الجمهورية الجزائرية الديمقراطية الشعبية

Ministry of Higher

Education and Scientific Research

Nour El Bachir University Center - El Bayadh –

Food hygiene

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2025-2026

FOREWORD

Second-year Master's course (LMD system), specializing in microbial biotechnology, El Bachir University Center in El-Bayadh.

Teaching Unit:

Fundamentals 1.

Subject: No. 1; Credits: 10; Coefficient: 5

Module responsible:

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Language of instruction:

French

Module description:

This course provides a comprehensive introduction to food hygiene and safety practices essential in the food industry. It is designed to equip participants with the knowledge and skills necessary to handle, prepare, and store food in a safe and sanitary manner, reducing the risk of foodborne illnesses.

Participants will learn about key topics such as personal hygiene, cross-contamination, cleaning and disinfection, pest control, food storage temperatures, and HACCP (Hazard Analysis and Critical Control Points) principles. Emphasis is placed on both theoretical knowledge and practical application in real-world settings, including kitchens, restaurants, catering services, and food production facilities.

The course is ideal for food handlers, kitchen staff, supervisors, and anyone working in the food industry who is responsible for ensuring food safety.

Objectives

Food hygiene is of paramount importance to ensure the safety of the commentator and restores their confidence in food.

This module will introduce students to the means and methods available to manufacturers and the government to improve, ensure, and verify food safety.

It will allow students to better understand hygiene issues and analyze the strategies implemented by manufacturers and public authorities to deal with food borne illness.

Understand the safety instructions and rules of use regarding protection of products and equipment in a biology laboratory.

Understand the importance of complying with hygiene and safety rules.

Learn the appropriate gestures, attitudes, and behaviors to approach zero risk.

Scientifically justify the application of the instructions given in a laboratory context.

Prerequisites:

- Knowledge of microbiology and biochemistry.

- Knowledge of Food Production and Processing.

Teaching Method:

Lectures, tutorials, and practical work.

Assessment Method:

A midterm exam on the material covered in the first semester is organized during the January session, and the practical work and tutorials are assessed through individual evaluation of the exercises performed and/or exercises similar to those completed during the session. Sometimes a semester-long project is included.

Course Recommendations:

Visit to a food production and processing plant

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I. Food hygiene

I. 1. Definition of food hygiene

Food hygiene is based on a set of practices to be adopted when handling food to eat safely and avoid health problems. Indeed, food can be a vector of disease since it is continually exposed to contamination risks during the various stages of the food chain **(FAO/WHO, 2015)**.

Food hygiene is based on three actions:

- Cleaning, - Disinfection and - Preservation

The Regulation of the European Parliament and of the Council on the Hygiene of Foodstuffs defines food hygiene as "the measures and conditions necessary to control hazards and ensure that a food is fit for human consumption, taking into account its intended use". Some also define it as the rational choice of foods that the body needs for its metabolism **(Multon *et al.*, 2013)**.

I. 2. The origin of microorganisms in food

Microorganisms can enter through several routes: food, air, water, animals, people, and their clothing. Once inside, germs can adhere to surfaces (cooking utensils, countertops, equipment, etc.). **(Todd *et al.*, 2009)**

I.2.1. Already exists in a food

Food is not sterile. Food can become host to many bacteria. But while some bacteria are essential for giving food taste, texture, or flavor, others can multiply in the food and alter it by causing organoleptic changes (unacceptable taste, odor, and appearance).

Smelling a product can sometimes provide information about its quality. The appearance, as well as the taste and texture of the food, can also provide information about its quality before ingestion and absorption by the body.

Products that give off an unusual or unpleasant odor or taste don't necessarily cause illness after consumption, but they do pose a higher risk than usual. That said, it all depends on the person eating the food and its level of spoilage. Generally, these products end up in the trash and are unlikely to be consumed. **(Matthews *et al.*, 2025.)**

I. 2.2. An accidental addition

I. 2.2. 1. People

Whether sick, healthy, or cured (healthy carriers), humans carry a large number of germs on their skin, in the digestive and respiratory tracts (nose, throat). Sneezing, coughing, contact with dirty hands, or even dander or perspiration promote the transfer of these microorganisms to food. **(Dawson, 2020)**

Similarly, hands that have been in contact with infected animals, whether sick or healthy, can carry pathogens responsible for diseases transmissible from animals to humans, which can contaminate kitchen surfaces and food **(ANSES, 2013)**. The germs in question are mainly *Staphylococcus*, *Streptococcus*, etc., which are carried by healthy skin or by wounds, abscesses, or boils. Poor hygiene can lead to the presence of intestinal bacteria on the skin (fecal contamination: *Salmonella*).

Aerosol contamination (coughing, sneezing, but also simply breathing) can also occur: germs of tonsillitis, sinusitis, both bacterial (*Streptococci*, *Staphylococci*, etc.) and viral.

Would a person get sick from eating a mouthful of these spoiled products? A product that smells bad, or has changed in taste or texture, is most likely contaminated by a higher than normal bacterial flora. The bacteria have encountered conditions favorable to their multiplication, which does not exclude the possibility that pathogenic bacteria had the same opportunity, provided they were already present in the food.

Furthermore, contamination can be linked to clothing; dirty clothing can also be a source of contamination **(Rawat *et al.*, 2015)**

I. 2.2. 2. Soil

Soil, and particularly topsoil, contains a very large number of microbial species of very diverse types (*Bacillus*, *Clostridium*, *Streptomyces*, *Corynebacterium*, spores and conidia of *Penicillium*, *Aspergillus*, *Mucor*, *Fusarium*, etc.). The products most exposed to soil microorganisms are fruits and vegetables, for which the problems posed by surface barrier microorganisms are also present.

Another particularity of soil microorganisms is their ability to transform natural compounds into products toxic to humans. **(Hou *et al.*, 2020)**

I. 2.2. 3. Water

Tap water must meet microbiological quality standards and limits and is subject to health monitoring. Distributed water can, in rare cases, carry pathogenic germs (flooding, distribution network malfunction,

etc.). In the absence of analysis demonstrating its potability, well or borehole water should not be used. In the event of microbiological contamination, the population is informed of the usage restrictions to follow while waiting for a return to normal conditions.

Freshwater and saltwater contain a variable number of microorganisms depending on the intensity of the pollution. Their natural flora consists of aerobic Gram-negative bacteria, including *Pseudomonas*, *Vibrio*, etc.

Water is used extensively in the food industry: this water can contain a variety of microorganisms and be a source of contamination.

Microorganisms found in water, in addition to the normal water flora, can have diverse origins: soil (*Streptomyces*, *Bacillus*, etc.), fecal matter (*Enterobacteria*, S*treptococci*, etc.), plants (fungal spores and conidia), animals, etc. Water can be a vector for pathogenic microorganisms: *Salmonella*, *Shigella*, *Yersinia*, *Vibrio*, *Listeria*, viruses, protozoa, etc. Microorganisms from the environment can be introduced directly or through vectors (insects).

Wastewater is a veritable breeding ground for microbial cultures, with flora of diverse origins and generally a high level of fecal germs. **(Tirado *et al.,* 2025)**

I. 2.2. 4. Air

Air can carry microorganisms that can contaminate kitchen surfaces and the food stored there.

Air and dust contain a very large number of suspended microbial cells. These are mainly bacteria, sometimes molds (*Aspergillus*, *Penicillium,* etc.) and more rarely yeasts (*Torulopsis*). Among the bacteria, Sporulantes and *Micrococcus* predominate.

Air characteristics can influence the further development of microorganisms in contaminated products. For example, humid air favors the growth of *Aspergillus repens*, while dry air allows the proliferation of several other molds (*Penicillium cyclopium* or *Pleospora enfectoria*, etc.) in sugar industry products. **(Moracanin *et al.,* 2019)**

I. 2.2. 5. Animals

Animals (dogs, cats, new pets, livestock, etc.) can transmit diseases to humans. Insects, and especially flies, are also sources of contamination, as they constantly move between waste, feces, and food **(ANSES, 2013)**.

I. 2.2. 6. Equipment

A contaminated surface can in turn contaminate any other surrounding surface, or more precisely, any surface with which it comes into contact. Hands are a surface that best allows the spread of microorganisms in the kitchen. Germs thus reach food or kitchen utensils.

Other surfaces can facilitate the circulation of microorganisms in the kitchen, such as sponges, dishcloths,

cutting boards, slicing, grating, and grinding equipment if they are not properly cleaned between uses, door or appliance handles, and any other object used for cleaning. **(Deflorio *et al.*, 2021)**

I. 2.3. Deliberate addition

The deliberate addition of microorganisms is necessary in the manufacture of food, such as bread, cheese, yogurt, etc.

I. 3. Contamination risks

Before discussing food hygiene, it is first necessary to identify the food safety hazards that must be controlled to prevent repercussions on consumer health. These hazards include the following substances **(Dardé, 2011)**:

-Chemical contaminants: natural or man-made substances that are found in food during processing, preservation, and distribution (pesticide residues, toxins, metals, etc.);
-Microbiological contaminants: the presence of pathogenic microorganisms;
-Physical contaminants: the presence of foreign bodies in food, such as sharp objects, etc.

However, the absence of food spoilage does not necessarily mean that there is no danger, and even food that appears to be in good condition and tastes good can be dangerous. Similarly, fresh, high-quality products are not necessarily synonymous with the absence of risk **(Leclerc, 2011)**.

I. 4. Effect of microorganisms on food

I. 4.1. Harmful effect of microorganisms

It is true that some foods are more at risk than others; some microorganisms grow preferentially in certain foods rather than others:

-Eggs and egg products: *Salmonella;*
-Poultry meat: *Campylobacter;*
- Cooked plants and starchy products: *Bacillus;*
-Milk and dairy products: *Staphylococcus aureus*
-Uncooked beef, raw milk cheese: *Escherichia coli*
-Raw meat: *Trichinella*

However, all foods remain susceptible to contamination by bacteria and viruses, which may be the result of cross-contamination **(Leclerc, 2011)**. One of the best-known examples of illness linked to improper handling of a high-risk food is Hamburger disease, which appeared in the United States in 1982 and was transmitted by ground beef steaks contaminated with the pathogenic bacterium *Escherichia coli* O157:H7. This difficult-to-detect bacterium is present in the digestive tract of cattle and can come into contact with and contaminate the meat during butchering. This disease caused numerous deaths in France in 2005, mainly among children, leading to the launch of an epidemiological investigation to trace the source of the contamination **(Dumas, 2005)**.

It is important to note that the same principle applies to other products such as milk, which can be contaminated by feces during milking, and to raw fruits and vegetables that may have come into contact with contaminated manure. **(Singhal *et al.*, 2020)**

I. 4.2. Different types of foodborne flora

I. 4.2.1. Pathogenic flora

a. Bacterial Infection: Mechanisms Leading to Disease Outbreaks

Bacteria primarily enter the body via the mucous membranes, which represent 400m² of surface area in humans. The oral and gastrointestinal mucous membranes are the most common entry routes for bacteria present in food.

Example: *Brucella* sp

Brucella sp are small, facultative, intracellular Gram-negative coccobacilli, measuring 0.6 to 1.5 µm in length and 0.5 to 0.7 µm in diameter. The cells are nonmotile, noncapsulated, nonspore-forming, and do not form flagella. The bacteria are strictly aerobes, catalase and oxidase positive (variable urease), but some strains grow best in an atmosphere containing 5 to 10% CO_2.

The optimal growth temperature is 34°C, but the tolerated temperature can vary between 20 and 40°C on suitable media, although *Brucella* sp are usually grown at 37°C. The pH required for growth varies between 6.6 and 7.4, with an optimal pH of 6.8.

Isolating *Brucella* sp from samples contaminated by other bacteria or fungi requires the use of selective media corresponding to basal media (such as Trypticase Soy, Tryptose, or Albimi broths or agars) to which antibiotics and antifungals are added **(Roux, 1994)**.

Furthermore, this *Brucella* sp isolation requires an incubation time of at least 3 to 4 days. The colonies are translucent, round with regular edges. Culture in liquid medium exhibits slight turbidity. The recently identified species *Brucella microti* and *Brucella inopinata* **(Hubalek *et al.*, 2007)** are distinguished from other species by their growth after only 24 hours of culture.

The outer membrane is mainly composed of phospholipids, proteins, and LPS. Two different forms of LPS are distinguished in *Brucella* sp: LPS-S for *Brucella* Smooth (S), characterized by a smooth appearance of colonies growing on the surface of solid media, and LPS-R for *Brucella* Rough (R), characterized by a rough appearance. *B. ovis* and *B. canis* are species that are naturally found in a rough state.

LPS-S consists of three entities: lipid A, the core, and the O chain (also called O antigen), whereas LPS-R does not contain the O chain. Lipid A, embedded in the outer membrane, represents the proximal part of LPS, the core its middle part, and the O chain its distal part, which is "free" in the external environment. Several biological roles are associated with the O chain, such as protection against complement-mediated lysis, or the survival and replication of *Brucella* sp in the host. Several studies have also cited LPS as a virulence factor. **(Cavaillon *et al.*, 2024)**

I. 4.3. Food spoilage flora

I. 4.3. 1. Flora selected by the nature of the food

• Lipolytic flora

This flora is capable of hydrolyzing TGs.

TG + 3H20 ----------> Glycerol + 3AG

Animal fats are mainly composed of unsaturated fatty acids that can oxidize in air, producing aldehydes and ketones (rancidity, rancid odor).

• Caseolytic flora

This flora is capable of hydrolyzing casein, so these enzymes are exoproteases. A bitter, sour odor is observed. This flora is primarily psychrotrophic; it is found in dairy products and especially cheeses.

• Lactic acid flora

Lactic acid bacteria are capable of fermenting sugar by producing lactate (= lactic acid). Main genera: *Lactobacillus* sp, *Streptococcus* sp, *Lactococcus* sp, *Leuconostoc* sp. This is a mesophilic flora, so at room temperature, milk becomes acidic due to protein precipitation (curdled milk). In the refrigerator, this flora becomes inactive, but the caseolytic flora prevails (which gives the milk a sour odor). **(Kao *et al.*, 2020)**

• Acetic acid flora

This is particularly sought after in wine and the "soft" part of grapes. Bacteria are capable of producing acetate from carbohydrates.

I. 4.3. 2. Flora selected by environmental parameters: Temperature

• Psychotropic flora

Optimal temperature: 25–35°C, therefore multiplying at room temperature and multiplying further at 4°C. The most common: *Bacillus* sp. Pathogens: *Listeria* sp and *Yersinia* sp.

• Temperature-resistant flora

Bacteria that withstand pasteurization at 63.5°C for 30 minutes, such as: *Enteroccocus faecalis*, *Bacillus* sp, *Clostridium* sp, *Micrococcus* sp, and environmental G(+).

