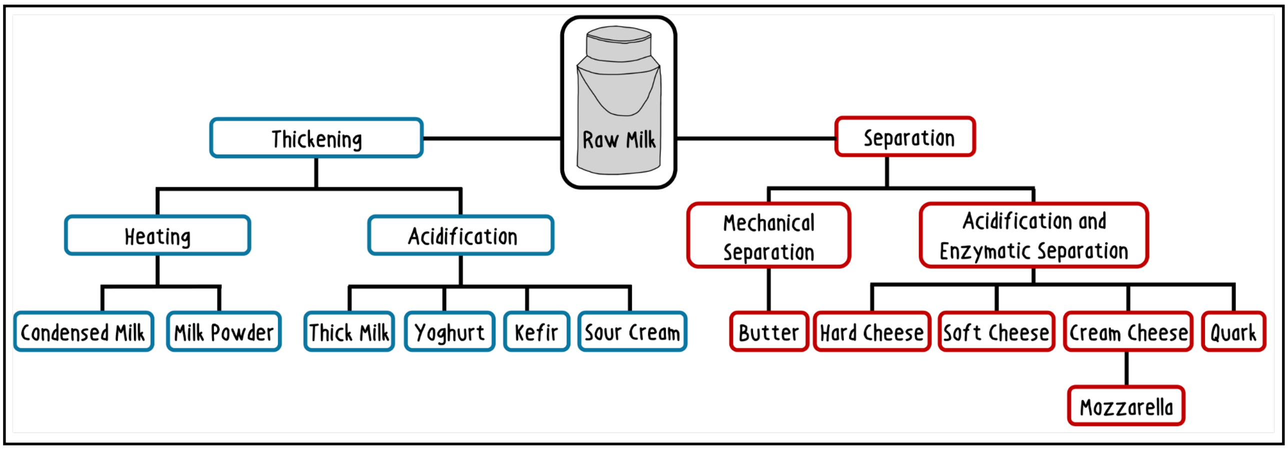
Knowledgebox II: Cow's milk products

In addition to drinking milk as a beverage, it can be used to make a wide variety of products—from butter and yogurt to cheese. It's hard to imagine supermarket shelves without them. But how are these products actually made?

How are dairy products manufactured?

The special thing about milk is that you can make many different foods from it without using many additional ingredients. What matters is how the components of milk are altered, combined, or separated. As a result, different tastes and consistencies emerge (picture 4). One possibility is to separate fat and liquid **mechanically** by stirring. If you stir cream for a long time, the fat particles will glue together, and the result is butter. What remains is buttermilk, the liquid part. Stirring breaks down the protective layer surrounding the fat droplets, causing the fat to clump together. Another method is to **thicken** milk by reducing water through heating. This is the way how condensed milk develops. By reducing almost the whole amount of water, you get powdered milk. To produce not just a thickened dairy product but also to alter its flavor, the milk can be **soured**. Hereby, **lactic acid bacteria** metabolize lactose into acid. The milk proteins gradually



Picture 4: Manufacturing different milk products.

accumulate, causing the milk to thicken slowly—at first, without the solid and liquid components separating clearly. Thus, products like yoghurt, thick milk, kefir, or sour cream develop. However, for many types of cheese, the milk must be thickened to the point where the proteins clump together completely and separate from the liquid part of the milk. This process is called **coagulation**. The solid part is the cheese **curd**, the liquid one the **whey**. If you let soured milk sit long enough or add large amounts of acid, this type of coagulation occurs. However, acid coagulation takes a long time, and both the curds and the whey taste sour. Therefore, **rennet** (an enzyme mix) is used; it separates milk quickly and gives cheese a mild taste.

The next paragraph tells you in detail how this works. However, for the rennet to work effectively, the milk still needs to be slightly acidified by lactic acid bacteria. Today, for most types of cheese, the milk is first acidified and then curdled using rennet. But why do the proteins clump together during cheese production?

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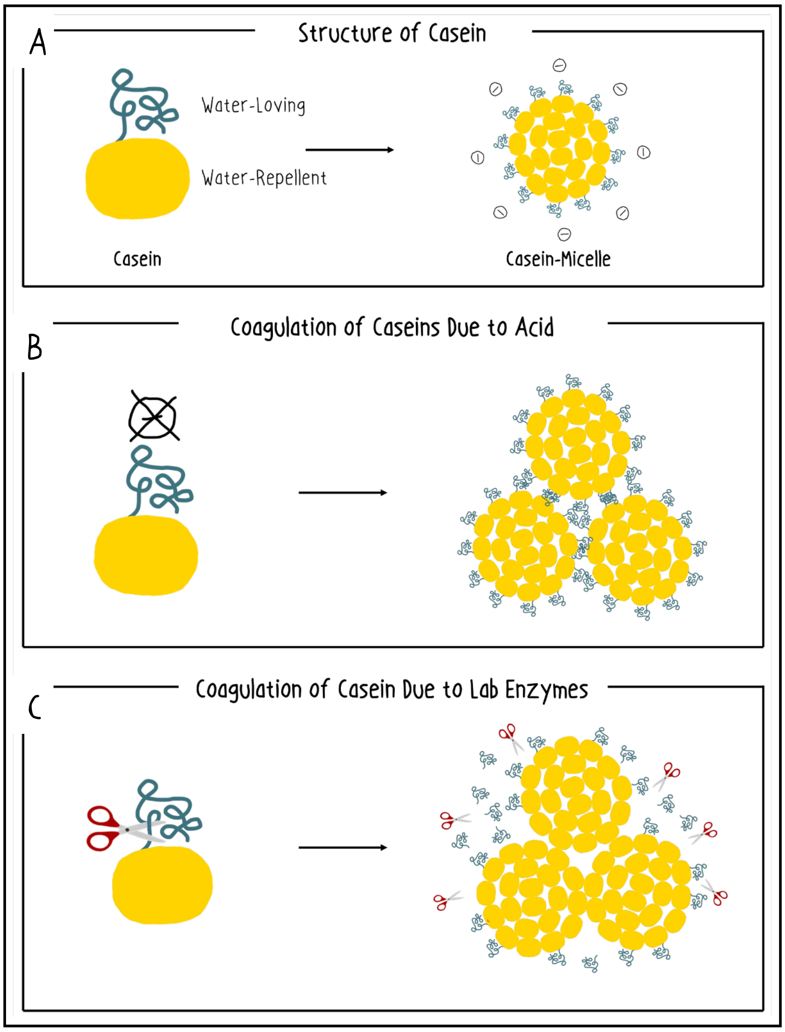
What role does the protein casein play in the production of dairy products?

