Chapter II:
2. Use of Microorganisms in Food Fermentations

2.1. Bread

The four ingredients used in bread-making are flour, water, salt, and yeast or sourdough. For 100 kg of flour, the ingredient proportions are 62 liters of water, 2 kg of yeast, and slightly less than 2 kg of salt. These proportions may vary depending on the type of bread desired. Traditional bread, for example, is often more hydrated, with between 65 and 70 liters of water per 100 kg of flour.

2.1.1. Characteristics of the Ingredients:

- **2.1.1.1. Flour**: It is the essential element. Firstly, mixing flour with water allows the formation of dough. Secondly, the quality, characteristics, and properties of flour have a direct impact on bread. Flour determines the bread’s taste, color, and texture. Bakers sometimes use carefully formulated ready-made blends from millers, called "mixes," designed to produce specific types of bread (such as multigrain bread). Each type of bread requires a specific type of flour.
- **2.1.1.2. Water**: By moistening starch and gluten particles, water enables the formation of an elastic gluten network that binds all other flour components together. Without water, the dough would not be able to retain carbon dioxide during fermentation. Water plays a crucial role in the dough’s plasticity and helps dissolve the salt.
- **2.1.1.3. Salt**: It plays a very important role in the "chemistry of bread." Salt enhances the dough’s consistency; when incorporated at the beginning of kneading, it slows down oxidation, thus preventing loss of flavor. It contributes to the bread’s taste, enhances the crust’s color, and affects its preservation.
- **2.1.1.4. Yeast**: Baker’s yeast is a microscopic fungus of natural origin, *Saccharomyces cerevisiae*. One gram of fresh yeast contains 9 to 10 billion cells. Yeast can survive with or without air, and its preferred food is simple sugar, glucose. This biological agent allows the dough to rise by transforming sugars into carbon dioxide and alcohols, which evaporate during baking. Without yeast, bread would be flat.

The storage conditions of yeast are very important: if stored at too low or, more critically, too high a temperature, it loses its fermentation power.

2.1.2. The Eight Stages of Breadmaking

Artisan bakers oversee the entire bread-making process, from production to final sale to the consumer. Each baker has their own technique, but the main stages of breadmaking remain the same.

2.1.2.1. Kneading:

The baker mixes all the dough ingredients. The gluten in the flour absorbs the water poured into the mixing bowl. The dough becomes elastic and traps air. If a mechanical mixer is used, it facilitates this crucial stage, but the baker must remain vigilant and closely monitor the process. Different kneading methods are possible. Slow-speed kneading produces a less developed bread, while intensified kneading, which is longer and has a higher rotation speed, results in very well-developed bread with a thin crust. Improved kneading, a balance between these two methods, lasts 10 to 15 minutes and includes rest periods of 2 to 3 minutes. For proper fermentation, the baker must ensure the dough reaches a final temperature of 23 to 25°C; if necessary, the water is cooled.

2.1.2.2. First Fermentation (Pointage):

Before dividing the dough, the baker lets it rest in the mixing bowl. This step, known as pointage, is crucial for developing the bread's aroma. The production of carbon dioxide begins, causing the dough to rise and its qualities to improve, making it more elastic. For traditional bread, this step takes longer. The baker's experience helps determine when the dough is ready, as every dough reacts differently based on daily variables (such as air humidity). The baker touches the dough with their fingertips and decides if it's time to move to the next stage.

Note: No machine, no matter how advanced, can replace the baker's hand.

2.1.2.3. Scaling (Pesée):

Once the dough has finished fermenting, the baker divides it into dough pieces (pâtons) of the desired weight. A dough divider is often used, though it slightly reduces the dough's flexibility. To compensate for this, the baker allows the dough to rest again.

2.1.2.4. Shaping (Façonnage):

By hand or with a machine, the baker shapes each dough piece to give it the desired bread form. This movement is called la tourne. The shaped loaves are placed on baking nets or in small cloth-lined baskets designed for their shape (long for baguettes, round for country loaves).