• Temperature-resistant spore-forming flora

This is a life form that can withstand 10 minutes at 100°C (in food). Above 80°C, there is no vegetative form, which is instantly destroyed. Genus: *Clostridium* sp and *Bacillus* sp.

The main target is the presence of *Clostridium* spores. There are two types of *Clostridium* sp:

- *Proteolytic or "putric" Clostridium:* a spoilage agent that causes putrefaction (deep, anaerobic degradation of proteins, with the release of H_2S, indole, and ammonia). They are particularly sought in meats and canned goods.

- *Butyric Clostridium:* which is responsible for butyric fermentation. They are sought in dairy products.

I. 4.4. Microscopic fungi

Molds and yeasts belong to the kingdom Mycetes (Fungi). The classification of fungi is very complex due to the number and diversity of species. Therefore, in the food industry, the distinction is simplified to their morphological types.

The cellular organization of fungi is called the thallus, and in microscopic fungi (micromycetes), it can be slimy (yeasts) or filamentous (molds). However, there are exceptions; some yeasts can form filamentous structures (pseudomycelium) under certain conditions (*Candida* sp, *Trichosporon* sp) **(Meyer *et al.,* 2004)**.

These organisms have high adaptability and are generally found at acidic pHs (many are acidophilic). Their temperature range is also wide, reaching low temperatures, close to 0°C. In terms of water activity, microfungi have the ability to withstand low aw. These physiological characteristics allow them to develop in difficult conditions and therefore offer them very strong competitiveness, explaining why they are found in all environments.

I. 4.4. 1. Molds

Molds are characterized by their membership in the filamentous microfungi, and are therefore multicellular. The filaments are more or less branched, and these structures form hyphae. All hyphae constitute the mycelium (figure 1).

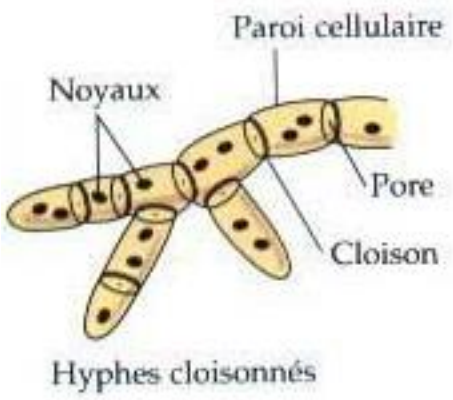


Figure 1: A septate hypha **(Meyer *et al.,* 2004)**.

Molds are primarily concentrated in the soil, and reproduction can be asexual (haploid) or sexual (diploid) (figure 2) **(Meyer *et al.,* 2004)**.

- ***Asexual reproduction*** is used for the dissemination of the species. The mold forms spores (conidia) which are disseminated into the environment.

- ***Sexual reproduction***, through genetic mixing, will allow the survival of the species in difficult conditions.

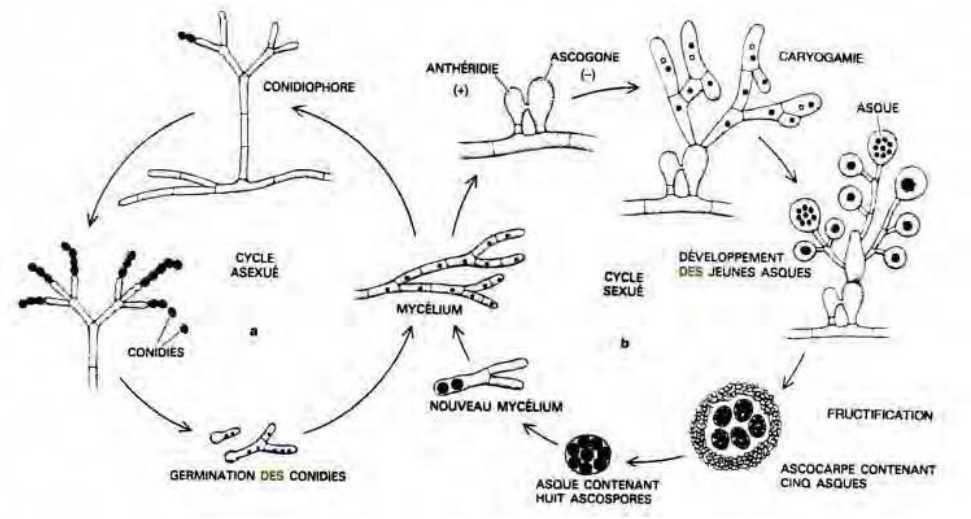


Figure 2: Mold reproduction diagram **(Meyer *et al.*, 2004)**.

Molds are organisms that require relatively undemanding culture conditions to thrive **(Meyer *et al.*, 2004)**.

- ***Temperature:*** growth is generally possible between 4 and 30°C, with the optimum temperature being between 18 and 28°C.

- ***pH:*** the optimum is between 4 and 6.5, but they can colonize very acidic environments (pH 2) via acidophilic molds, as well as basic ones (up to pH 11).

- ***aw:*** some molds can tolerate a water activity of 0.2-0.3.

- ***Oxygen:*** they have the ability to grow in a very wide range (from ambient air to products containing only traces of oxygen). However, molds do not grow anaerobically.

As previously seen, molds are saprophytic soil microorganisms. They are therefore easily found on food (especially fruits and grains). Molds then act in a positive or negative way **(Meyer *et al.*, 2004)**.

- These microfungi are capable of producing a wide range of hydrolytic enzymes (lipases and proteases), and are therefore found in the food industry, particularly in the ripening and processing of products (particularly *Penicillium* sp). Molds are therefore integrated into the manufacturing processes of cheese, soy sauce, and alcoholic beverages. Furthermore, some molds are used in biotechnology for the formation of food flavorings.

- However, molds can grow on the surface of food unintentionally. In addition, some molds produce mycotoxins that can be toxic to humans (aflatoxin production by *Aspergillus flavus*). There are also toxins

that are non-toxic to humans, such as penicillin (discovered in 1940), an antibiotic produced by certain molds of the *Penicillium* genus (*Penicillium notatum*, *Penicillium chrysogenum*, etc.).

I. 4.4.1.1. Mold Development

Molds must obtain the water, nutrients, and minerals necessary for the synthesis of their own matter from the surrounding environment. They absorb these nutrients through the walls of their vegetative system. They are said to be absorbotrophs.

Different lifestyles exist: symbiosis with plants, parasites of animals or plants, and some also grow on organic waste or contaminate food products (this third category constitutes saprophytes) **(Meyer *et al.*, 2004)**.

I. 4.4. 1.2. Growth conditions

Molds derive their carbon and energy from organic carbon molecules. Mold growth also requires sufficient oxygen, a temperature between 5 and 25°C (growth is possible between 0 and 60°C, but outside of optimal temperatures, it will be slower), and sufficient humidity **(Leyral and Vierling, 2007)**.

I. 4.4. 1.3. Growth and multiplication

Hyphal growth is strictly apical, involving lysis of the apical wall and synthesis of new cell wall material (using hydrolases and synthases contained in vesicles often originating from the Golgi apparatus or the endoplasmic reticulum).

Sexual or asexual reproduction occurs through spores, tiny living particles. These are dehydrated cells with a reduced metabolism, surrounded by thick protective walls that isolate them from the surrounding environment. They are produced in very large numbers and can survive for a long time (several months or even years). They are deposited and will germinate when conditions (mainly humidity) become favorable. Spore germination is the origin of the vegetative form of molds; Indeed, their development includes a vegetative phase, involving growth and nutrition, and, almost simultaneously, a reproductive phase during which spore formation occurs.

I. 4.4. 1.4. Mold involvement in the food industry

Molds often possess significant lytic properties (cellulolytic, pectinolytic, amylolytic, proteolytic, etc.), making them dangerous degradation agents but also sometimes useful allies (particularly during cheese ripening or enzyme production) (table 1). The harmful effects occur at several levels:

- Fungi with phytopathogenic activity, which are harmful to the production of raw food materials such as fruits and vegetables.
- Saprophytic molds, which contaminate food and degrade its quality.

As regards the "positive" action of molds, we note their great usefulness in agriculture (the filamentous fungus *Botrytis* sp is the cause of "noble rot" in Sauternes grapes), as well as in industry (production of cheeses as we have previously specified, production of molecules with pharmacological activity, industrial enzymes.

Table 1: Comparison of the different roles of molds

Useful molds	Spoilage molds
Penicillium roqueforti: production of blue-veined cheeses	*Aspergillus flavus*: produces highly toxic and carcinogenic compounds (aflatoxins)
Penicillium camemberti: production of bloomy-rind cheeses	*Aspergillus terreus*: contributes to the decomposition of organic matter
Penicillium album: surface flora of sausage, covering of certain cheeses	*Alternaria dauci*: infects the leaves of cultivated carrots
Mucor: surface flora of certain cheeses, manufacturing of various products	*Fusarium avenaceum*: responsible for the deterioration fruit
Aspergillus Oryzae: Fermentation of rice and soy products	

I. 4.4.1.5. Mold taxonomy

The main mold genera include *Penicillium* and *Mucor* ; Mucor does not have rhizoids and grows on all moist substrates, including grains of rice, rye, and wheat. These molds are involved in saccharification processes (transformation of starchy or cellulosic substances into fermentable sugars). As for the genus *Penicillium*, the spore-forming apparatus has a shape similar to that of a paintbrush, with a green or white thallus.

Species belonging to the genus *Penicillium* grow in soils, foodstuffs, decomposing organic matter, seeds, etc. They are widely used in industry, particularly for cheese production (*Penicillium roqueforti*, *Penicillium camemberti*), but also for the production of metabolites: the manufacture of penicillin-type antibiotics **(Leyral and Vierling, 2007)**.

There are species that are intermediate between yeasts and molds; they should be classified as yeast-like fungi. This is the case for species of the genus *Geotrichum*.

Furthermore, filamentous fungi can be divided into several groups, depending on the presence of a cell wall in their vegetative system, their spore type.

I. 4.4.2. Yeasts

Yeasts are fungi for all or part of their vegetative cycle. Some have been known for over 4,300 years for their fermentation potential. A number of fermentation processes and products derived from them rely on the use of yeasts. They can be grouped into seven categories:

- Baker's yeasts and bread products;

- Brewer's and beer yeasts;
- Winemaking and wine yeasts;
- Distiller's and spirits yeasts;
- Food yeasts;
- Yeast-derived products (autolysates, etc.);
- Industrial ethanol and fuel.

I. 4.4.2.1. General characteristics and taxonomy

The terms spherical, globose, ovoid, elongated, and cylindrical are often used to describe the vegetative forms of yeast. The mitochondria of yeast cells contain DNA, RNA, RNA polymerase, and respiratory enzymes. They degenerate into promitochondria during fermentation or when the medium contains more than 5% glucose; there is then no further synthesis of cytochromes aa3 and b, and the cell no longer respires.

The following table lists the main species encountered along with their most important synonyms **(Leyral and Vierling, 2007)**.

Table 2: Classification of the main industrial yeast species

Classification	Espèces
Ascomycotina	*Kluveromyces fragilis*
Hemiascomycètes	*Saccharomyces cerevisiae* (Syn. *S. ellipsoideus, S. italicus*)
Endomycétales	*Saccharomyces exiguus*
Saccharomycetoideae	*Torulaspora delbrueckii* (Syn. *S. rosei*)
	Zygosaccharomyces bailii (Syn. *S. bailii*)
Deuteromycotina	*Candida utilis*
Blastomycètes	*C. krusei*
Cryptococcaceae	*C. lipolytica*
	Tichosporan cutaneum

I. 4.4.2.2. Role in food (yeast production)

In addition to their significant enzymatic power, which allows yeasts to be biological catalysts capable of recovering agricultural and agri-food by-products, yeasts are produced for their biomass, which is rich in amino acids, which could be used as alternative nitrogen sources in animal or human food (SOP: Single-Cell Organism Proteins).

Molasses and sulfite liquors are the main carbon sources used for yeast production. Potato waste and starch production residues are also good substrates. Furthermore, it is feasible to produce yeast on a fermentation substrate based on date juice **(Leyral and Vierling, 2007)**.

II. Food microbiology (further reading and specification)

II.1. Microbiology of meat, poultry, dairy products, and seafood

II.1.1. Microbiology of meat and poultry

From a nutritional point of view, meat is a substance rich in water, high-value proteins, and fats, but it contains very little carbohydrate (glycogen). Meat is a favorable substrate for the

growth of microorganisms, primarily proteolytic microorganisms, which cause adverse changes in odor, color, and texture.

Microbial growth, however, is hampered by the compact structure, the presence of integuments, and especially by cold storage. Meat evolves without any microbial action. After the death of the animal, physicochemical and enzymatic changes are observed: there is first a phase of rigor mortis, followed by a maturation phase.

II.1.1.1. Meat flora

II.1.1.1.1. Original flora

The flesh of a healthy, living animal is virtually sterile. In a sick animal, there may be direct contamination via the lymphatic system. The meat is therefore likely to contain pathogenic germs from the animal, and these germs will very often be pathogenic to humans. Meat can also become contaminated at the time of slaughter from the flora of the animal's intestine, skin, or mucous membranes. Parasites, particularly cestode helminths (taeniasis or "tapeworm"), nematodes (*Trichinella spiralis*, the agent of trichinosis), and protozoa (*Toxoplasma gondii*, the agent of toxoplasmosis transmitted by infected meat), as well as pathogenic bacteria, may therefore be present in meat, such as salmonellosis and typhoid fever caused by *Salmonella* or listeriosis caused by *Listeria monocytogenes*.

II.1.1.1.2. Contamination flora

A. Contamination flora due to slaughter and primary processing

Contamination originates from the animal, the handler, or the equipment. Meat can become contaminated during various stages (slaughter, bleeding, evisceration, dressing (i.e., removing the hide), scalding for pigs, showering, etc.). Cross-contamination may occur between "clean" and "dirty" areas (waste, viscera) of the plant. The animal contaminating flora comes from the skin (*Micrococcus*, *Pseudomonas* including *P. fluorescens*, *P. fragi*, and other germs of the common Gram flora - staphylococci including *S. aureus*, *Lactobacillus*, *Streptomycetes*) or from the digestive tract (coliforms including *Escherichia coli*, *Clostridium perfringens*, fecal streptococci, possibly pathogenic *Enterobacteriaceae* such as *Salmonella* sp

and *Shigella* sp). This flora contains common germs and germs harmful from a health point of view. In the case of pigs, scalding water is a vector and a frequent reservoir of contamination **(Bazinet and Castaigne, 2011)**.