To understand why proteins, clump together during the production of many dairy products, we need to take a closer look at the milk protein casein. It is not a single protein, but a group of several proteins composed of different subunits. These subunits have different properties—one subunit readily binds to water, while the other avoids binding (Picture 5A). When these parts come together in an aqueous environment like milk, they arrange themselves so that the water-loving (hydrophilic) parts face outward and the water-repelling (hydrophobic) parts face inward. This creates tiny particles called **micelles** (Picture 5A). The outer shell of these micelles is negatively charged, so the particles repel each other—similar to the same poles of a magnet. This keeps them dispersed in the milk, and the milk remains liquid and uniform (Picture 5A). When conditions change—for example, when **lactic acid bacteria** make the milk sour—the shell loses its negative charge. The micelles then no longer repel each other, stick together, and the milk becomes thicker (Picture 5B). This is how yogurt is formed, which is slightly tart and creamy. In cheese production, the casein micelles are supposed to clump together so strongly that they separate from the liquid part of the milk. **Lactic acid bacteria** and the enzyme **rennet** are used for this purpose. **Enzymes** are proteins that have different functions depending on their type. One group is called **proteases**. These enzymes can break down proteins by cleaving the bonds between the individual building blocks of the proteins. The enzymes found in rennet belong to this group. They recognize specific structures in casein and cleave it at specific sites (Picture 5C). In the process, a hydrophilic part of the protein is removed, which normally forms a protective shell on the surface of the micelles. This shell ensures that the micelles repel each other and maintain their distance. When it disappears, the micelles can more easily come into contact and bind together. They come together to form large clumps (Picture 5C). In the process, the total surface area of the proteins decreases. Since water is primarily bound to the surface of the proteins, less water can now be retained. As a result, some



Picture 5: Casein clumps together during cheese production. A: Due to the hydrophilic and hydrophobic parts of casein, it assembles into micelles. B: When the milk is acidified, casein loses its charge and the micelles come together. C: Rennin enzymes cleave off the hydrophilic part of casein, causing the micelles to clump together.

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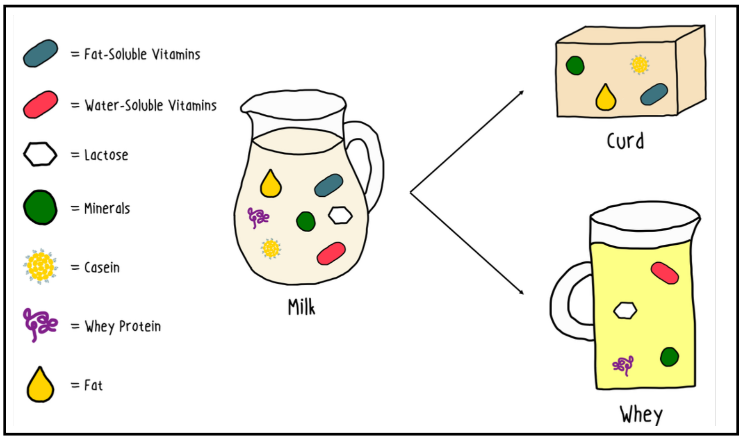
of the water separates from the protein clumps. This creates solid clumps, the curd, and the liquid, known as whey, which is separated off. But how does the curd turn into cheese?

How does curd turn into cheese?

After coagulation, the curds are cut into small pieces. The smaller the pieces, the more whey can drain out, and the firmer the cheese will be later on. The curds are then pressed to remove additional liquid. In the production of hard cheese, this is followed by a salt bath, which draws out even more water from the cheese and gives it its characteristic flavor. The cheese is then stored to mature. During this time, bacteria transform the cheese, and in some varieties, mold is added. Different lactic acid bacteria, temperatures, and the addition of other bacteria or mold result in many different types of cheese with distinct flavors.

Which components of the milk are found in cheese and which in whey?

When milk is separated into curds and whey, the components are distributed unevenly across two phases (Picture 6): a solid phase and a liquid phase. The solid phase, the curd, forms when casein cross-links to create a sort of network. Large portions of the milk fat remain trapped in this network, as do some of the minerals. The fat-soluble vitamins are also largely enclosed in the curd, as they are bound to the milk fat. How much water remains trapped depends on the subsequent processing—this results in different types of cheese. Whey, on the other hand, is the liquid portion that drains from this network. It consists mainly of water and primarily contains the substances that cannot be incorporated into the solid structure. These include, in particular, milk sugar (lactose) as well as whey proteins, which are too small and too soluble to be retained in the curd. Water-soluble vitamins and some of the minerals also pass into the liquid. Thus, the same milk yields two very different products: a firm, textured cheese and a thin, yet nutrient-rich whey.