2.1.2.5. Proofing (Apprêt):

Proofing is another resting period where the shaped dough undergoes further fermentation. During this stage, the yeast continues feeding on the sugars in the dough, releasing carbon dioxide. Trapped within the gluten structure, the gas causes the dough to rise, tripling its volume. The proofing time depends on temperature, yeast quantity, kneading method, and initial fermentation time, ranging from one to four hours. Some bakers use temperature-controlled proofing chambers.

2.1.2.6. Loading into the Oven (Enfournement):

Meanwhile, the oven is preheated to 250°C. Before placing the dough inside, the baker injects steam to keep the bread from drying out, ensuring a thin, golden crust. Before baking, the baker scores the dough's surface with a blade to allow carbon dioxide to escape.

2.1.2.7. Baking (Cuisson):

Baking time varies depending on the bread's shape and weight, ranging from 12 to 50 minutes. In the early stages of baking, the dough continues to rise as the crumb forms and solidifies. The crust hardens and develops its final color.

2.1.2.8. Unloading from the Oven (Défournement):

The bread is carefully removed from the oven as it is fragile while still hot. It must cool properly to allow moisture and carbon dioxide to escape. During this period, the loaves should be stored in a dry, well-ventilated room.

2.1.3.Role of Microorganisms in Breadmaking

Bread is made from flour, yeast or sourdough, salt, and water. Sourdough is a fermenting dough with an acidic reaction. It contains an acidifying microflora, primarily composed of yeasts (*Saccharomyces cerevisiae*).

The yeast *Saccharomyces cerevisiae* plays a crucial role during the kneading of the bread dough. It produces chemical substances that modify the structure of gluten, thereby altering the dough's texture. The yeast breaks down sugars and converts them into carbon dioxide and alcohol, causing the dough to rise. During baking, the carbon dioxide bubbles remain trapped, while the alcohol evaporates. This process gives the bread its final texture.

2.2. Cheese Making

Cheeses are made from different types of milk (sheep, cow, goat), either pure or mixed. Originally, cheese making was a way to preserve milk, allowing only the solid part of the milk to be stored after transformation. The process involves several steps depending on the characteristics of the final product desired.

2.2.1. What is Lactic Fermentation?

Lactic fermentation is a chemical reaction between bacteria and milk. The bacteria consume lactose (the sugar in milk) and produce lactic acid, which gradually coagulates casein (the milk protein). The fermentation process stops when the mixture is refrigerated

2.2.2. Steps of Cheese Making

2.2.2.1. Coagulation: Lactic ferments or rennet are added to the milk to cause coagulation, dividing it into two parts:

- *Curd* (solid part), used to make cheese.
- *Whey* (liquid part), often used as animal feed.

2.2.2.2. Draining: The curd contracts, and the whey is drained. This separation happens naturally but can be accelerated by stirring, cutting, or heating.

2.2.2.3. Molding: The curd is shaped using perforated molds or by pressing it into cloth-lined wooden or other material frames.

2.2.2.4. Pressing: This step removes excess water by applying weight to the mold. It is optional and depends on the type of cheese being produced.

2.2.2.5. Salting: Salt is either incorporated into the cheese or applied to its surface to control the development of specific microorganisms.

2.2.2.6. Aging: For fresh cheeses, the process ends after draining. However, for other cheeses, the aging process begins, lasting from several days to months. During this period, fermentation transforms the curd into cheese, giving it its flavor and aroma. Cheese is carefully handled (turned, brushed, etc.), and temperature and humidity significantly affect aging, determining the final taste and texture.

This final stage occurs in a cellar, where the development of aging agents is monitored and controlled depending on the type of cheese being produced.

2.2.3. Examples of Microorganisms Used in Cheese Making

- **Camembert** : *Penicillium camembertii*
- **Munster**: Washed with salted water, *Brevibacterium linens*
- **Roquefort**: *Penicillium roqueforti*, requires oxygen for growth
- **Emmental**: Two bacteria work together—one (a lactic bacterium) produces lactate, while the other (a propionic bacterium) consumes it and releases CO_2, forming characteristic holes in the cheese. Microorganisms also play a role on the cheese surface.