B. Contamination flora due to subsequent handling

Meat can be contaminated during storage and subsequent handling by numerous germs from the air, soil, handlers, and possibly washing water: cross-contamination between pieces of meat can occur. These most commonly include *Pseudomonas* and other Gram-negative germs, spore-forming bacteria such as *Bacillus* (including *B. cereus*), *Clostridium* (including *C. perfringens* and possibly *C. botulinum*), coliforms and *Enterobacteriaceae* that can be pathogenic (*E. coli*, *Salmonella*, *Shigella*), staphylococci, *Listeria*, yeasts, coryneform bacteria (*Brochothrix thermosphacta*), and mold spores (*Cladosporium*, *Sporotrichum*, *Geotrichum*, *Thamnidium*, etc.). Insect contamination can be significant under certain conditions (exposure during sale) **(Delarras, 2007)**.

C. Evolution of flora and meat degradation

Raw meat is subject to the action of its own enzymes and that of microorganisms. The action of enzymes is desirable because it causes the meat to tenderize: this process is called meat ripening. However, the action of enzymes often has harmful consequences from a microbiological point of view because it promotes the development of germs.

The invasion of tissues by microorganisms depends on several factors: the animal's state of health and fatigue, the microbial load of the animal and particularly its intestines, the method of killing and rendering, and the meat storage conditions.

Germs develop depending on the physical characteristics (surface area exposed to air, cut, etc.) and chemical characteristics (pH, water content, etc.) of the meat and the external conditions (aeration, temperature). The spoilage caused will depend on these different factors. Due to low-temperature storage conditions, psychrophilic germs are the preferred agents of meat spoilage, mainly resulting in superficial alterations. At higher temperatures, "deep" putrefaction is favored **(Bazinet and Castaigne, 2011)**.

E. Aerobic spoilage

This is spoilage that occurs mainly on the surface.

• **Viscosity (or stickiness):** this is due to the growth of bacteria (*Pseudomonas*, *Achromobacter*, *Streptococcus*, *Leuconostoc*, *Bacillus*, *Micrococcus*, and *Lactobacillus*), and more rarely yeasts or molds **(Delarras, 2007)**.

• **Discoloration and greening:** discoloration results from oxidation under the action of lactobacilli, *Leuconostoc*, and yeasts. Greening is linked to the production of H_2O_2 and H_2S, which modify myoglobin, and is caused by *Lactobacillus*, *Brochothrix*, etc.

• **Pigmentation:** these are caused by colored or diffusible pigment bacteria (*Photobacterium*,

Flavobacterium, *Pseudomonas*, *Micrococcus*, *Serratia*, etc.), yeasts (*Rhodotorula*, etc.), and molds (*Cladosporium herbarum*, *Sporotrichum carvis*, *Penicillium*, etc.).

• **Changes in organoleptic characteristics:** these occur through the rancidity of fats (*Pseudomonas*, yeasts, molds), and the release of compounds responsible for undesirable tastes and odors (lactic acid bacteria, souring agents, yeasts, *actinomycetes*). *Brochothrix thermosphacta* is a psychrophilic bacterium that is often involved: it releases volatile fatty acids with an unpleasant odor **(Bazinet and Castaigne, 2011)**.

• **Mold:** this is caused by *Thamnidium*, *Mucor*, *Rhizopus*, etc., molds sometimes associated with yeasts, and is most commonly found in dry environments. This surface damage generally does not spread inward unless the meat is physically damaged (mechanically tenderized meat or ground meat). It is most often slow and limited. It generally does not have a major impact from a health perspective, unless the damage is very severe.

• **Putrefaction:** surface putrefaction can be caused by certain species of *Pseudomonas* as well as *Enterobacteriaceae* **(Delarras, 2007)**.

b. Anaerobic degradation

This develops when the meat has been ground, deboned, cut deeply, packaged in plastic wrap, and in all cases where anaerobic conditions are present.

• **Sourification:** this is caused by bacteria whose metabolism releases organic acids (formic, acetic, lactic, butyric, propenoic, etc.) or by bacteria with non-putrefactive proteolytic activity.

The main agents are lactic acid bacteria, coliforms and other *Enterobacteriaceae*, *Clostridium butyricum*, aerobic-anaerobic *Bacillus* (including *B. cereus*), *Staphylococcus*, etc.

• **"Bone stench":** this is linked to the presence of *Bacillus* and *Clostridium* and occurs in carcasses that are refrigerated too slowly. Microbial action, coupled with enzymatic changes, generates malodorous compounds **(Bazinet and Castaigne, 2011)**.

• **Putrefaction:** this is caused by proteolytic bacteria that release sulfur compounds, ammonia, amines, skatole, and indole: these are proteolytic, putrid, and sulfite-reducing *Clostridium* (*C. sporogenes*, *C. perfringens*), certain species of *Proteus*, and other Gram-negative, anaerobic, and proteolytic bacteria from the common flora (putrid fermentation).

II.1.2. Microbiology of dairy products

Milk, due to its composition, is a choice food: it contains fats, lactose, proteins, mineral salts, vitamins, and 87% water. Its pH is 6.7. It is a very favorable substrate for the development of microorganisms. Milk is used in many forms and is the raw material for many food products **(Delarras, 2007)**.

II.1.2.1. Milk flora

II.1.2.1.1. Original flora

Milk contains few microorganisms when collected under good conditions from a healthy animal (less than 10^3germs/mL). These are mainly saprophytic germs of the udder and milk ducts: micrococci but also lactic streptococci (*Lactococcus*) and *Lactobacillus*. Raw milk is protected against bacteria by inhibitory substances called "lactenins," but their action is very short-lived (about 1 hour) **(Buchin and Beuvier, 2000)**.

Other microorganisms can be found in milk from sick animals: they are generally pathogenic and dangerous from a health perspective. These can be mastitis agents, i.e., udder infections: pyogenic streptococci (*Streptococcus*), pyogenic corynebacteria, *Staphylococcus*, etc. They can also be general infection germs that can pass into the milk in the absence of udder abnormalities: *Salmonella*; *Brucella*, the agent of Malta fever, and, exceptionally, *Listeria monocytogenes*, the agent of listeriosis; *Mycobacterium*, the agent of tuberculosis; *Bacillus anthracis*, the cause of anthrax; *Coxiella burnettii*, the cause of fever, and some viruses. **(Chatelin and Richard, 1983)**.

Common udder germs do not pose a health hazard but can grow abundantly in milk. Others can cause serious illnesses or poisonings which are generally limited by veterinary monitoring of producing animals.

II.1.2.1.2. Contamination flora

Milk is contaminated by microbial inputs from various sources:

- Animal feces and integuments: coliforms, *Enterococcus*, *Clostridium*, possibly pathogenic enterobacteria (*Salmonella*, *Shigella*, *Yersinia*), etc.
- Soil: *Streptomyces*, *Listeria*, spore-forming bacteria, fungal spores, etc.;
- Bedding and feed: varied common flora, particularly *Lactobacillus*, *Clostridium butyricum* (silage) **(Buchin and Beuvier, 2000)**.
- Air and water: various flora including *Pseudomonas*, spore-forming bacteria, etc.;
- Milking and milk storage equipment: *Micrococcus*, yeasts, and lactic acid flora with *Lactobacillus*, *Streptococcus* (*Streptococcus*, *Lactococcus*, *Enterococcus*), *Leuconostoc*, etc. This flora is often specific to a factory;
- Milk handlers: *Staphylococcus* in the case of manual milking, but also germs from sputum, fecal contamination, etc.;
- Various vectors (especially insects): Fecal contamination flora.

Among these microorganisms, there are some that are harmless, others that are dangerous from a health point of view, and others that can cause the deterioration of milk.

II.1.2.2. Flora development and milk degradation

Many microorganisms can grow abundantly in milk, causing changes in texture and taste. These changes depend on the milk's storage conditions (aeration, temperature) and the treatments it has undergone.

• **Sourning and acidification with coagulation:** the normal pH of milk is 6.6. Most milk microorganisms

are capable of fermenting lactose, producing acidification that leads

to casein coagulation. This coagulation occurs at pH 4.6 and above. It is facilitated by heating the acidified milk. The microbial fermentations responsible for acidification are homo- or hetero-lactic. The organisms involved vary depending on the type of milk contamination and the storage temperature. From 10°C to 37°C, the most frequently involved organism is *Lactococcus lactis* (formerly *Streptococcus lactis*), with less frequent associations with coliforms, *Enterococcus*, *Micrococcus*, and *Lactobacillus*. Above 37°C, the causative organisms are *Streptococcus thermophilus*, *Enterococcus faecalis* (formerly *Streptococcus faecalis*), or *Lactobacillus bulgaricus* **(Buchin and Beuvier, 2000; Chatelin and Richard, 1983)**.

When milk has been pasteurized, acidification is produced by thermotolerant organisms or resistant spore-forming organisms (*Clostridium*, *Bacillus*). When heterofermentative lactic acid bacteria intervene, gas is released, which can lead to the formation of a honeycomb curd.

• **Proteolysis:** this is favored by prolonged storage at low temperatures. Proteolysis can manifest itself directly through the odor and a slight alkalinization of the milk: the germs incriminated are *Micrococcus*, *Alcaligenes*, *Aeromonas*, *Bacillus*, *Clostridium*, *Pseudomonas*, and other common Gram-negative bacteria. These microorganisms intervene directly or through the action of their thermostable enzymes. For *Pseudomonas fluorescens*, for example, a load greater than 5.10^6/mL must have been reached for the proteolytic enzymes to have an effect on the milk. Proteolysis can also develop on curds resulting from acidification: it then causes the digestion of this curd.

• **Stringing:** this can be due to non-bacterial agents (excess cream, coagulation of lactalbumin by heating), indirect microbial action (transmission of leukocytes and fibrin into the milk following mastitis), or direct microbial action. It is then caused by the mucilaginous capsules of bacteria such as *Alcaligenes viscosus*, *Micrococcus*, *Enterobacter*, or *Leuconostoc*, which thrive at low temperatures **(Chatelin and Richard, 1983)**.

• **Other degradations:** *Pseudomonadaceae* and spore-forming bacteria (*Bacillus cereus*) can denature fat by oxidation of unsaturated fatty acids, hydrolysis, or both. Other germs, *Pseudomonas fluorescens* or *Alcaligenes faecalis*, can cause significant alkalinization with the formation of urea, ammonia, and carbonate. *Lactococcus lactis* var *maltigenes* can give milk a caramel flavor. Finally, pigmented microorganisms can cause unwanted discolorations: blue (*Pseudomonas syncyanea*), yellow (*Flavobacterium*), or red (*Brevibacterium erythrogenes*).

II.1.3. Microbiology of Seafood

The microbiology of the aquatic environment significantly influences the microbiology of fish, mollusks, and crustaceans.

The water in rivers, lakes, and ponds contains a significant amount of flora. These waters can be extremely polluted by human and animal waste and therefore contain pathogenic germs (*Salmonella*, *Shigella*, *Vibrio*, *Clostridium perfringens*, etc.). Seawater contains flora similar to that of freshwater, but this flora is adapted to salinity conditions. Excluding coastal waters, the sea is often less polluted than most river waters. The flora of seawater varies depending on many factors: proximity or distance from the coast, surface area or

depth, temperature, currents, etc. It contains germs belonging to the Gram-saprophyte flora (*Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Vibrio*, *Halobacterium*, *Photobacterium*, etc.) and the genera *Micrococcus*, *Sarcina*, *Corynebacterium*, etc. In coastal areas, the micrococci load can be very abundant, and *Enterobacteriaceae* (coliforms, *Proteus*, and sometimes *Salmonella*) are also found. *Clostridium botulinum* is common in certain maritime areas (type E in the Baltic Sea). In addition, viruses (bacteriophages and enteroviruses) and animal and plant parasites can be found in seawater **(Caplice and Fitzgerald, 1999)**.

II.1.3. 1. Microbial flora of fish

The meat of fish and crustaceans is rich in water, histidine, non-protein nitrogen, phosphorus, and vitamins. It contains few carbohydrates. The flora of these products is strongly influenced by that of the aquatic environment. In addition, a specific flora is found. Normally, the flesh of fish or crustaceans is sterile: the contaminated areas are the gills, the mucus covering the skin, and the digestive tract. In crustaceans, in addition to the digestive tract, the shell supports significant microbial pollution.

The surface flora of saltwater fish and crustaceans consists of bacteria belonging to the genera *Pseudomonas*, *Achromobacter*, *Aeromonas*, *Flavobacterium*, *Serratia*, *Sarcina*, *Proteus*, *Vibrio*, *Bacillus*, *Corynebacterium*, etc. The flora is more or less psychrophilic depending on the usual water temperature. In freshwater, we also find *Lactobacillus*, *Alcaligenes*, *Streptococcus*, and *Brevibacterium*. This surface flora varies greatly in terms of quantity (from 10^2 to 10^6 cells/cm^2). The intestinal flora is always composed of bacteria belonging to the genera *Achromobacter*, *Pseudomonas*, *Flavobacterium*, *Escherichia*, *Clostridium*, *Vibrio*, etc. Contamination occurs through the environment and handling. Fish sometimes have their own pathogenic flora, including bacteria (*Vibrio parahaemolyticus, Mycobacterium balnei*, *Nocardia asteroides*, etc.), viruses, fungi (*Ichthyophorus*, *Saprolegnia*, etc.), and helminths. The diseases caused by these germs in fish are important from an economic point of view; they are less so from a health point of view for consumers.

II.1.4. Microbial spoilage

Spoilage of aquatic products is due to tissue enzymes and microorganisms. The latter play a very important role. Many factors determine the modalities of microbial spoilage:

- Fish variety, flesh pH, fat content;
- Fish habitat, type and extent of bacterial contamination;
- Fishing and storage conditions: aerobic or anaerobic packaging that can result in tissue crushing;
- Presence or absence of heading and gutting, which, if carried out under poor conditions, have a high incidence of contamination;
- Microbiological quality of ice and wash water; storage temperature, etc.

As with meat, cross-contamination can occur between the upstream and downstream stages of a processing chain. The impact of these factors is similar to that of other meat products. Microbial degradation comes from the surface flora and the intestinal flora: the latter can invade the tissues after autolysis of the viscera, hence the importance of rapid evisceration. At low temperatures, the most active germs are *Pseudomonas*, *Achromobacter*, *Aiteromonas* and *Flavobacterium*; at ordinary temperatures, *Micrococcus* and *Bacillus* are

involved. In other cases, coliforms, *Proteus*, *Clostridium*, etc. are involved. These germs are responsible for bad taste, bad odors, souring, coloring or discoloration, degradation and especially putrefaction of proteins and fats. Spoilage most often results in the release of ammonia and amines such as trimethylamine (TMA), which can be measured globally (or total volatile basic nitrogen (TVBN). H_2S, dimethyl sulfide, methyl mercaptan, and other foul-smelling compounds are also produced. Toxic amines such as histamine can be formed from amino acids (this is common in certain fish families: tuna, scombridae, and clupeidae) **(Chauvin, 2005)**.

II.2. Technological floras (meat products, dairy products)

Floras of technological interest are used in various industries, particularly in the food industry, for a variety of purposes. They evolve and contribute to the development of aromatic distinctiveness, health benefit, and even safety.