Picture 6: Distribution of the main components of milk between curds and whey.

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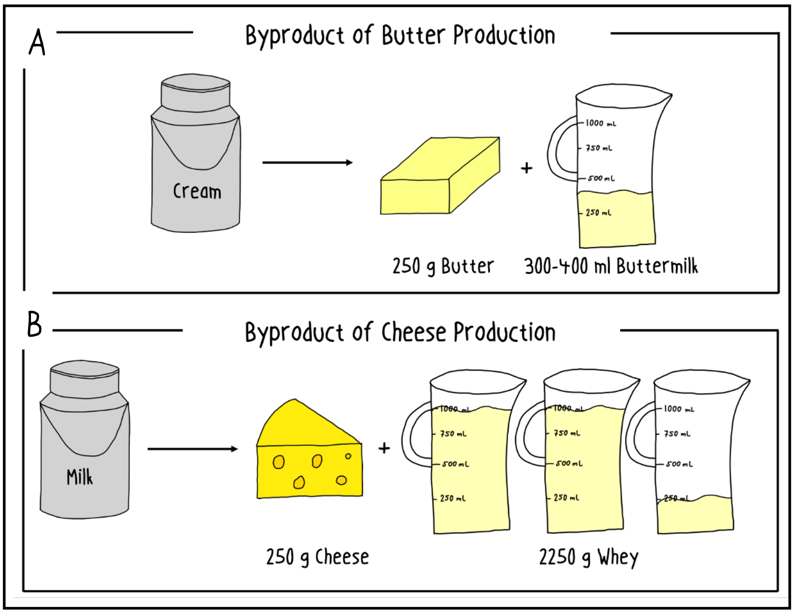
Knowledgebox III: How to process whey and milk leftovers

The production of cow's milk products often generates large quantities of byproducts. These include, for example, buttermilk from butter production and whey from cheese production. For a long time, these byproducts were primarily used as animal feed and rarely utilized in food products or for other purposes. But is that still the case today?

What are the typical byproducts produced in a dairy, and what are they made of?

When the liquid separates from the solid part of milk, one might think the liquid consists only of water. However, since buttermilk and whey still contain many valuable components of milk, efforts are made to put them to good use. But how much whey and buttermilk are actually produced? The amount of buttermilk produced during butter production depends on the fat content of the cream used. If whipping cream with about 30% fat is used, the production of approximately 250 g of butter yields about 300 to 400 ml of buttermilk (Picture 7A). The amount of whey produced cannot be generalized either. It depends primarily on the type of cheese being made. However, the production of 250 g of hard cheese yields about 2.25 l of whey (Picture 7B). This example shows that whey, in particular, is produced in large quantities. That is why there are already many clever ideas for putting it to good use.



Picture 7: Byproducts of the production of A: butter und B: hard cheese.

How are these byproducts currently being reused?

The simplest way to use whey and buttermilk is to sell them as **beverages** (Picture 8). To make their sour taste more appealing, they are often mixed with fruit. However, there is a particular problem with whey: the large quantities produced during cheese manufacturing cannot be consumed solely as a beverage. A popular method is therefore to process the whey into whey powder (Picture 8). Whey consists of about 94% water and around 6% solids, such as protein, lactose, and minerals. During the production of **whey powder**, the water is removed, leaving only the solid components. Whey powder has a major advantage: it has a much longer shelf life than fresh whey and requires less storage space. It can also be used as a substitute for milk in numerous foods. The powder can be mixed with varying amounts of water. This prevents too much liquid from diluting the product. But whey offers even more possibilities. Before it is dried, experts often first extract specific valuable substances from it. These substances can then be used specifically for certain products and often sold at a higher price. A particularly well-known example is **whey protein** (Picture 8). It is especially popular in the **fitness industry**. The liquid that remains after the whey proteins are removed is called **permeate** (Picture 8). But the permeate is also useful. Lactose can be extracted from it

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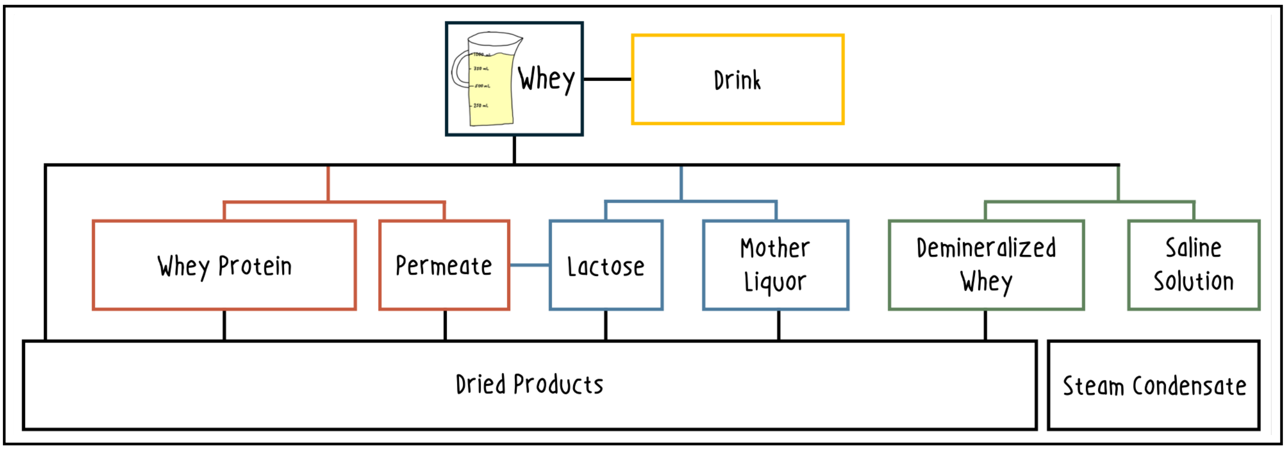
(Picture 8). This is used, for example, in food products or baby formula. After the lactose has been removed, a liquid remains. This is called **mother liquor** (Picture 8). Whey can also be used in another way. Some of its minerals can be removed. This whey is then called **demineralized whey** (Picture 8). It can be used, for example, as food for sick people. A **saline solution** is produced as a byproduct (Picture 8). However, there are few other uses for this salt solution. Many products