Camembert

Emmental

2.2.4. Factors Controlling the Development of Microorganisms

The development of these microorganisms depends on:

- **pH**: Microorganisms are more or less sensitive to acidity. This factor is used as early as the coagulation stage to prevent the growth of pathogens.
- **Salting**: This affects the amount of free water available for microorganisms.
- **Humidity (Hygrometry)**: This refers to the moisture content in the air (the amount of water in gaseous form). In cheese-aging cellars, humidity is usually high (around 80–95%). Lowering humidity limits the growth of bacteria, then yeasts, and finally molds.
- **Oxygenation**: Proper aeration promotes the development of aerobic microorganisms.
- **Temperature**: The temperature in the cellar is maintained between 10 and 12°C to support microbial activity.

2.2.5. Conclusion

In cheese production, all types of microorganisms play a role. They serve various functions, including protecting the cheese from harmful microorganisms by acidifying the environment. During aging, they are essential in transforming the cheese, giving it its distinct taste, texture, and character.

2.3. Milk:

2.3.1. Milk composition

Milk is a complex liquid primarily composed of water and four key constituents, whose proportions vary depending on species and breeds. For example, the average composition of cow's milk is as follows:

- **Water : 87.5%**
- **Carbohydrates : 4.9%**
- **Lipids: 3.6%**
- **Proteins: 3.4%**
- **Minerals: 1%.**

2.3.2. Dairy products

Dairy products can be made from cow's milk, goat's milk, or sheep's milk. There is a wide variety of dairy products, including:

- **Cream**, either raw or pasteurized, obtained after skimming the milk.
  - *"Cream"*: at least 30% fat content in milk
  - *"Light cream"*: at least 12% fat content
- **Butter**, obtained after cream maturation and churning. It must contain 82% fat, 16% water, and 2% non-fat dry matter.
- **Yogurt** (or *yoghurt*), obtained by fermenting milk with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria. Pasteurization is mandatory. Fermented milk using other bacteria is not considered yogurt.
- **Fresh cheeses** (*White cheese , a small creamy fresh cheese*, etc.), which are non-aged cheeses obtained through lactic fermentation.
- **Dairy desserts**, composed of at least 50% milk (*dessert creams, rice pudding*, etc.).
- **Cheeses**, made by coagulating milk, draining the curd, and sometimes undergoing an aging process. There are eight families of cheese:
  - Processed cheese
  - Fresh cheese
  - Soft cheese with a bloomy rind
  - Soft cheese with a washed rind
  - Blue cheese
  - Cooked pressed cheese
  - Uncooked pressed cheese
  - Goat cheese.

2.3.3. Milk Quality and Yield:

The quality of milk collected on farms can be analyzed based on the following criteria:

- **Physical quality**: The milk must be free from any impurities.
- **Chemical quality**: Fat and protein content.
- **Bacteriological quality**: Enumeration of total aerobic mesophilic flora, which should be as low as possible.
- **Absence of pathogenic germs** (*Brucella, Listeria*, etc.).

2.3.4. Factors Influencing Yield Improvement:

Milk yield improvement mainly depends on the following factors:

✓ **Quality of raw materials**: Optimization of animal feed, livestock selection, milking hygiene, and milk storage cleanliness.

✓ **Control of manufacturing processes**: Monitoring acidification, temperature, etc.

✓ **Proper adjustment and functioning of equipment** (e.g., skimmers).

The **conversion yield** is a crucial criterion for assessing the profitability of a dairy production unit.

	Dairy Products	**Milk Quantity Required**
Cow's Milk:	**1L of cream**	**10L of milk**
	1kg of butter	**20L of milk**
	1kg of fresh cheese	**2-3L of milk**
	8 pots of yogurt (125g each)	**1L of milk**
	1kg of aged pressed cheese	**10-12L of milk**
Goat's Milk:	**1kg of fresh lactic cheese**	**6-7L of milk**
	1kg of semi-dry lactic cheese	**8L of milk**
	1kg of dry lactic cheese	**9.5L of milk**
	1kg of pressed cheese	**10-11L of milk**
Sheep's Milk:	**1kg of lactic cheese**	**3-4L of milk**

Once collected from farms, the milk is transported to the dairy where it will be processed into consumer milk and dairy products. However, before being processed, the milk arriving at the factory must go through a reception process. The reception takes place in a dedicated room and involves two operations: measuring and pouring.