III. Fermentation

III.1. Definition

Fermentation is a metabolic process that generally converts carbohydrates into acids, gases, or alcohols to extract some of their chemical energy while reoxidizing the coenzymes reduced by these reactions.

III.2. Types of fermentation

III.2.1. Lactic fermentation

III.2.1.1. Definition

It is an essential step in the production of cheese and yogurt, as well as many fermented plant products (silage, sauerkraut, olives, pickles, etc.) and charcuterie (sausage, ham, etc.). In addition to their organoleptic role (acidification, aromatic by-products), lactic fermentation plays a major role in stabilization (by lowering pH and promoting antibiosis) and, above all, a major role in food quality: lactic ferments are sources of growth factors. Lactic acid is prepared industrially for use as a food additive. Depending on the case, it is used. Homo- (*Streptococcus*, *Lactococcus*, certain *Lactobacillus*, etc.) or heterolactic bacteria (*Leuconostoc*, other *Lactobacillus*, etc.) or a mixture, and much more rarely, molds.

Technological floras can naturally impart a specific flavor to foods. This is the case, for example, for the taste of butter, which is opposed by the lactic acid bacteria of diacetyl. Recently, diacetyl has also been used as a natural flavoring to replace the use of butter itself in the formulation of a food product. Another example of an aromatic molecule: lactones, which can be used to obtain several flavors, such as fruity flavors, for example, or even meaty flavors.

III.2.1.2. Fermented milk

III.2.1.2.1. Acidified milk

It is produced by adding *Lactobacillus casei* subsp. *acidophilus* to milk. Indeed, this bacterium causes acidification of the environment by producing lactic acid from the lactose used. Acetaldehyde is also produced, giving fermented milks their distinctive taste.

In addition, *Bifidobacterium* sp. may be added. This bacterium is present in our intestines, and its addition improves the product's digestibility.

Production is carried out using skimmed milk previously heated for 1 hour at 93°C. The product is then cooled to a temperature of 37°C, at which point the *Lactobacillus* is inoculated to initiate lactic acid fermentation **(Branger *et al.*, 2007; Shiby and Mishra, 2013)**.

III.2.1.2.2. Alcoholic fermented milks

These milks undergo a double fermentation: lactic acid fermentation like other fermented milks, but also alcoholic fermentation. To achieve this, several microorganisms are used: lactic acid bacteria and yeasts **(Branger *et al.*, 2007; Shiby and Mishra, 2013)**.

III.2.1.2.3. Examples

III.2.1.2.3.1. Kefir

This is the most famous of the alcoholic fermented milks.

A. Fruit kefir

Fruit kefir (or water kefir) is a refreshing, sparkling, and watery beverage originating from muslim populations settled in the north Caucasus. This beverage is produced from sugar, lemon, and dried fruits such as figs, as well as fruit kefir cultures called kefir grains. It differs from milk kefir, where kefir grains are added to milk.

Kefir was widely consumed since its discovery, but disappeared after world war II. With the emergence of many healthy and beneficial organic products, it is making a comeback for its health benefits. Indeed, the word kefir is of Turkish origin and means "to feel good".

B. Kefir grains and composition

Kefir should not be confused with kefir grains (figure 3). Kefir grains are used to seed the beverage to produce kefir. They appear as small, transparent grains (2 to 6 mm). Several scientific studies have demonstrated that the matrix of fruit kefir grains contains a polysaccharide called kefiran, which embeds the microflora. Kefiran is composed of D-glucose and D-galactose and is responsible for the cohesion of the grains.

Figure 3: Fruit kefir and kefir grains

The microflora of kefir grains consists of multiple lactic acid bacteria, acetic acid bacteria, and yeasts. These bacteria were identified by PCR (Polymerase Chain Reaction) and 16S DNAr sequencing, and yeasts were identified by Fourier Transform Infrared Spectroscopy (FTIR) (table 3) **(Franzetti *et al.*, 1998 ; Galli *et al.*, 1995 ; Horisberger, 1969 ; Lutz, 1999 ; Neve *et al.*, 2002)**.

Table 3: Bacteria and yeasts present in kefir grains **(Gülitz *et al.*, 2011)**

Genus	Species	
Lactic acid bacteria	* *Lactobacillus casei* * *Lactobacillus hordei* * *Lactobacillus nagelii*	* *Lactobacillus hilgardii* * *Leuconostoc mesenteroides* * *Leuconostoc citreum*
Acetic acid bacteria	* *Acétobacter fabarum*	* *Acétobacter orientalis*
yeasts	* *Lachancea fermentati* * *Saccharomyces cerevisae*	* *Zygotorulaspora Florentina* * *H. valbyensis*

According to Gülitz et al. **(2011)**, the fruit kefir consortium is composed of approximately 10^8 *Lactobacillus*, 10^6 acetic acid bacteria, and 10^6 yeasts per gram of kefir grains.

C. Symbiotic interactions of kefir microorganisms

The various microorganisms, yeasts and bacteria, in kefir live in symbiosis. This is the coexistence of two different species, the association of which is beneficial for both species. There is a metabolic dependency between them, resulting in exchanges necessary for their development.

To study the synergy between yeasts and bacteria in kefir, it developed a co-culture model system. This study demonstrated that co-culture increased cell yield. This is why the interaction between the various microorganisms in kefir is defined as mutualistic, meaning that they each benefit from this relationship.

For example, *Zygotorulaspora florentina* releases arginine, which is necessary for the growth of *Lactobacillus nagelii*, and the growth of *Zygotorulaspora florentina* is possible thanks to the acidification of the medium by the *Lactobacillus*.

D. Kefir production process

Ingredients and their necessary contributions

The bacteria and yeasts present in kefir grains require carbon compounds, nitrogen compounds, minerals, and vitamins to grow. Sugar is the carbon source, and therefore the energy source, present in the growth medium. Dried fruits are also a source of energy, particularly by providing fructose, but they also provide the vitamins, minerals, and nitrogen compounds necessary for the development of microorganisms. Lemon provides the acidity of kefir.

Production Technology

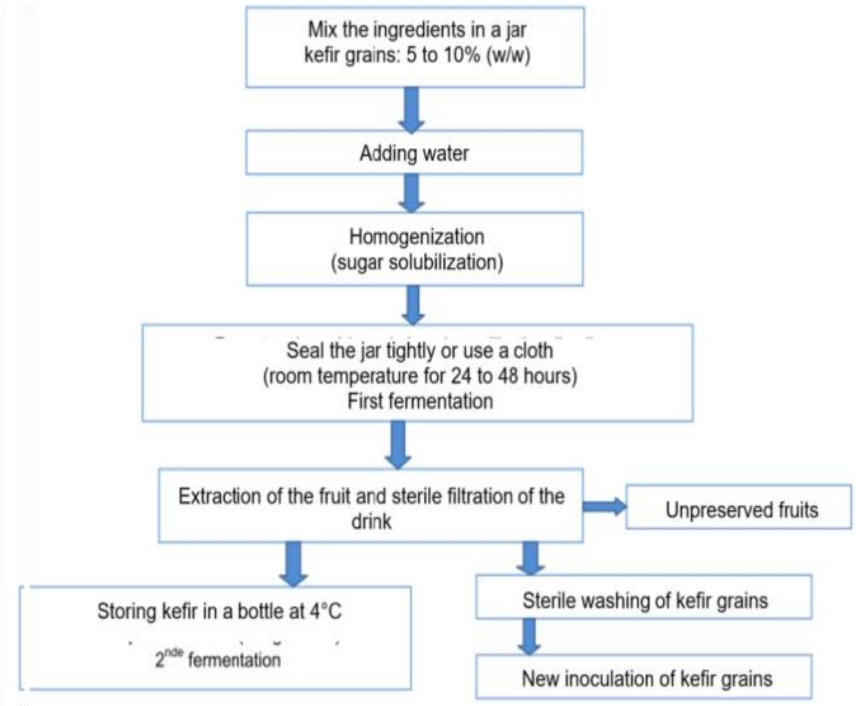


Figure 4: Fruit kefir production process

Kefir can be prepared from bottled mineral water or tap water. Tap water must first be boiled to remove any contaminants, then cooled before production to avoid destroying the microorganisms in the grains. The fruits used must be washed, and utensils should preferably be made of glass, wood, or plastic, as metal can alter the taste of the drink.

Hygiene must also always be maintained by using sterile equipment **(Bauwens, 2010)**. According to the traditional kefir production process (figure 4), kefir grains (5 to 10%, w/v) are mixed with dried fruit (4 dried figs per 1 L), lemon (half a lemon cut into quarters per 1 L), and sugar (50g per 1 L) in a jar. Then, water is added. The first fermentation takes place for 24 to 48 hours at room temperature. After this, the grains and fruit are separated from the resulting kefir. It is placed in a bottle where the second fermentation takes place. It can be stored at 4°C for up to one month. The kefir grains are dried for future use.

It is possible to suspend the proliferation of the grains during a period when the kefir is not being produced.

If this period is short, such as a few days, the grains should be placed in a container, covered with water, and a little sugar added. The container should be covered with a cloth and placed in the refrigerator. For a longer period, the kefir grains should be left to dry in a cloth for about a week in a dry, warm place. The dried grains can be stored in an airtight container. To reactivate the grains, it is necessary to soak them in regularly renewed water **(Bauwens, 2010)**.

E. Fermentation and environmental conditions

The lactic acid bacteria present in kefir grains are heterofermentative. They ferment carbohydrates into lactic acid, ethanol, and carbon dioxide (figure 5). These bacteria help slightly acidify the environment and produce aromatic substances. The yeasts also break down the carbohydrates present in the beverage into ethanol and CO_2 through a fermentative metabolism. The resulting fermentation is called alcoholic and its reaction is as follows:

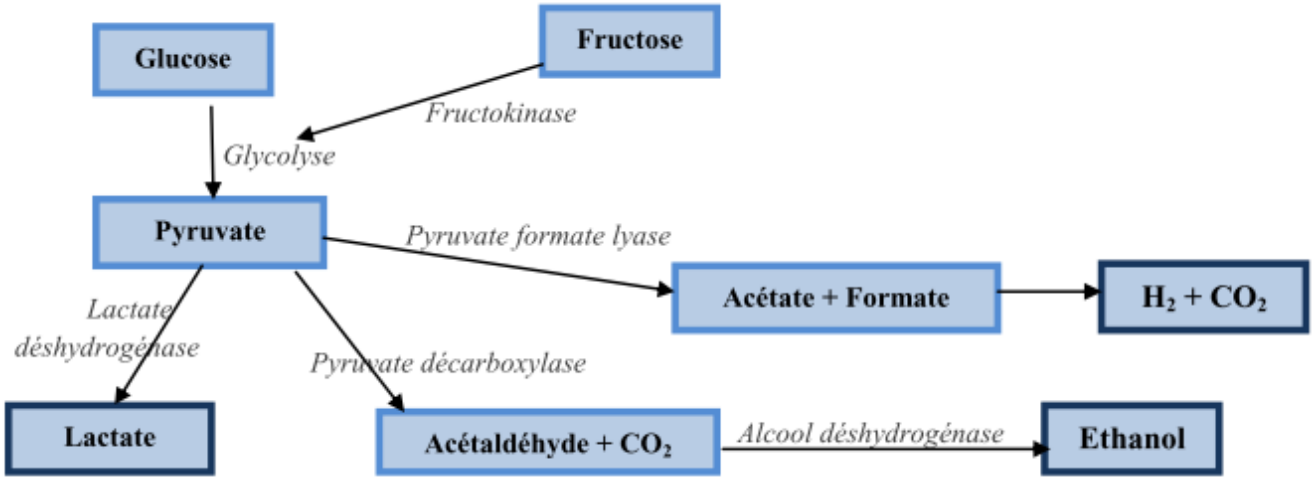


Figure 5: Diagram of glucose metabolism by kefir bacteria

The first carbon dioxide bubbles can be observed after a few hours. After 48 hours, the kefir can be consumed. It contains between 0.5 and 2% alcohol. For a very low-alcohol drink, such as for children, ferment for only 24 hours and cover the jar with a cloth. Indeed, the longer the fermentation, the more alcoholic and acidic the kefir will be. During alcoholic fermentation, other compounds (higher alcohols, acids, etc.) may be produced, but in smaller quantities. These products are responsible for the flavors of the kefir, i.e., all the olfactory and taste sensations experienced when tasting it **(Stadie *et al.*, 2013)**.

A second fermentation occurs spontaneously when the filtered kefir is poured into an airtight bottle. It continues to ferment without the grains for 24 to 48 hours. This second fermentation enhances the flavor and digestibility of the beverage.

The medium develops at room temperature, which corresponds to the optimal temperature for the growth of yeast and bacteria in kefir grains (between 18 and 25°C). The pH of the medium should be between 3 and 4.6, which is acidic **(Kebler, 1921)**.

The acidity of the environment, necessary for growth, is provided by the lemon. Under these conditions, and during fermentation, the bacteria and yeasts will multiply, and the kefir grains will increase in size. The grains will then divide and separate to continue their growth.

F. Nutritional benefits of kefir

Fruit kefir grains can be considered probiotics, meaning they are beneficial to the body. First of all, thanks to the bacteria and yeasts in the kefir grains, the drink is brimming with multiple vitamins (B, C, D) and minerals, which help combat problems such as stress and depression.

Furthermore, kefir has diuretic, cleansing, and regenerative properties. For example, it aids digestion, lowers cholesterol levels, restores intestinal flora and transit, and many other benefits.

To obtain the best possible benefits, it is necessary to drink kefir 1 to 3 times a day, up to ½ liter. During convalescence, it will allow the body to regenerate. Numerous scientific articles have demonstrated the nutritional benefits of kefir. Many studies focus on milk kefir but few on fruit kefir **(Stadie *et al.*, 2013)**.

Nevertheless, a study conducted by Bolla et al. **(2013)** aimed to highlight the benefits of kefir on intestinal flora. To do this, they infected hamsters with *Clostridium difficile*, a Gram-positive anaerobic bacterium that causes diarrhea. One group of seven hamsters received a placebo, while another received a mixture of kefir microorganisms. At the end of the treatment, it was observed that 6 of the 7 animals receiving the placebo developed diarrhea, while only 1 animal treated with kefir did. The results of this study therefore prove that kefir can prevent diarrhea caused by *C. difficile*.

Kefir is composed of yeasts and bacteria that adapt to the ingredients used to make the drink, resulting in a preparation that is never identical. Furthermore, it is possible to vary the basic recipe by swapping one component, such as water for tea, or by adding other fruits, such as apricots, dried dates, or fresh fruit. Thus, each preparation is unique. This drink is not a "miracle product," but it helps restore our body's balance during times of stress or deficiency, for example. Finally, it's important to remember that the grains proliferate very quickly. Therefore, don't hesitate to share kefir grains with friends and family so that kefir can be passed down from generation to generation to benefit from its healing properties **(Kurtzman and Robnett, 2003)**.