Picture 8: Products that can be produced from whey.

created during whey processing are subsequently dried to extend their shelf life. This process produces various powders. Drying also produces something else: **vapor condensate** (Picture 8). This is water that forms during evaporation and later condenses back into a liquid. Milk residues are still present in this water. Therefore, it cannot simply be drained away. It must first be purified. But are the vapor condensate and the salt solution the only byproducts that remain?

What's left over from whey?

Even though there are many ways to use whey today, there are still leftovers. This is because not all products are equally popular. By-products, in particular, are often in less demand than the main products. Whey proteins are especially popular in the fitness industry. Permeate, on the other hand, is used by only a few people. That's why there's often more permeate left over than is sold. Even when lactose is extracted from the permeate, the remaining whey is not fully utilized. Furthermore, not all dairies produce all products. Consequently, not all opportunities for further processing whey are always exploited. This demonstrates that while whey is a valuable raw material with versatile applications, components still remain unused. New uses must be found for these components in order to utilize as many substances as possible completely and in their most valuable form. But does it actually make sense to use all substances to the very end? This is precisely what the consideration of sustainability in economic production processes addresses.

How can whey be turned into a sustainable food product?

From 2025 to 2027, Infinite Roots and the Institute for Technical Biocatalysis will be conducting research on this—they're using whey as a nutrient source for fungal mycelium and developing a new food product—available on Kniffelix.de starting in 2027!

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Knowledgebox IV: Sustainability in dairy industry

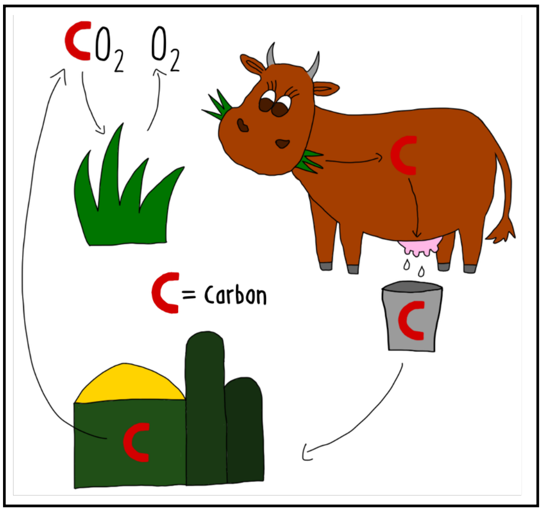
To understand why milk byproducts are valuable, you first need to know how much of a burden the dairy industry places on the environment.

What impact do milk production and dairies have on the environment?

Milk production consumes a lot of resources. Cows need feed, water, pasture, housing, and care. Growing feed crops like corn or soybeans takes up a lot of land, often in monocultures, which depletes the soil, reduces biodiversity, and requires more fertilizer and pesticides. Cows themselves produce greenhouse gases such as methane (CH_4) and nitrous oxide (N_2O). Dairy processing also consumes a great deal of energy: milk must be cooled, heated, separated, and pumped. Processes involving high temperatures, such as the preservation of milk, require a particularly large amount of energy. In addition, a significant amount of wastewater is generated, containing milk residues and traces of cleaning agents, which must be treated at great expense before it is released into the environment. The production of milk alone places a heavy burden on the environment—and its further processing into dairy products adds to this burden. This makes it all the more important that as much of the milk as possible is utilized.

How are milk byproducts processed today?

Milk byproducts are often used in biogas plants. There, microorganisms break down the materials and produce biogas, which consists mainly of methane (CH_4) and carbon dioxide (CO_2). The methane is burned to generate electricity and heat—either directly for the dairy or for the power grid. What makes biogas special is that the energy comes from substances that previously absorbed CO_2 from the air. In the case of milk, this happens through the cows' feed. The CO_2 that the plants absorbed while growing is thus stored in the milk and the byproducts (Picture 9). When the methane is burned, this CO_2 is released back into the air. Unlike with coal or oil, biogas does not release old CO_2 that has been stored in the ground for centuries. Biogas is therefore more climate-friendly. Nevertheless, CO_2 enters the atmosphere, and too much of it harms the environment. Furthermore, the valuable nutrients in the milk residues are lost through decomposition. Proteins, sugars, and minerals consist of many different chemical building blocks and therefore have high material value. CO_2 and methane, on the other hand, are simple molecules—they provide energy, but they cannot be used to produce food or valuable materials. A truly sustainable approach would be not to break down these by-products, but to continue using them. Research and creative ideas can help turn today's "waste" into tomorrow's valuable raw materials. At the same time, the dairy industry can become more sustainable by making each stage of production more energy-efficient and environmentally friendly.