The milk is then examined, tasted, and, if necessary, a sample is taken to measure its acidity, ensuring that acidic milk unfit for consumption is set aside.

2.3.5. Milk Treatments

Raw milk, as collected from the farm, can only be stored in the refrigerator for up to 48 hours and is only safe for consumption if boiled. Therefore, to extend its shelf life and make it directly consumable, this milk, which is transported to the dairy in a refrigerated truck (4°C), must undergo thermal and physical treatments.

2.3.5.1. Standardization

Standardization is the first treatment applied to raw milk. Since milk composition varies depending on diet, seasons, and cow breeds, its fat content (FC) can range from 30 to 70g/L.

A standardizing separator is used to unify the composition of milk from different farms and to adjust the fat content according to legal requirements for consumer milk and dairy products. This process involves separating the cream from the milk by centrifugation and then reintroducing it according to the required fat content:

Whole milk is obtained by reintroducing more than 3.25% fat.

Semi-skimmed milk is obtained by reintroducing approximately 1.7% fat.

Skimmed milk is obtained by reintroducing 0.1% to 0.3% fat.

Standardization allows milk to be artificially adjusted to a fixed fat content while ensuring it retains sufficient nutritional value.

2.3.5.2. Homogenization

This treatment, which follows standardization, aims to prevent fat from rising to the surface, which could disrupt milk flow or cause deposits on the packaging during subsequent thermal preservation treatments. The process is simple: it involves breaking fat globules into fine, uniform particles using pressure.

Depending on the required shelf life and bacteriological condition, the standardized and homogenized milk then undergoes thermal treatments: either pasteurization or sterilization.

2.3.5.3. Pasteurization

Pasteurization is a thermal treatment designed to eliminate pathogenic germs that cause diseases. There are two types of pasteurization:

Low-temperature slow pasteurization, where the milk is heated for 30 minutes at a temperature between 63 and 65°C or for 5 minutes at 68°C.

High-temperature pasteurization, where the milk is heated for 15 seconds at a temperature between 72 and 75°C and then immediately cooled.

Since pasteurized milk is not sterile, it must be immediately cooled to 3°C. Afterward, the milk is pressurized, meaning it is stored at normal atmospheric pressure and bottled. This results in pasteurized milk that can be stored in the refrigerator for up to 7 days.

2.3.5.4. Sterilization

Unlike pasteurization, this thermal treatment destroys all milk germs, whether pathogenic or not. Milk sterilized using the classic method is heated to 115°C for 15 to 20 minutes and can be stored for 4 months. As for Ultra-High Temperature (UHT) sterilized milk, it is heated to 140°C for 2 to 3 seconds and can be stored for 3 months.

Ultimately, all these thermal and physical treatments make it possible to produce a wide range of consumer milk products available in stores.