III.2.1.2.3.2. Koumis

This alcoholic fermented milk is similar to kefir, but the latter was originally made from mare's milk. Today, it is made from cow's milk. Lactic acid bacteria ferment the lactose and release lactic acid, and yeasts use the lactose to produce ethanol; this is called alcoholic fermentation. However, the alcohol content remains low, around 2%. During its production, the milk is heated to 28°C and inoculated, making sure to incorporate air at the same time. The product is then incubated for 2 hours **(Branger *et al.*, 2007; Shiby and Mishra, 2013)**.

III.2.1.2.3.3. Buttermilk

Buttermilk is made in two ways:

- either from whey. This whey is recovered after churning fresh milk during butter production.

- or from fresh milk directly with the addition of ferments.

These added ferments belong to the *Lactococcus lactis* species. They enable the production of lactic acid and thus the acidification of the product. In addition, they also allow bacteria such as *Leuconostoc cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* to develop the flavor of the product. It produces diacetyl, which gives buttermilk its nutty flavor. It is produced from pasteurized, low-fat milk (2% fat) at approximately 22°C **(Branger *et al.*, 2007; Shiby and Mishra, 2013)**.

III.2.1.2.3.4. Sour cream

It is obtained from cream (18% fat) that has undergone fermentation by homofermentative lactic acid bacteria. Fermentation lasts for 9 hours at 20°C **(Branger *et al.*, 2007; Shiby and Mishra, 2013)**.

III.2.2. Acetic acid fermentation

This fermentation is unique in that it is carried out by aerobic strains (*Acetobacter*, *Gluconobacter*). The presence of oxygen is necessary for the formation of reduced coenzymes (NADH, H^+). However, it is a fermentation because the bacteria cannot completely oxidize the substrate. The ethyl alcohol (ethanol) present in the medium will only be partially oxidized to acetic acid. The reaction is as follows:

Ethanol (C_2H_6O + ~~O_2~~) ~~>~~ Acetate (CH_3COOH) + H_2O + energy

The best-known application of this fermentation is the production of vinegar. Vinegar is made from a substance containing alcohol (wine, cider). To obtain vinegar, simply leave a wine exposed to the air; *Acetobacter* then colonizes the surface and produces acetic acid. Another application of acetic acid fermentation is the production of sauerkraut **(Branger, 2004; Bourdichon *et al.*, 2012)**.

Acetic acid comes from the oxidation of alcohol by atmospheric oxygen. Wine, beer, cider, and all fermented alcoholic liquids in general turn sour upon contact with air. Louis Pasteur drew on the experiments of vinegar makers of his time and the effects of fermentation to determine the nature of the ferment used. He discovered that to make a new vinegar, simply mix vinegar with wine. In his thesis Matsushita et al. **(2005)**, he shows that the ferment is a living being that he calls *Mycoderma aceti* (vinegar flower) "one would think one had before one's eyes a mass of small grains or small globules. This is not the case." With a few spots of *Mycoderma aceti* deposited on an alcoholic surface, he noted the next day or the day after that the surface was covered with a veil formed by the *Mycoderma*. He observed the multiplication in all directions of these mycodermas and subsequently carried out numerous experiments to show that *Mycoderma aceti* was the only ferment in the production of vinegar. The simplified acetic fermentation reaction is:

CH_3-CH_2-OH + O_2 → CH_3-COOH +H_2O + Energy

In 1864, Pasteur scientifically established the vinegar-making process. Several reactions actually occur to form acetic acid (figure 6):

-Formation of acetaldehyde from ethanol, catalyzed by alcohol dehydrogenase, resulting in the formation of two protons and two electrons.
-Hydration of acetaldehyde.
-Formation of acetic acid, catalyzed by aldehyde dehydrogenase, resulting in the formation of two protons and two electrons.
- The electrons are transferred to the membrane, where an electron transporter system (similar to that of mitochondria) is located. The oxygen is then reduced to H_2O.

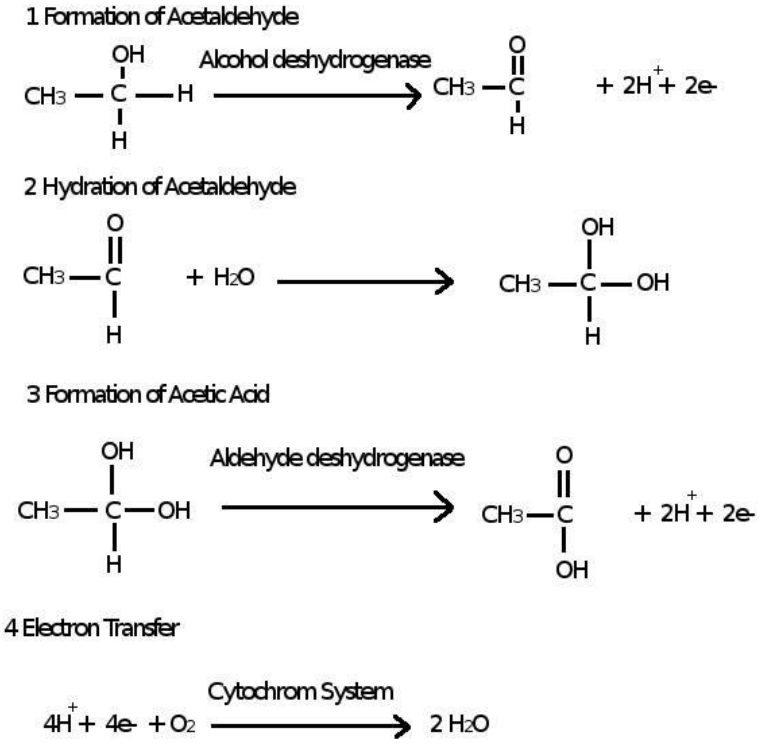


Figure 6: Acetic acid formation reactions **(Matsushita *et al.*, 2005)**

III.2.2.1. Example (vinegar production):

There are several methods for producing vinegar:

- The German method: this consists of a mixture of alcohol and beech wood shavings, which contain the bacteria necessary for fermentation. The mixture is placed in a barrel ventilated from the bottom up. The vinegar is collected at the bottom of the barrel **(Lopez, 2013)**.

- The Orléans method: this involves growing a culture of *Acetobacter aceti* by mixing wine and vinegar

in a ventilated barrel. The bacteria are then present mainly at the air-liquid interface, i.e., on the surface. This is a static culture method. Today, this method is used to produce traditional, high-quality vinegar **(Roig, 2012)**.

Since Pasteur's work, the *Acetobacter aceti* bacterium has been cultivated in a rationalized way for industrial vinegar production. The fermentation process is thus accelerated, formerly 3 weeks, it is now possible to produce large quantities of vinegar in 24 hours **(Lopez, 2013)**.

The industrial method involves the use of a bioreactor operating with a high level of aeration and bacteria immersed in the culture solution.

Vinegar can be made from a variety of raw materials, including grapes, rice, apples, berries, grains, whey, or honey.

Legislation regarding the designation of vinegar varies by country: in Europe, the acetic acid concentration must be at least 60 $g.L^{-1}$ and in the United States, it must be at least 40 $g.L^{-1}$ **(Roig, 2012)**.

III.2.3. Propionic fermentation

This is carried out by anaerobic bacteria belonging to the genera *Clostridium* or *Propionibacterium*. The bacteria ferment glucose, producing propionate and acetate. The reaction is as follows:

3Glucose ($C_6H_{12}O_6$) → 4Propionate ($C_3H_6O_2$) + 2Acetate (CH_3COOH) + 2CO_2 + energy

The ripening stages of cooked cheeses often involve this fermentation in order to give flavor to the product **(Meyer *et al.*, 2004)**. Butyric acid is synthesized by saccharolytic Clostridia such as *Clostridium butyricium* which uses glucose in the following way:

4 Glucose ($C_6H_{12}O_6$) + 2 H_2O → 2 Acetate (CH_3COOH) + 3 Butyrate ($C_4H_8O_2$) + 8 CO_2 + 10 H_2 + energy

This fermentation is characteristic of expired cans; it releases a strong rancid odor and the can swells due to the release of hydrogen. In the food industry, this fermentation, if controlled and limited, can be desirable. A typical example is its use in the manufacture of Emmental cheese, which allows holes to be created **(Meyer *et al.*, 2004)**.

*** Sourdough:** A natural yeast discovered around 4,000 years ago in Egypt. It is a symbiotic culture of yeasts and lactic acid bacteria in a mixture of wholemeal flour and water.

III.2.3.1. Propionic acid bacteria

These are Gram-positive bacteria that ferment lactates to produce acetic and propionic acid, as well as CO_2. They contribute to the flavor and opening of hard-pressed cheeses (Emmental, Comté, and Gruyère) **(Hermier *et al.*, 1992)**. They are necessary for the ripening of hard-pressed cheeses.

III.2.3.2. Role in cheese production

A second fermentation by lactic acid bacteria can occur. For example, hard-pressed cheeses such as Gruyère are seeded with bacteria of the genus *Propionobacterium*, which enable propionic acid fermentation. Lactic acid is transformed into propionic acid, acetic acid, and carbon dioxide. Both acids give cheese its distinctive flavor, while carbon dioxide is responsible for the formation of holes **(Branger *et al.*, 2007)**.

Propionic acid fermentation uses a wide variety of substrates: sugars, glycerol, lactic acid, and malic acid. Propionic acid fermentation, with lactic acid as its substrate, plays a major role in cheesemaking. The bacteria responsible for this process are the genus *Propionibacterium* (figure 7) **(Corrieu and Luquet, 2007)**.

These bacteria are divided into two categories: cutaneous and dairy, the latter being used in cheesemaking. Although they can grow on a wide variety of carbon sources and have no particular requirements for nitrogen sources, they nevertheless require an essential supply of minerals, biotin (vitamin B5), and pantothenic acid. This fermentation leads to the formation of propionate, acetate, and CO_2 from glucose or lactate and occurs anaerobically.

These ferments are commonly used in the cheese industry, particularly for the production of cooked pressed cheeses such as Emmental. The carbon dioxide released by propionic fermentation causes the cheese to open, i.e., the formation of holes in the cheese. Furthermore, the products of this fermentation contribute to enriching the flavor of these cheeses. Thus, cheese ripening is carried out at temperatures that allow the development of propionic acid flora (12°C or 24°C) **(Corrieu and Luquet, 2007)**.

At the end of ripening, high propionic acid levels (200mg/100g of cheese) are characteristic of cooked pressed cheeses. On average, a Gruyère cheese contains 222 mg of propionic acid per 100 g of cheese **(Caplice and Fitzgerald, 1999)**.

Bacteria of the genus *Propionibacterium* also have the ability to survive in different environments, ranging from cheese to fermented milk: they are resistant to fairly significant temperature variations (from 4°C during the storage of cold-ripened cheeses to 44°C during the production of fermented milk) **(Caplice and Fitzgerald, 1999)**.

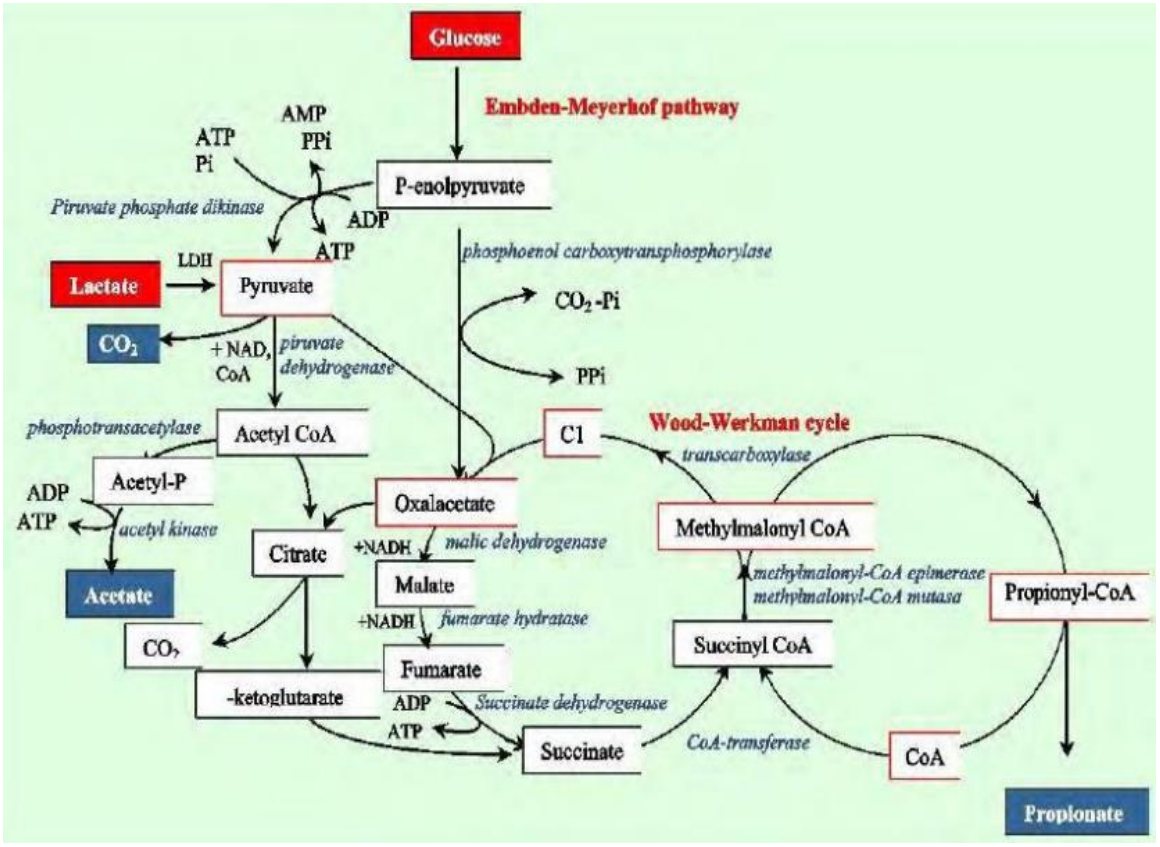


Figure 7: Propionate synthesis route **(Caplice and Fitzgerald, 1999)**

III.3. Industrial implementation of fermentation

To implement a process such as fermentation, a food company must:

III.3.1. Plan operations and procure equipment and facilities

This step is crucial because the goal is precision manufacturing processes so that the product meets expectations and is reproducible. It is necessary to procure the appropriate equipment and facilities depending on the type of product desired. **(Ray and Bhunia, 2025).**

III.3.2. Select microorganisms

When microorganisms are used in food products, they must meet certain conditions: they must not pose any risk to human or animal health or the environment, they must grow rapidly, and they must be easy to use

There are several selection criteria for microorganisms, such as substrate specificity, cost, culture type, growth rate, etc. **(Ray and Bhunia, 2025).**

III.3.3. Implement controls

Controls of raw materials, microorganisms, methods, and other important points must be implemented. This requires analyzing the manufacturing process and identifying critical points. Problem-solving procedures must be considered **(Branger, 2004)**.