Picture 9: Circle of carbon dioxide in milk

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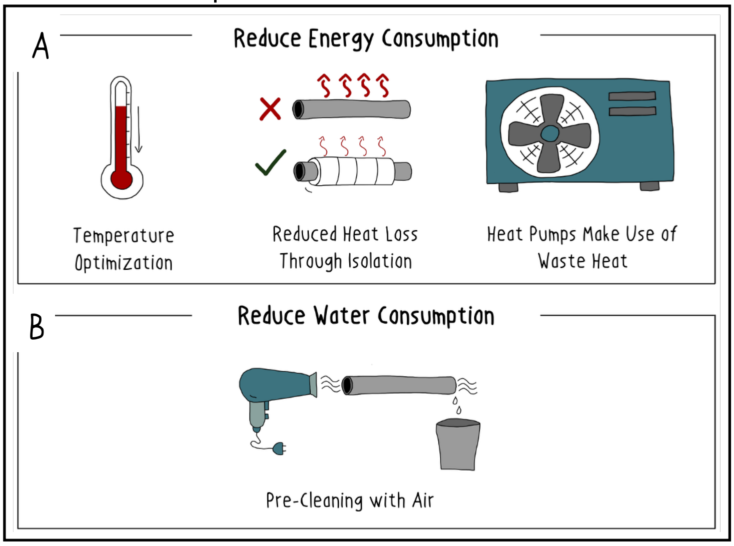
How can a dairy make its production processes more sustainable?

One key factor is reducing **energy** and **water** consumption. When it comes to energy consumption, it's worth reviewing process temperatures. Many steps in cheese production have been carried out at specific temperatures for generations. Today we know that in many cases, slightly lower temperatures are sufficient—and this saves a lot of energy (Picture 10A). Since cooling also requires energy, these temperatures should also be reevaluated. Another important measure is to prevent heat loss. When **heat** is **lost**, it must be replenished, which requires additional energy. Well-**insulated pipes, boilers, and machines** help reduce such losses (Picture 10A). Additionally, waste heat can be utilized. Waste heat is heat generated, for example, during the operation of machines. Since this waste heat is often not hot enough for many industrial processes, heat pumps can raise it to a higher temperature and thereby make it usable (Picture 10A). If heat pumps are powered by electricity from renewable sources, this is particularly environmentally friendly. A great deal of energy can be saved when **heating** and **cooling** are considered together. In a dairy, for example, cold milk is first heated, and after cheese production, the warm whey is cooled again. Energy is required for both processes. However, a so-called **heat exchanger** can be used to combine the two processes: Here, the whey transfers its heat to the milk and cools itself in the process, thereby saving both heating and cooling energy. There are also opportunities to **save water**, for example through water treatment and reuse or through more efficient cleaning processes. If the excess milk in the pipes is first removed with a strong airstream, less milk remains in the pipes, and less water is needed for cleaning (Picture 10B). However, implementing such changes is often complex. Dairies have grown over many years, and the individual steps are closely interlinked. Even small changes can have a major impact on the overall process and costs, as well as cause complications. This can quickly disrupt the production flow.



Picture 10: Methods for improvement of environmental balance of dairies. A: Measures for reduction of energy consumption. B: Measures for reduction of water consumption.

Sustainable change requires long-term planning and can only be achieved through a combination of measures that address manufacturing processes as well as the use of water and heat. The goal is to utilize raw materials as fully as possible and transform them into valuable products, thereby preventing waste or low-quality byproducts and conserving the environment and resources.

How is whey turned into a sustainable food product?

This is exactly what Infinite Roots and the Institute for Technical Biocatalysis will be researching from 2025 to 2027—they are using whey as a nutrient for fungal mycelium, which is then processed into a new food product. Starting in 2027, you can explore this exciting topic on Kniffelix.de.

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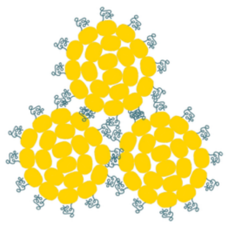
Experiment: Curd

Experiment introduction

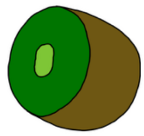
Question:
How does the coagulation of milk proteins differ when caused by acidic pH value versus enzymes?

Coagulation by acidic pH-Value

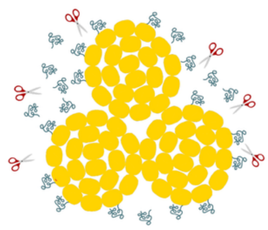
Vinegar essence

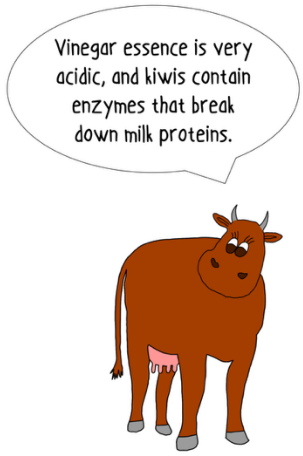


Coagulation by proteases (enzyme)