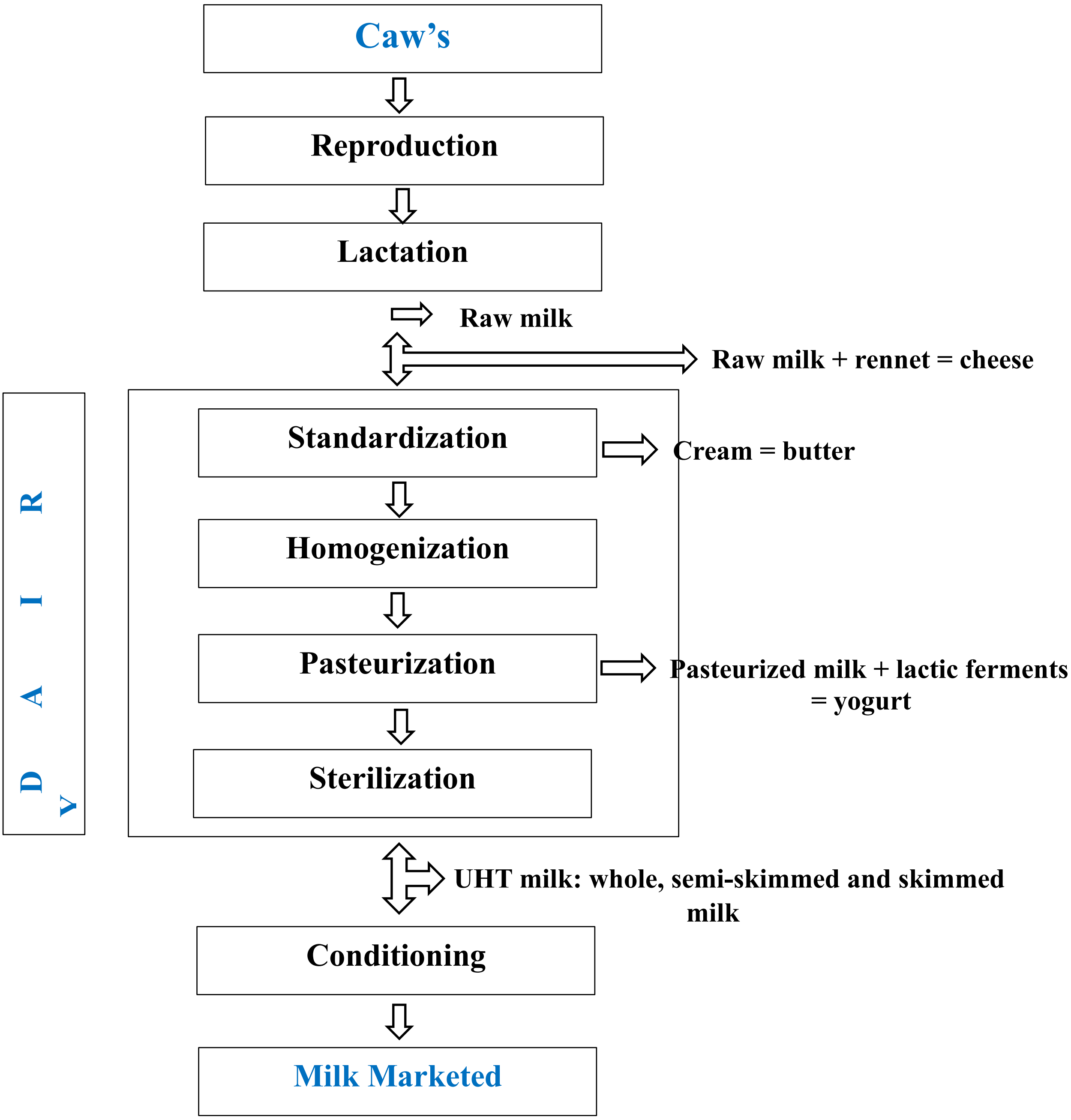

Fig 01: Cow's milk cycle to marketing

2.4. Others

2.4.1. Lacto-Fermented Vegetables

Lactic fermentation is not only used for preserving dairy products; it also allows for the preservation of mushrooms and various vegetables such as cabbage, beetroot, carrot, beans, onion, etc. This technique involves preserving vegetables by promoting the development of lactic acid bacteria, which acidify the environment and inhibit the growth of undesirable organisms.

For fermentation to occur, all the necessary conditions for the development of lactic acid bacteria must be met. Vegetables must provide sugar, B-group vitamins, and minerals. Since fermentation takes place in an anaerobic environment, oxygen must be eliminated; to achieve this, vegetables are often covered with salted water (as salt inhibits bacteria responsible for vegetable decomposition). Finally, the temperature should be between 18 and 22°C at the beginning of fermentation.

Fermentation then proceeds through three phases:

- **Pre-fermentation**, lasting 2–3 days, during which various microorganisms develop, leading to the decomposition and softening of the vegetables.
- **Fermentation**, which begins when lactic acid bacteria dominate over other microorganisms.
- **Storage**, when the pH drops below 4. At this stage, undesirable microorganisms can no longer develop, and new flavors emerge.

The vegetables can then be preserved for at least a year, even if the temperature rises above 10°C. This preservation method is not only economical, as it requires no energy input, but also beneficial to health since lactic acid bacteria produce numerous vitamins and lactic acid, which has various digestive benefits.