IV. Factors influencing the growth of microorganisms and the growth curve

Depending on environmental conditions, bacteria can grow more or less rapidly and in greater or lesser quantities. All bacteria have the same growth curve; it is not unique to each bacterium. It is the optimal physicochemical conditions for cultivating microorganisms that differentiate them.

IV.1. The bacterial growth curve

The bacterial growth curve (figure 8) can be divided into several distinct stages, common to all bacteria **(Guespin-Michel, 2011)**.

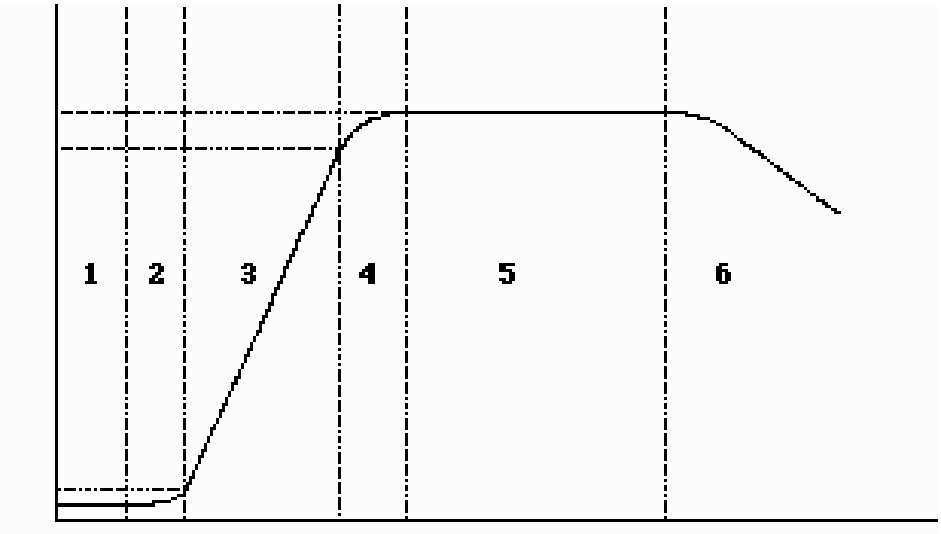


Figure 8: Bacterial growth curve

1 - Lag phase: there is no apparent growth. The lag phase explains the delay between the moment of contamination and bacterial growth in the product.

2 - Acceleration phase: the growth rate increases.

3 - Exponential phase: the growth rate is constant, the population doubles at regular intervals.

4 - Decline phase: the synthesis rate begins to gradually decrease.

5 - Stationary phase: the synthesis rate is zero, bacterial growth stops.

6 - Decline phase: the rate of lysed cells increases until it exceeds growth.

However, the duration of these stages varies depending on the bacteria. These durations can also depend on the physicochemical conditions of the culture medium for these microorganisms.

IV.2. Physicochemical conditions for cultivating microorganisms

Various parameters can be taken into account when cultivating microorganisms. These parameters can influence the increase or decrease in the risk of bacterial growth.

IV.2.1. Temperature

There are different categories of bacteria based on their temperature behavior (table 4):

Table 4: types of bacteria based on their temperature resistance

	Minimum temperature	Optimal temperature	Maximum temperature
Mesophilic bacteria	15°C - 20°C	30°C - 37°C	40°C - 43°C
Psychrophilic bacteria	-15°C	20°C	10°C
Thermophilic bacteria	25°C	45°C	50°C

IV.2.2. pH

In general, the optimal pH for bacterial growth is between 6.5 and 7.5. However, some bacteria can tolerate much more acidic pH values, corresponding to the pH of certain foods. Indeed, some strains of *Escherichia coli* can be grown in nutrient broth with a pH between 4.4 and 9. Furthermore, there are acidophilic bacteria whose optimal pH is 2; these are sulfoxidizing bacteria **(Delarras, 2007)**. The pH of foods is between 2 and 9. Therefore, the vast majority of bacteria can grow in all types of food. **(Gong *et al.*, 2024)**

IV.2.3. Aw, the amount of available water

Aw represents the amount of water available for microorganisms to carry out hydrolysis reactions that could deteriorate food. The minimum threshold required for bacteria is generally between 0.75 and 0.95 **(Delarras, 2007)**. This corresponds to the Aw of foods, which is between 0.60 and 1 for non-dry foods. This is why some foods are dehydrated to preserve them, such as milk powder, with an available water content of less than 0.60. Thus, microorganisms cannot, or only minimally, deteriorate it.

V. Microorganism control procedures and food preservation

V.1. Preservation methods

V.1.1. On an industrial scale

On an industrial scale, there are several processes that allow food to be preserved for longer periods of time. These processes follow strict regulations imposed by law. EC Regulation 2073/2005 constitutes the minimum set of criteria that must be taken into account **(Leyral and Vierling, 1997)**.

V.1.1.1. Heat treatment

Depending on the desired goal -to destroy bacteria or prevent their proliferation- the same temperatures are not used (figure 9).

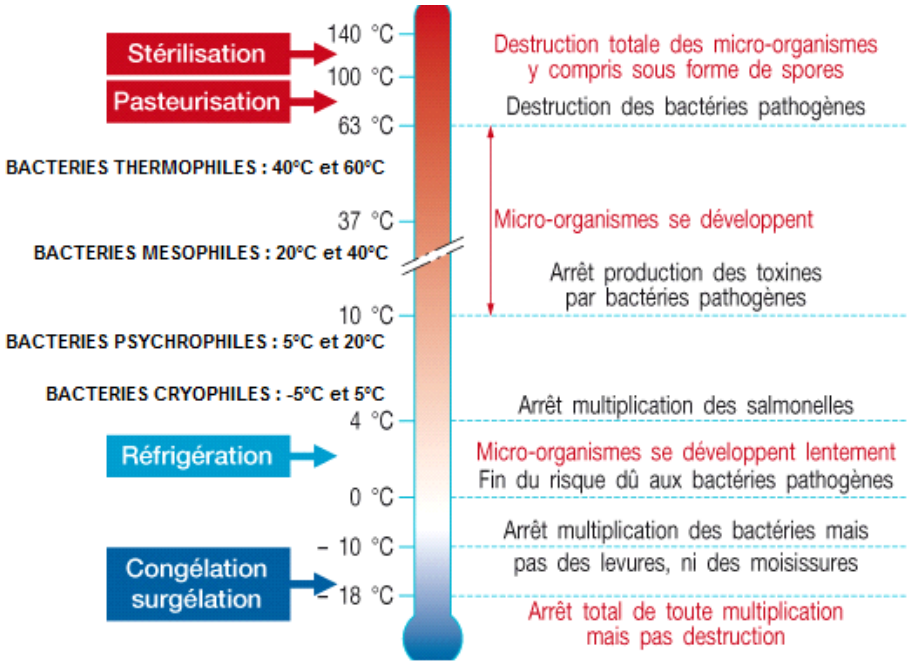


Figure 9: Effect of temperature on microorganisms

A. Heat Treatment

-Canning is one of three heat sterilization processes. It involves preserving food in a container that is impervious to liquids, gases, and microorganisms at any temperature below 55°C. This container must be treated at a temperature high enough to destroy or completely inhibit the food's enzymes, microorganisms, or toxins (table 5). Once this treatment is complete, the food can be stored at room temperature as long as the container is unopened **(Leyral and Vierling, 2007)**.

Table 5: Example of canning scales

Food	Duration (min)	Temperature (°C)
Natural green beans	2 à 4	121
Stewed peas	10 à 15	121
Sardines in oil	2 à 4	121
Natural Frankfurters	3 à 4	121
Molds	6 à 10	121

- UHT (Ultra High Temperature) sterilization concerns liquid products that must be heated uniformly in bottles. Temperature ranges range from 135 to 150°C for 1 to 5 seconds **(Leclerc, 2011)**.

Pasteurization refers to a heat treatment that more or less completely destroys microorganisms in their vegetative form. Unlike canning, these foods must then be stored at 3°C (table 6) **(Leyral and Vierling, 2007)**.

Table 6: Pasteurization scales

Food	Duration (min)	Temperature (°C)
Milk	30	62
Dessert cream	30	71
Bottled apple juice	30	77
Beer	1 à 2	82-88

The effectiveness of a sterilization process depends on: the initial microbial load, the heating time, and the chosen temperature.

Indeed, prolonged heating at moderate temperatures destroys vitamins and is not sufficient to destroy microorganisms, whereas short heating at high temperatures destroys bacteria while preserving vitamins **(Leyral and Vierling, 2007)**. Conversely, cold is used to slow or stop the growth of microorganisms without destroying those initially present.

B. Preservation by cold temperature

- Refrigeration thus inhibits the development of mesophilic germs, which develop at temperatures above 20°C, by maintaining the temperature between 0 and 4°C. The low temperature reduces the fluidity of the bacterial cytoplasmic membrane. The enzymatic reactions and exchanges necessary for its metabolism are slowed. Temperatures close to 0°C do not eliminate bacteria, but they slow the multiplication of spoilage flora and pathogenic bacteria. However, *Listeria monocytogenes* and other spoilage bacteria still multiply weakly at temperatures close to 0°C.

- Freezing, however, inhibits not only the growth of mesophilic microorganisms but also that of psychrotrophic and cryophilic microorganisms. All development stops completely. However, a rise in temperature is enough to restart bacterial growth, hence the need to maintain the cold chain **(Leclerc, 2011)**.

V.1.1.2. Other preservation methods

- When cold is not enough to slow the growth of certain bacteria, it is possible to deprive them of oxygen by placing the food in a vacuum (i.e., under an airtight film from which the air has been previously expelled).

- Dehydration also allows food to be preserved. Indeed, deprived of water, microorganisms no longer proliferate. Nevertheless, spores and some vegetative forms persist.

All of the following processes aim to reduce the water available to the bacteria.

- Candiing is used to bind the fruit's water to sugar in order to reduce its availability to the bacteria **(Leclerc, 2011)**.

- Preservatives can also be added to the medium (an additive incorporated into a food to slow the growth of the microorganisms present). These include sodium chloride, which has inhibitory properties on microorganisms when highly concentrated. Indeed, it will form bonds with water, reducing its availability

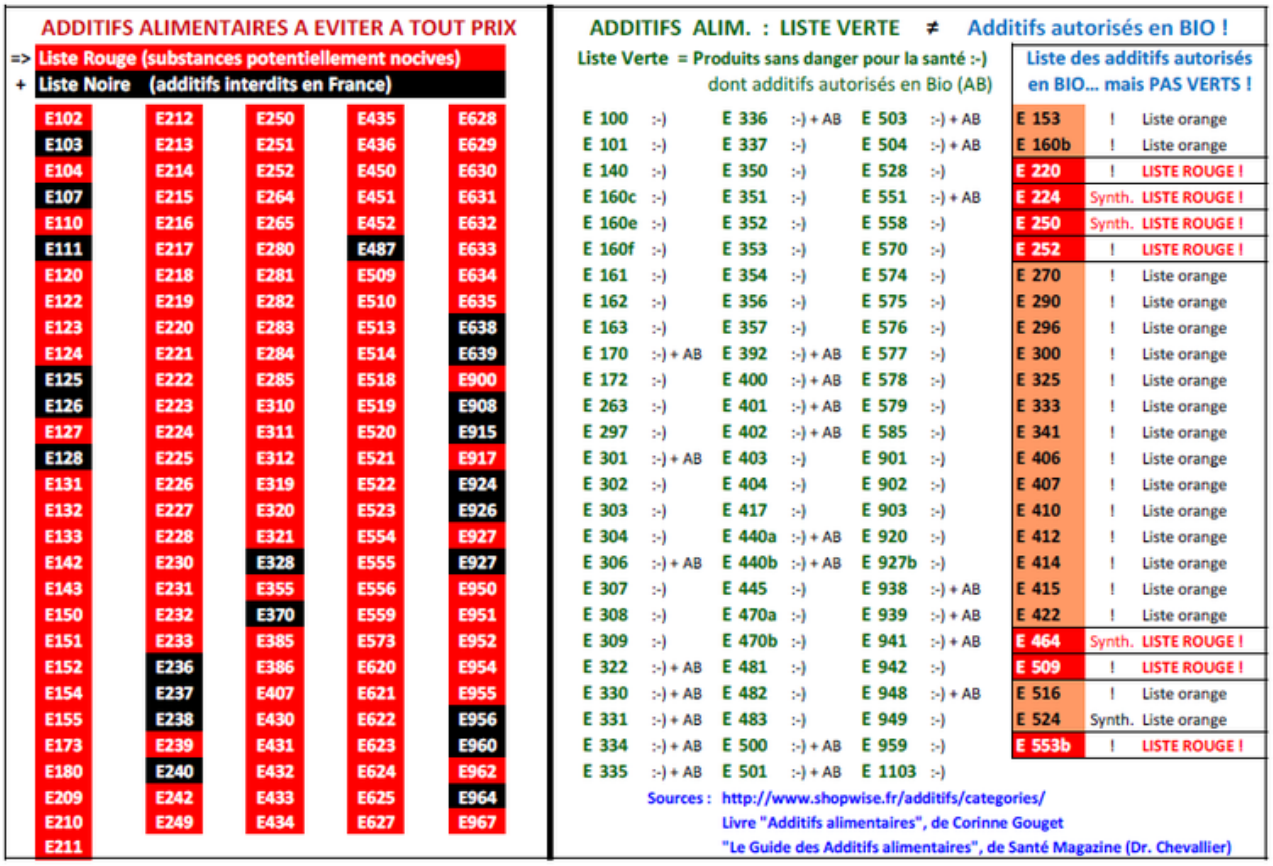
to the bacteria. This curing process is used to preserve charcuterie. However, some additives can have side effects. Sulfur dioxide, for example, which is added to wine to inhibit bacteria and mold, can cause headaches and discomfort (after drinking too much of it) **(Leyral and Vierling, 2007)**.

V.1.1.3. Preservation techniques using food additives

Food additives include preservative additives, or chemical preservatives (E200 to E297), which are used to extend the shelf life of food. Chemical preservatives do not have the ability to make a product healthy if it was not healthy before its processing, nor to improve the quality of a poor product; they can only preserve the product's initial characteristics longer than usual **(Guespin-Michel, 2011)**.

Food additives are intentionally added in small quantities to a food during its preparation to ensure better preservation or to compensate for the loss of sensory qualities. They can be of natural origin (mineral, plant, or animal), derived from the processing of natural substances, or obtained synthetically. Generally, natural molecules are often too fragile or too expensive for industrial production. They therefore give way to synthetic products. The term "additive" refers to any substance (not consumed as is) that is not a normal constituent (ingredient) of food and whose intentional addition serves a purpose that can be categorized into three categories: technological, organoleptic, and nutritional. Their use is regulated and limited to a maximum concentration of 1% except in a few special cases.