Kiwis

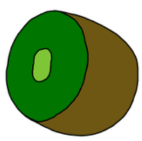




Materials

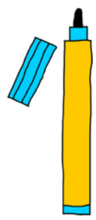
Whole milk

Kiwis



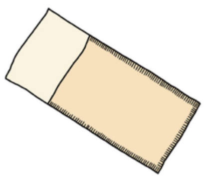
Vinegar essence

Waterproof marker

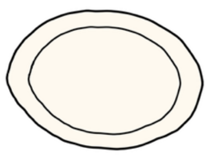


Tea spoon

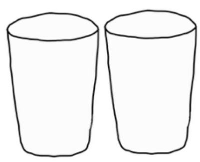
Tea bag



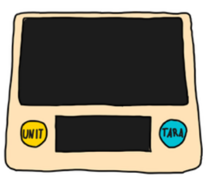
Plate



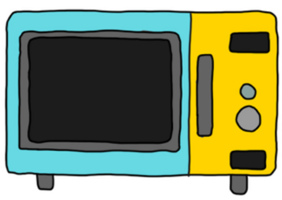
Glasses



Scale



Microwave



Hand Blender

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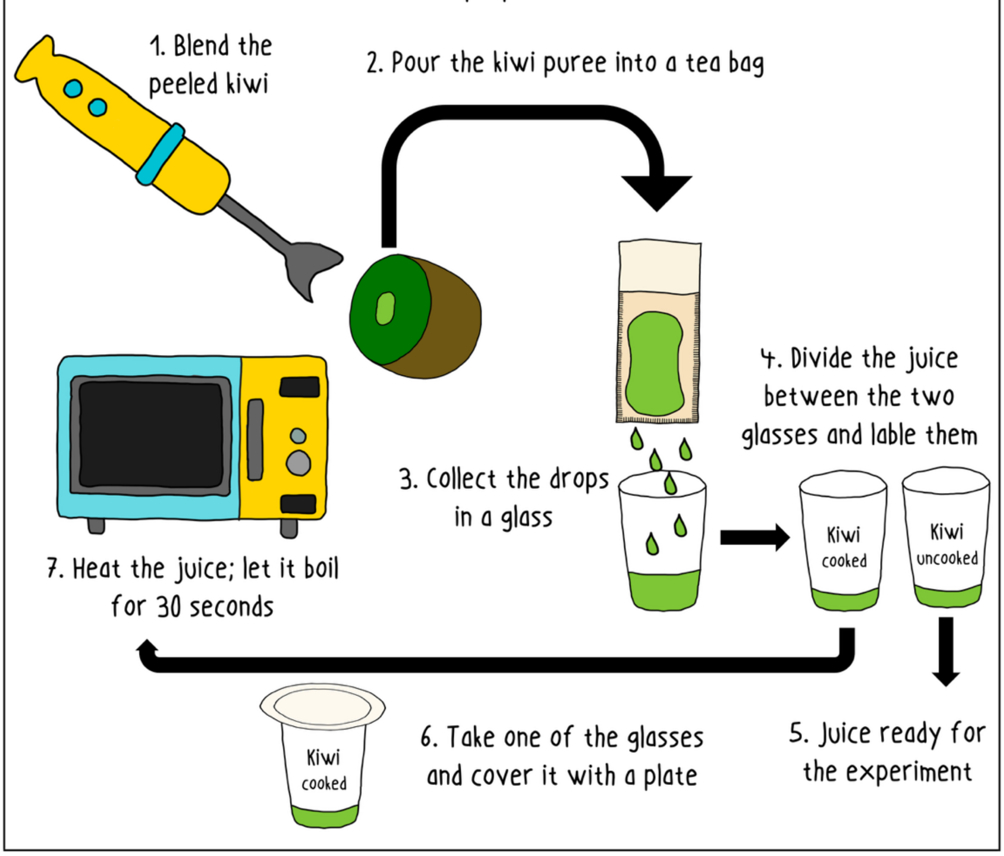
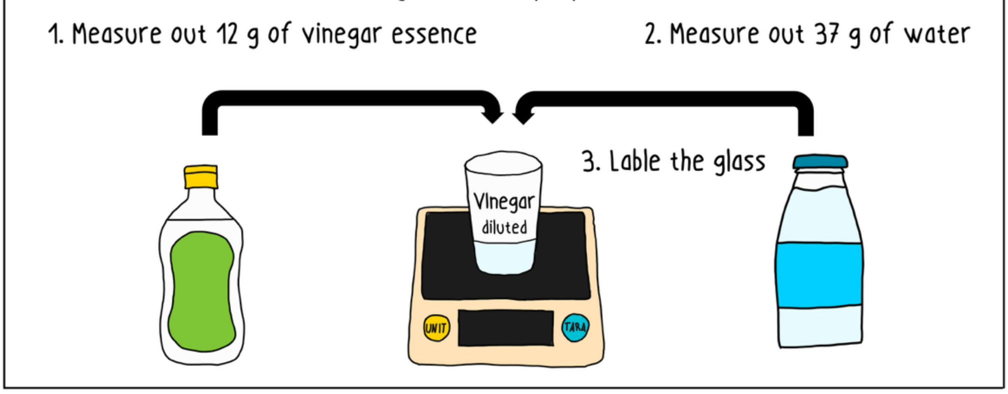
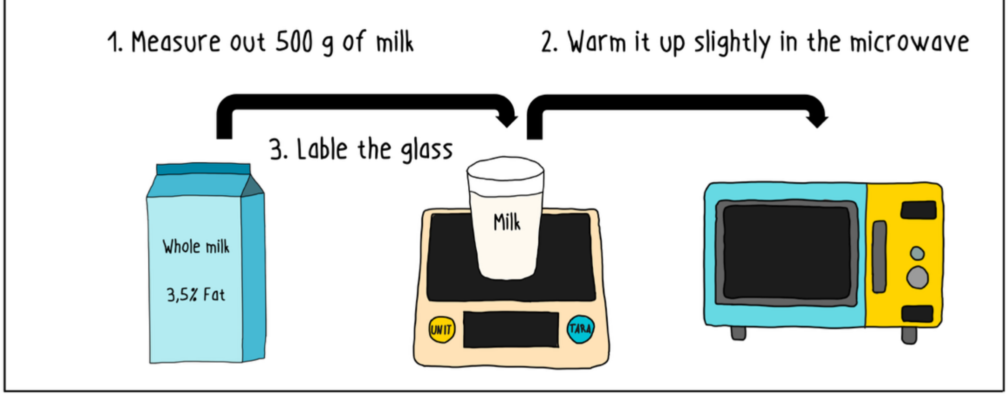
Kiwi preparation