2.4.2. Acetic Fermentation

Acetic acid is formed by the oxidation of alcohol through the oxygen in the air. Wine, beer, cider, and generally all fermented alcoholic liquids turn sour when exposed to air. Louis Pasteur, relying on the experiments of vinegar makers of his time and on the effects of fermentation, determined the nature of the ferment involved. He demonstrated that the ferment is a living organism, which he named *Mycoderma aceti* (vinegar mother). He observed its multiplication in all directions and conducted numerous experiments to prove that *Mycoderma aceti* was the sole ferment responsible for vinegar production. The simplified acetic fermentation reaction is:

$$CH_3\text{-}CH_2\text{-}OH + O_2 \rightarrow CH_3\text{-}COOH + H_2O + \text{Energy}$$

2.4.2.1. Vinegar Production

There are several methods for producing vinegar, one of which is the **Orléans method**. This method involves cultivating *Acetobacter aceti* by mixing wine and vinegar in a ventilated barrel. The bacteria primarily develop at the air-liquid interface, meaning on the surface. This is a static culture method. Today, this method is used to produce traditional and high-quality vinegar.

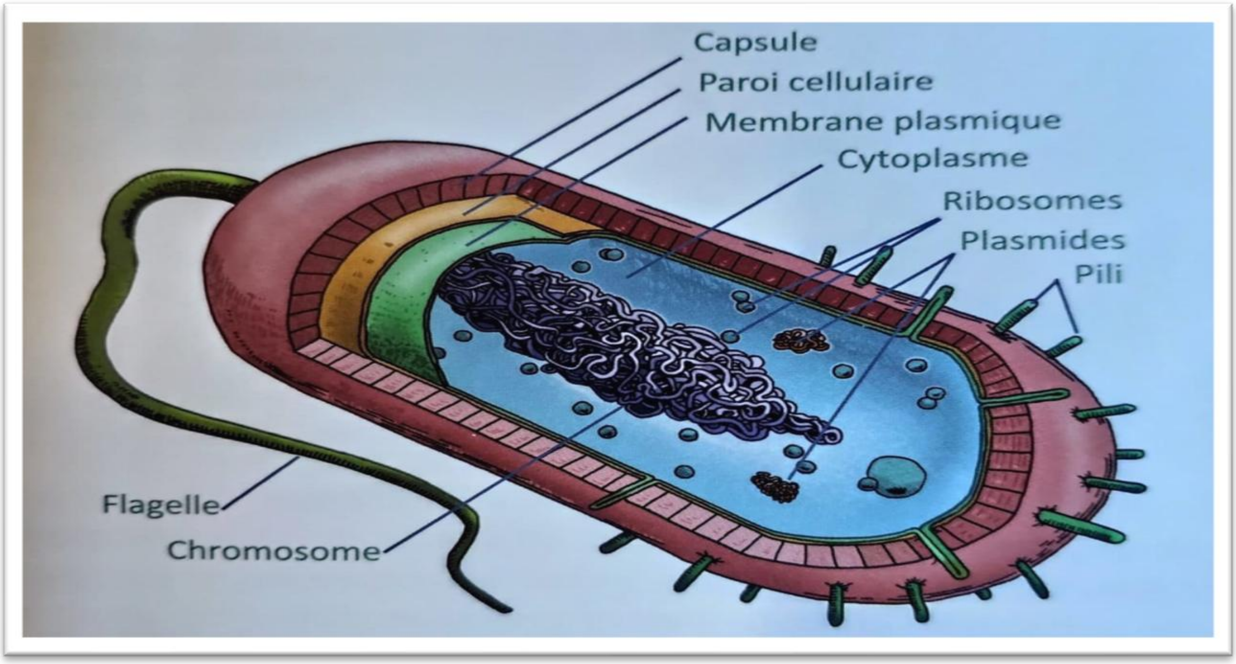

Fig 02: the bacterium *Acetobacter aceti*

Since Pasteur's research, the bacterium *Acetobacter aceti* has been cultivated in a controlled manner for industrial vinegar production. The fermentation process has been accelerated; whereas it previously took three weeks, it is now possible to produce large quantities of vinegar in just 24 hours.