Nitrites and nitrates are often used in the preservation of cured meats, meats, and, more rarely, canned fish. They inhibit the growth of *Clostridium botulinum*. They can also help stabilize the color of meat products by complexing myoglobin. Acute toxicity: LD50 = 75-100 mg/kg. Toxicity is due to the methemoglobinizing effects of nitrites. Methemoglobinemia is defined as the transformation of myoglobin into methemoglobin (figure 10).



ADDITIFS ALIMENTAIRES A EVITER A TOUT PRIX

=> Liste Rouge (substances potentiellement nocives)

\+ Liste Noire (additifs interdits en France)

E102	E212	E250	E435	E628
E103	E213	E251	E436	E629
E104	E214	E252	E450	E630
E107	E215	E264	E451	E631
E110	E216	E265	E452	E632
E111	E217	E280	E487	E633
E120	E218	E281	E509	E634
E122	E219	E282	E510	E635
E123	E220	E283	E513	E638
E124	E221	E284	E514	E639
E125	E222	E285	E518	E900
E126	E223	E310	E519	E908
E127	E224	E311	E520	E915
E128	E225	E312	E521	E917
E131	E226	E319	E522	E924
E132	E227	E320	E523	E926
E133	E228	E321	E554	E927
E142	E230	E328	E555	E927
E143	E231	E355	E556	E950
E150	E232	E370	E559	E951
E151	E233	E385	E573	E952
E152	E236	E386	E620	E954
E154	E237	E407	E621	E955
E155	E238	E430	E622	E956
E173	E239	E431	E623	E960
E180	E240	E432	E624	E962
E209	E242	E433	E625	E964
E210	E249	E434	E627	E967
E211				

ADDITIFS ALIM. : LISTE VERTE ≠ Additifs autorisés en BIO !

Liste Verte = Produits sans danger pour la santé :-)

dont additifs autorisés en Bio (AB)

E 100	:-)	E 336	:-) + AB	E 503	:-) + AB	E 153	!	Liste orange
E 101	:-)	E 337	:-)	E 504	:-) + AB	E 160b	!	Liste orange
E 140	:-)	E 350	:-)	E 528	:-)	E 220	!	LISTE ROUGE !
E 160c	:-)	E 351	:-)	E 551	:-) + AB	E 224	Synth.	LISTE ROUGE !
E 160e	:-)	E 352	:-)	E 558	:-)	E 250	Synth.	LISTE ROUGE !
E 160f	:-)	E 353	:-)	E 570	:-)	E 252	!	LISTE ROUGE !
E 161	:-)	E 354	:-)	E 574	:-)	E 270	!	Liste orange
E 162	:-)	E 356	:-)	E 575	:-)	E 290	!	Liste orange
E 163	:-)	E 357	:-)	E 576	:-)	E 296	!	Liste orange
E 170	:-) + AB	E 392	:-) + AB	E 577	:-)	E 300	!	Liste orange
E 172	:-)	E 400	:-) + AB	E 578	:-)	E 325	!	Liste orange
E 263	:-)	E 401	:-) + AB	E 579	:-)	E 333	!	Liste orange
E 297	:-)	E 402	:-) + AB	E 585	:-)	E 341	!	Liste orange
E 301	:-) + AB	E 403	:-)	E 901	:-)	E 406	!	Liste orange
E 302	:-)	E 404	:-)	E 902	:-)	E 407	!	Liste orange
E 303	:-)	E 417	:-)	E 903	:-)	E 410	!	Liste orange
E 304	:-)	E 440a	:-) + AB	E 920	:-)	E 412	!	Liste orange
E 306	:-) + AB	E 440b	:-) + AB	E 927b	:-)	E 414	!	Liste orange
E 307	:-)	E 445	:-)	E 938	:-) + AB	E 415	!	Liste orange
E 308	:-)	E 470a	:-)	E 939	:-) + AB	E 422	!	Liste orange
E 309	:-)	E 470b	:-)	E 941	:-) + AB	E 464	Synth.	LISTE ROUGE !
E 322	:-) + AB	E 481	:-)	E 942	:-)	E 509	!	LISTE ROUGE !
E 330	:-) + AB	E 482	:-)	E 948	:-) + AB	E 516	!	Liste orange
E 331	:-) + AB	E 483	:-)	E 949	:-)	E 524	Synth.	Liste orange
E 334	:-) + AB	E 500	:-) + AB	E 959	:-)	E 553b	!	LISTE ROUGE !
E 335	:-) + AB	E 501	:-) + AB	E 1103	:-)			

Liste des additifs autorisés en BIO... mais PAS VERTS !

Sources : http://www.shopwise.fr/additifs/categories/

Livre "Additifs alimentaires", de Corinne Gouget

"Le Guide des Additifs alimentaires", de Santé Magazine (Dr. Chevallier)

Figure 10: Food additives

V.1.1.4. Controlled Atmosphere Preservation

A. Vacuum packaging

Placing a food under vacuum significantly reduces the amount of air surrounding it. This prevents oxidation of the food. Furthermore, the bacteria in the food are then deprived of oxygen and most of them are no longer able to develop normally (only a few bacteria can develop without oxygen) **(Bureau and Multon, 1989)**.

B. Modified atmosphere packaging

When packaging a product, the air surrounding it is replaced by a gas or gas mixture that depends on the type of product. For example, for salads sold in airtight packaging, the air contained in the packaging is modified (figure 11) **(Bureau and Multon, 1989)**.

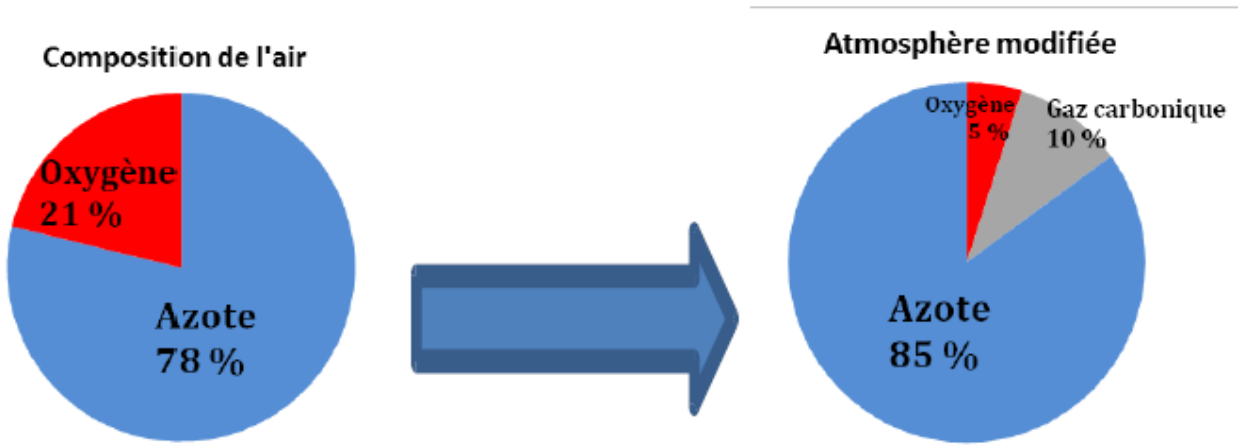


Figure 11: Comparison between gas mixtures

Leaving very little oxygen prevents the normal development of bacteria. However, a small amount is required for food cells to breathe. The 10% carbon dioxide plays an important role. When this gas enters the food cells, it is partially transformed into carbonic acid, which is a mild bactericide and therefore helps eliminate some of the bacteria. Finally, this atmosphere is supplemented by an inert gas, nitrogen **(Bureau and Multon, 1989)**.

B.1. Preservation by water removal

- Dehydration and drying

This technique involves partially or completely removing the water contained in a food. The water is then no longer available to bacteria, which are unable to proliferate. For example, drying fruit can preserve it for longer **(Hong and Gross, 2001)**.

- Freeze-drying

The principle of this process is to freeze a product and then place it under vacuum. Thus, the water it contains quickly passes from a solid to a gaseous state (this is called sublimation). This rapid elimination of water (much faster than drying or dehydration) allows flavors, aromas, and nutritional qualities to be well preserved. And since there is no longer any water, there is no longer any bacterial proliferation. Once rehydrated, the product regains almost its original texture. However, this method is more expensive than drying or dehydration. It is used in particular for feeding astronauts **(Hong and Gross, 2001)**.

- Salting

Salting consists of subjecting food to the action of salt, either by directly covering it with salt (dry salting) or by immersing it in a salt solution (brining). This technique is mainly used for preserving charcuterie and fish.

Let's take fish, for example. Fish contains water. Since salt concentrations tend to balance between two surfaces, some water will escape from the fish to decrease the external salt concentration, and some salt

will penetrate the fish to increase the salt concentration. The following highly simplified diagrams illustrate this phenomenon **(Chan *et al.*, 2022,).**

Creating this balance of salt concentrations eliminates some water from the fish. However, a certain amount remains. But when the salt ions (Cl^- and Na^+) penetrate the fish flesh, they will separate and each bind with water molecules, with which they have a strong affinity. See the diagram below **(Besson, 1993)**.

The water molecules contained in the fish will therefore be strongly attracted to the ions and will no longer be available to the bacteria. Since the bacteria are deprived of water, they will no longer be able to develop normally **(Besson, 1993)**.

V.1.2. Domestic scale

Some microbial poisonings are caused by food prepared at home, in restaurants, or in institutions **(China *et al.*, 2002)**. The main causes of these accidents are:

o Improper refrigeration of food;
o Failure to maintain the cold chain;
o Consumption of raw food;
o Prolonged food storage;
o Food handling by infected employees;
o Insufficient reheating of food;
o Maintaining food at inappropriate temperatures;
o Use of contaminated ingredients;
o Improper cleaning of work surfaces.

Food contamination causing poisoning can also occur in the food industry; however, this contamination is generally only reported in restaurants because it affects more people. **(Lennard *et al.*, 2020)**

So how can we prepare a meal while remaining hygienic? In other words, how can we eat safely?

V.2. Means of combating microorganisms

V.2.1. Kitchen equipment, utensils, and other objects: cleaning, disinfection, heat treatment

Cleaning kitchen utensils and equipment eliminates most of the dirt and germs present on their surfaces. Drying these surfaces after cleaning limits, or even eliminates, bacterial growth. Subsequent disinfection may be recommended on dirty surfaces or in the homes of at-risk individuals (babies, seniors, pregnant women) to reduce the number of microorganisms. Sodium hypochlorite, better known as bleach, remains the best and most inexpensive disinfectant available to everyone. Bleach is a very good disinfectant for sponges and other cleaning utensils. However, bleach should never be heated or even mixed with an acid (e.g., a descaling agent), otherwise it releases toxic chlorine gas. Utensils, work surfaces, dishes, and any other cleaned surface should be left to dry or wiped with a single-use paper towel or cloth **(ANSES, 2013)**.

Heat remains a very effective way to destroy most organisms (temperatures above 60°C). The current trend is to lower temperatures in dishwashers and washing machines for energy-saving reasons. It is therefore recommended to regularly wash at least 60°C, especially when the laundry or dishes are heavily soiled. It is also important to follow the operating instructions for water treatment appliances. Poor maintenance of these appliances can be a source of microorganisms.

V.2.2. Food preservation using the cold chain

Preserving a food allows the product to remain edible after a certain period of time without altering its organoleptic properties (odor, taste, color) and while limiting the risk of bacterial proliferation during primary production, processing, and home cooking. The cold chain is a way to preserve food; it is maintained at low temperatures, thus slowing the proliferation of microorganisms. **(Bai *et al.,* 2023)**

V.2.3. How to optimize food preservation in the refrigerator?

The refrigerator is divided into different zones, each characterized by a temperature range. Food must be properly distributed in the refrigerator. Organizing the refrigerator is one of the best practices to adopt to maximize food safety and avoid food waste. However, it must be accompanied by other practices **(ANSES, 2013)**:

- Regularly clean the refrigerator with a disinfected sponge and lightly bleached water.
- Respect the best-before dates on the labels of packaged products because additives simply slow the growth of microorganisms, so after a certain time they reach potentially toxic levels.

It is important to distinguish between:

-The best-before date (BBD), indicated by the words "use by..." on refrigerated products, means that the products remain edible until this date provided they are kept at a sufficiently low temperature and the packaging is neither damaged nor opened.
-The best-before date (BBD), indicated by the words "use by...", means that the food can still be consumed but its taste may be diminished.

Rehydrated foods must be refrigerated if not consumed immediately and consumed within 48 hours. Long-term cooking of dishes does not exempt you from this rule as they may contain spores, which are heat-resistant forms. **(De la cruz *et al.,* 2021)**

Leftovers should be consumed promptly or discarded if they are picnics, meals, or buffets that have been left at room temperature for too long.

Raw and cooked foods should be separated and packaged; however, cardboard overwrap, for example, should be removed to allow proper circulation of cold air **(Achour *et al.,* 1999)**.

V.3. Theoretical basis of cleaning and disinfection

Cleaning and disinfection operations aim, respectively, to remove soiling, destroy microorganisms, and eliminate residues of used chemicals. They are among the most important operations in the food and organic industries. The quality of finished products can be affected by the presence of foreign agents due to untimely microbial growth. This growth can occur from organic matter residues present in processing equipment at the end of manufacturing or from deposits formed during the processing of certain products such as milk **(AFNOR, 1989)**.

V.3.1. Cleaning

The term "cleaning" refers to all processes aimed at removing dirt or stains from a surface. In other words, cleaning is "a process of eliminating, not spreading, waste and particulate, biological, and chemical contaminants generally generated by the activity itself (personnel, process, product) and deposited on a surface. **(Frota *et al.*, 2020)**

" The **AFNOR standard (1995**) defines cleaning as "an operation that consists of removing all visible or invisible dirt from a given surface."

V.3.1.1. Cleaning methods

There are three types of cleaning:

- Manual cleaning: involves removing residues through mechanical action, with or without the chemical action of products such as detergents and disinfectants. The main advantage of this type of cleaning is that it targets critical areas of the equipment that are difficult to reach with other types of cleaning. The main disadvantage is the lack of reproducibility of the method. The effectiveness of this type of cleaning is ensured by the operator's proper application of cleaning procedures.

-Semi-automatic cleaning: requires minimal operator involvement. It involves a series of manual and automatic operations; cleaning with industrial washing machines is the best example.

-Automatic cleaning: this type of cleaning requires no human intervention; it is performed by spraying or recirculating fluids, and does not require any disassembly of the equipment.

V.3.1.2. Cleaning parameters

Cleaning effectiveness results from the combined implementation of four factors:

- Chemical action: it is produced by the use of a detergent when necessary. The detergent helps reduce traces of contaminants below the acceptable limit. This action depends on the product chosen, its dosage, and the quality of the water used **(AFNOR, 1989)**.