Vinegar essence preparation



Milch vorbereiten



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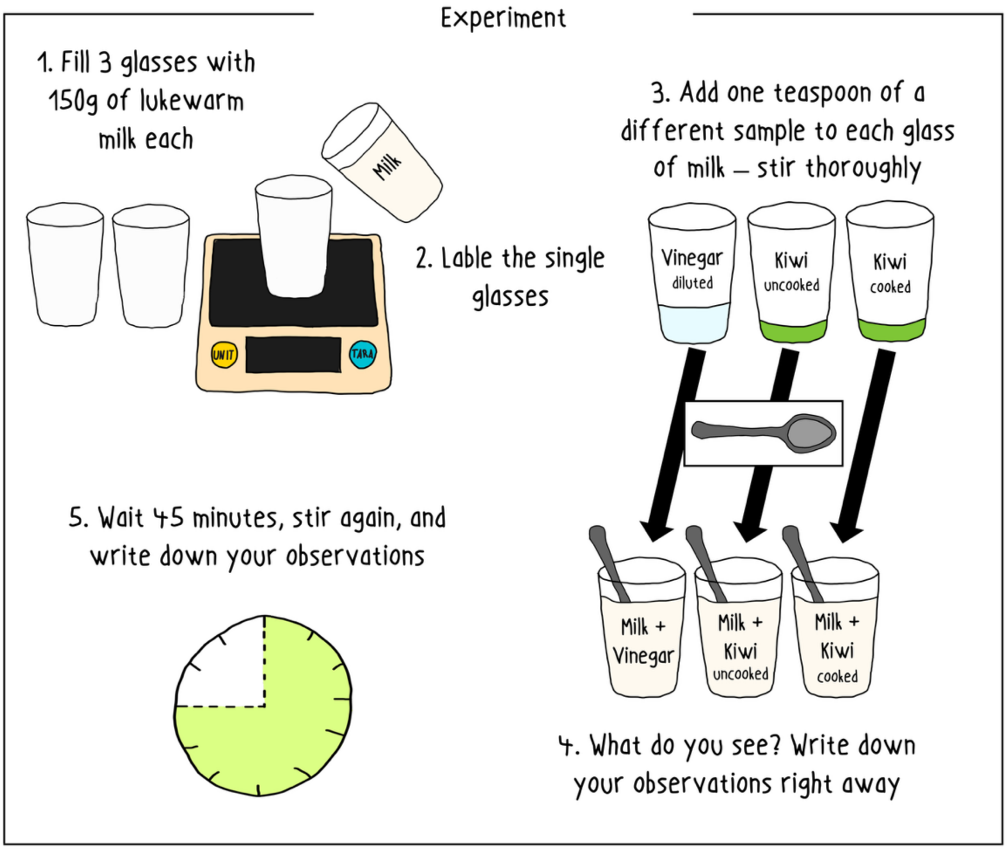

Go to the next page to see our observations

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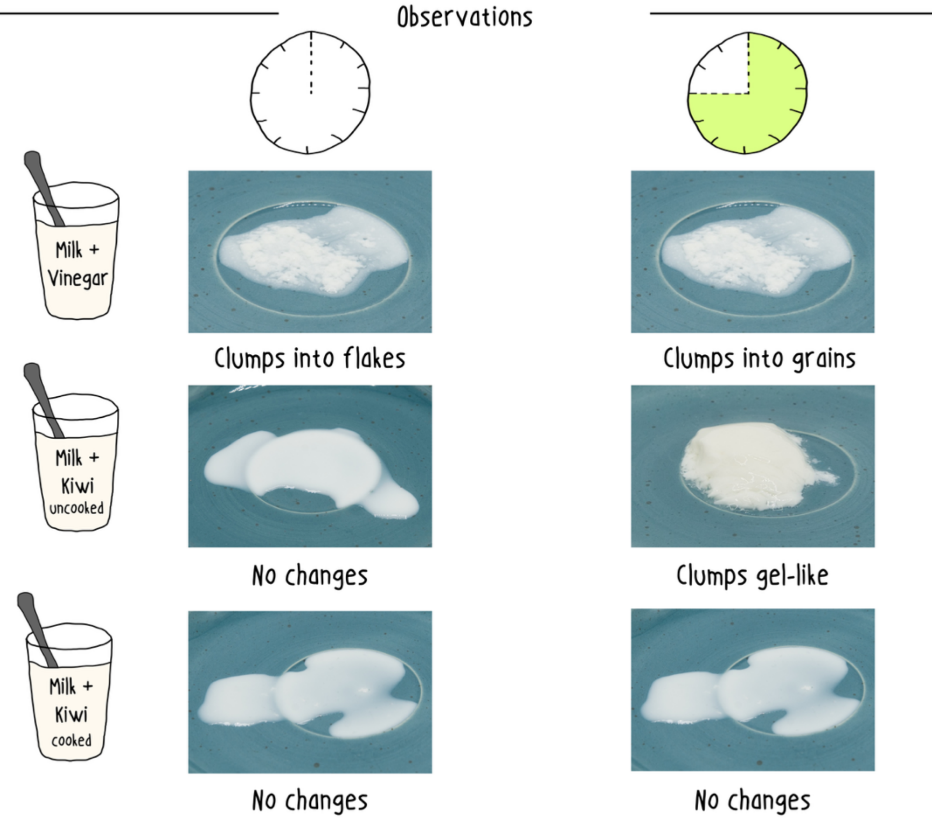
Observations