The industrial method involves using a bioreactor that operates with a high level of aeration and bacteria immersed in the culture solution. Industrial vinegar production follows different processes, summarized in the following diagram:

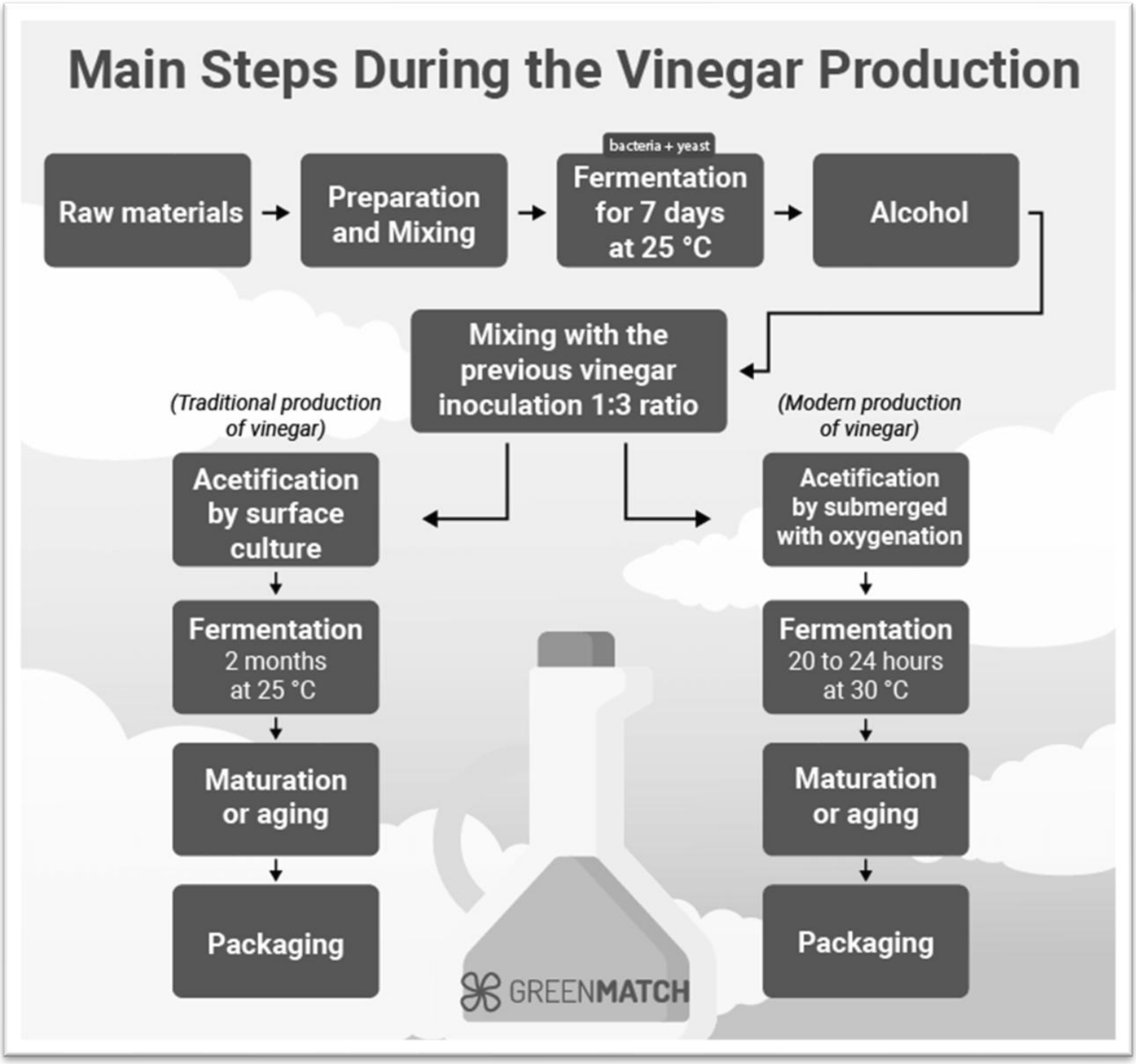

Fig 03: Diagram showing the different stages of vinegar production.

Vinegar can be made from various raw materials, including grapes, rice, apples, berries, cereals, whey, or honey.

Legislation regarding the designation of vinegar varies by country:

- In Europe, the acetic acid concentration must be at least **60 g/L**.
- In the United States, it must be at least **40 g/L**.