Analysis

Key observation: Acids and enzymes cause the milk proteins to clump together

Differences:

1. **Observation: Vinegar** causes the milk to curdle **immediately**, forming **flakes.**
   Explanation: Vinegar immediately lowers the pH, causing the proteins to clump together without forming a network. This results in flaky clumps.

2. **Observation: Kiwi enzymes** cause the milk proteins to clump **slowly**, resulting in a **gel-like** consistency
   Explanation: The enzyme actinidin in the kiwi gradually breaks down the milk proteins. They slowly adhere to one another and form a network before clumping together. This results in a gelatinous mass.

3. **Observation: Heated kiwi** shows **no change** in the milk
   Explanation: Heating destroys the actinidin. Since the milk does not change afterward, the acid in the kiwi alone is not strong enough to cause the milk proteins to clump together. The change is therefore caused by the enzymes.

Conclusion: The rate of protein change determines the structure of the curd

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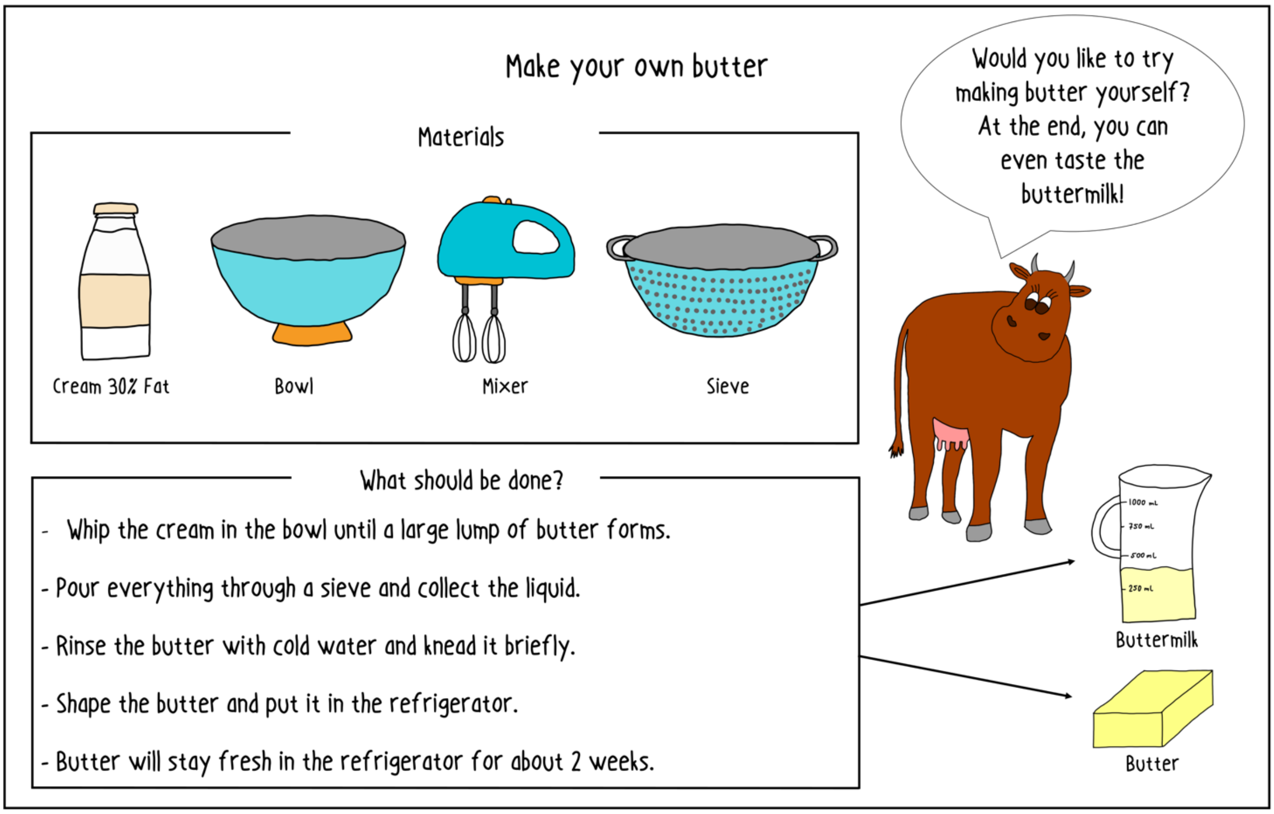
Experiment: Make your own butter



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