- Mechanical action: it plays an important role and can be manifested by friction, the abrasiveness of cleaning materials (wipes, microfibers), the jet distance, and the angle of impact. It allows for the renewal

of the cleaning solution in contact with the surface to be cleaned, thus facilitating the dispersion of the soiling in the detergent solution **(Belloin, 1993)**.

- Temperature action: it can accelerate or inhibit the cleaning effect of certain products. It accelerates chemical reactions and promotes the penetration of surfactants. It also acts on soiling by promoting its detachment from surfaces.

- Time action: the duration of chemical action, which corresponds to the contact time between the detergent product and the surfaces to be cleaned and the duration of the mechanical action, are significant.

The combined action of these four factors is called the "Sinner circle" represented in figure 12.

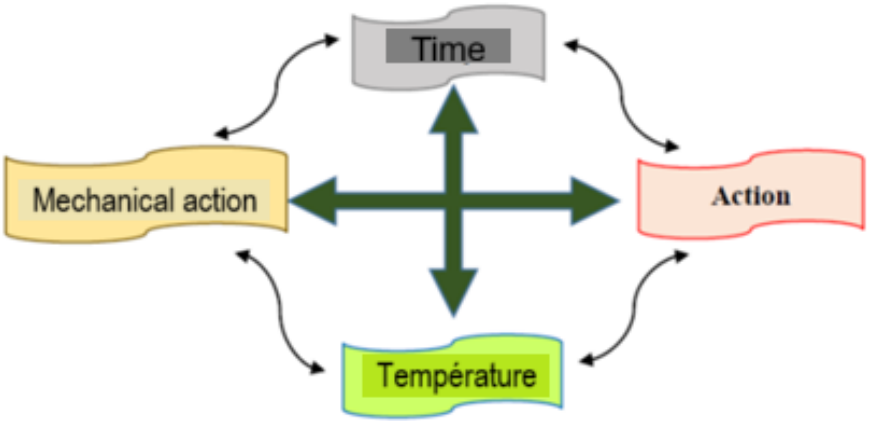


Figure 12: the "Sinner circle"

V.3.1.3. Cleaning mechanism

- Detergent selection

The ISO 862 standard defines detergent as the "physical principle" by which equipment is cleaned. This term broadly refers to cleaning.

Detergency involves a physicochemical process by which dirt or stains are detached from their substrate or support and dissolved or dispersed. Thus, a detergent is a chemical compound which, combined with physical factors such as temperature, contact time, and mechanical action, removes the soil effectively and quickly. A detergent must meet the following criteria:

- The nature of the soiling will determine the choice and type of detergent to use. A simple classification initially helps guide and direct the selection of a detergent. We can distinguish three main types of soiling (figure 13) **(Kluger *et al.*, 1981)**:

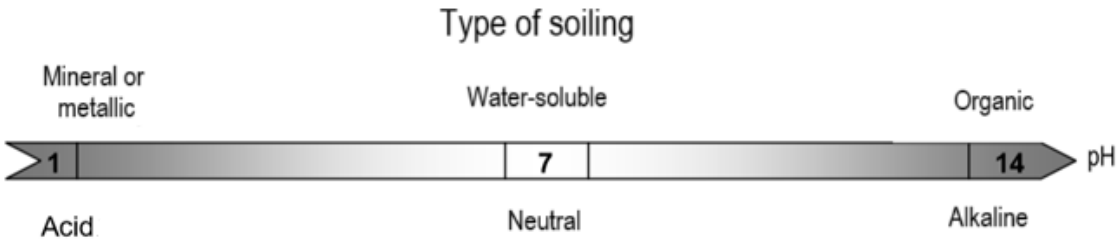


Figure 13: Choice of detergent according to the soiling

- The nature of the surface will influence the cleaning speed. Kluger et al. **(1981)** determined the cleaning capacity of different surfaces (table 7):

Table 7: Cleaning efficiency according to the nature of the surface **(Belloin, 1993)**

Eficiency

Cleaning efficiency (base of 100)	Surface type
100	Glass
80	Stainless steel
70	Aluminum
30	Rubber
20	Plastic material

Roughness

V.3.2. Disinfection

Disinfection is the process of chemically destroying microorganisms and/or inactivating unwanted viruses carried by contaminated environments **(Leveau and Bouix 1999)**. The general action of a disinfectant involves three stages:

- Attachment of the disinfectant to the cell envelope, alteration, and crossing of this envelope;
- Alteration of the cytoplasmic membrane and corresponding cellular dysfunction (exchanges, respiratory phenomena, etc.);
- Alteration of cellular constituents.

V.3.2.1. Choosing a disinfectant in the food industry

To ensure effective disinfection, the product should meet the following requirements:

- Have a very broad spectrum of activity;
- Have a long-lasting effect;
- Have equal effectiveness in the presence of soil residue;
- Be used at low concentrations;

- Be inexpensive;
- Be able to be used under very different pH and hardness conditions;
- Be non-corrosive to surfaces;
- Leave no residue after rinsing;
- Be safe, even at high concentrations, for human metabolism **(Diagne, 2013)**.

No single product combines all of these properties; the choice of a disinfectant substance from among those described will be based on a number of criteria that can be summarized as follows:

a. The legislative perspective

According to Amghar, disinfectants (basic products or developed formulations) must correspond to the positive list communicated previously **(Leveau and Bouix, 1999)**.

b. The spectrum of activity

b1. The control guidelines for technical disinfection products, as used in the food industry, are currently based primarily on the (3 x 5) test. The 3 x 5 test involves placing five bacterial suspensions in contact (for 5 minutes at a temperature of 20°C) with different concentrations of the disinfectant under study. The effective concentration is the one that determines a reduction of 10^5 in the initial bacterial population **(Martin-Delory, 1994)**.

b2. The manufacturer can select disinfectants based on other characteristics:

- Fungicidal activity. A reduction of 10^4 live mold spores and live yeast vegetative cells belonging to four specific species types is required, in 15 minutes at 20°C or 5 minutes at 50°C.

- Sporicidal activity. In this case, a reduction of 10^5 spores belonging to three species types is required in 1 hour at 21°C and/or 5 minutes at 75°C.

Interfering substances: When using disinfectants, manufacturers are sometimes faced with surfaces still contaminated by food products. These contaminants may appear:

- when the cleaning preceding the disinfection process is insufficient;
- when cleaning and disinfection processes are combined.

c. Application technique

The different methods for applying disinfectants are:

- Immersion - soaking;
- Circulation;
- Foam application;
- Spraying;
- Brushing;

- Fogging.

V.3.3. Control of cleaning and disinfection in the food industry

V.3.3.1. Surface inspections

Several methods are used:

- Swab methods: these are widely used, particularly for inspecting equipment in cutting workshops, in cheese factories on multi-molds, trays, risers, etc., at the level of valves, seals, agitator blades, etc. They are generally used to search for specific contaminating germs and are therefore qualitative. However, they can be made quantitative using a guide. Accuracy is limited by the surface condition of the support and by the resuspension of germs in the sterile liquid tube **(Diagne, 2013)**.

- Rinse methods: these are increasingly used for inspecting circuits, but also tanks or cheese equipment, bottles, etc. For circuits, they do not allow the localization of persistent contamination points.

- Pouring methods: these are specific to the testing of small containers (bottles, etc.), but require experience to achieve a consistent pouring medium.

- Impression methods: these are very well suited to flat surfaces. Germs are removed from surfaces by direct imprinting with the culture medium. These methods allow us to determine the distribution of germs on disinfected surfaces.

V.3.3.2. Ambient air monitoring

- Petri dishes with suitable media

Monitoring can be performed by opening a petri dish for a set time. Live germs give rise to colonies. This practice is unreliable since it relies solely on the sedimentation of germ-carrying particles **(Diagne, 2013)**.

- Air collection devices

A defined volume of air is sampled for a unit of time; the air is projected onto a petri dish containing a culture medium.

This technique provides good indications of ambient air contamination.

V.3.3.3. Other monitoring methods

ATP-metric methods have become widely used in the verification of cleaning and disinfection operations. ATP or bioluminescence measuring devices are based on measuring the number of photons emitted following a reaction involving the luciferin-luciferase complex and ATP. This principle indirectly makes it possible to assess the presence of organic matter (if it contains ATP) and therefore to characterize the cleaning effectiveness. The number of photons emitted is, in fact, proportional to the amount of ATP present in the sample **(Diagne, 2013)**.

VI. Food quality control

VI.1. Hygiene control in the agri-food industry

VI.1.1. Definition

A set of conditions and measures necessary to ensure food safety and wholesomeness at all stages of the food chain. We therefore see that food hygiene has two components: Food safety; Food wholesomeness.

VI.1.1.1. Food safety

The term "security" (in Latin, securitas) refers to a confident and calm state of mind of one who believes oneself to be safe from danger. This term is now used to guarantee food safety under the concept of "food security" **(Diagne, 2013)**.

Security refers to "a confident and calm state of mind of one who believes oneself to be safe from danger". For our purposes, this term is used to guarantee the safety of food under the concept of "food security" **(Boutou, 2014)**. It is the assurance that food will not cause harm to the consumer when prepared and/or consumed according to its intended use. Food security is an experience that refers to the safety of food supplies.

VI.1.1.2. Food safety

The concept of safety is different from that of safety. It applies more to the intrinsic characteristics of the product, namely taste, smell, texture, presentation, with the presence of spoilage microbes (bacteria, yeasts, and molds), for example.

The concept of safety is therefore stronger than that of wholesomeness, but the results are the same: losses. In one case (unhealthy), the product may be lost, and in the other case (unsafe), the consumer's trust may be lost **(Diagne, 2013)**.

These two components of hygiene are inseparable. The concept of healthiness is different from that of safety. It applies more to the intrinsic characteristics of the product, namely taste, odor, texture, presentation, and the presence of spoilage microbes (bacteria, yeasts, and molds). In other words, it is the assurance that food, when consumed according to its intended use, is acceptable for human consumption.

The concept of safety is therefore stronger than that of wholesomeness, but the results are the same: losses. In one case (unhealthy), the product can be lost, and in the other case (unsafe), the consumer can be lost **(Moll *et al.*, 1998)**.

Food hygiene refers to the safety and wholesomeness of food; these two components of hygiene are inseparable.

VI.2. 5M Method

How are microorganisms introduced into food?

Microorganisms are naturally present everywhere; they play an essential role in nature. Since the Neolithic period, foods fermented by naturally occurring microorganisms were recognized for their improved preservation, and developments have continued ever since. Some microorganisms are accidentally present in food and can cause food spoilage or pathologies that can even lead to death. It is interesting to understand how these microorganisms are introduced into food, whether they are useful for production or not **(Foucaud-Scheunemann and Helinck, 2009)**.

Regardless of the microorganism, it is always introduced into food during one of the 5Ms: Labor, Equipment, Environment, Raw Material, Method. Floras are divided into initial, exogenous, or technological floras. **(Chaabna *et al.*, 2024)** (figure 14).

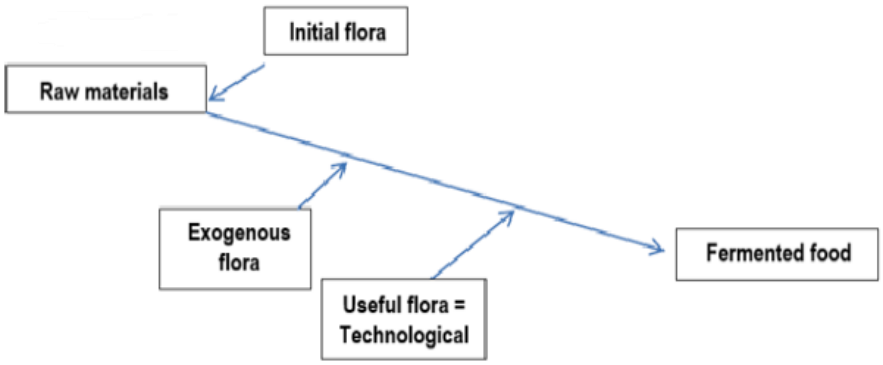


Figure 14: Floras are differentiated into initial, exogenous or technological flora

VI.2.1. Initial flora

This refers to the flora naturally present in the food. This flora is introduced during the manufacturing process when the product is added, which corresponds to the 5 M level of "raw material".

- Raw material:

Foodstuffs are not sterile and are in contact with the external environment; they therefore carry microorganisms that are introduced during manufacturing (figure 15). These microorganisms may or may not be of interest for future processing (for example, milk microorganisms used to make cheese such as Comté).

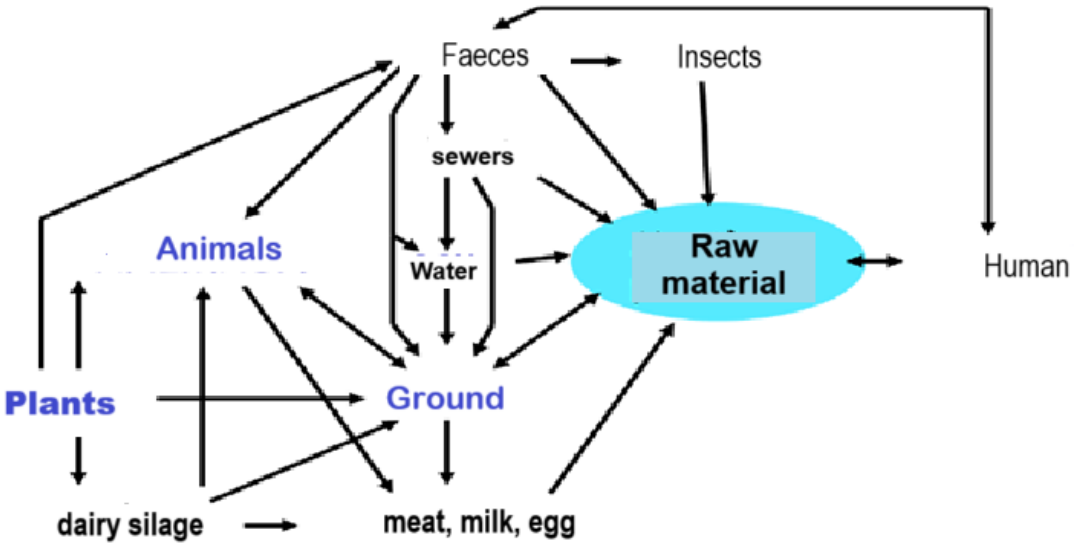


Figure 15: Origin of microorganisms present in the raw material **(Beuchat, 1996)**

VI.2.2. Exogenous flora

Exogenous flora is the so-called contamination flora. It can be introduced during the intervention of "labor", during the use of "equipment," or due to the "environment."

- Labor

Staff carry many germs (figure 16), and can therefore contaminate food through their clothing, saliva, hair, and other elements. Worker hygiene is therefore a very important issue **(Schneider, 2013)**.

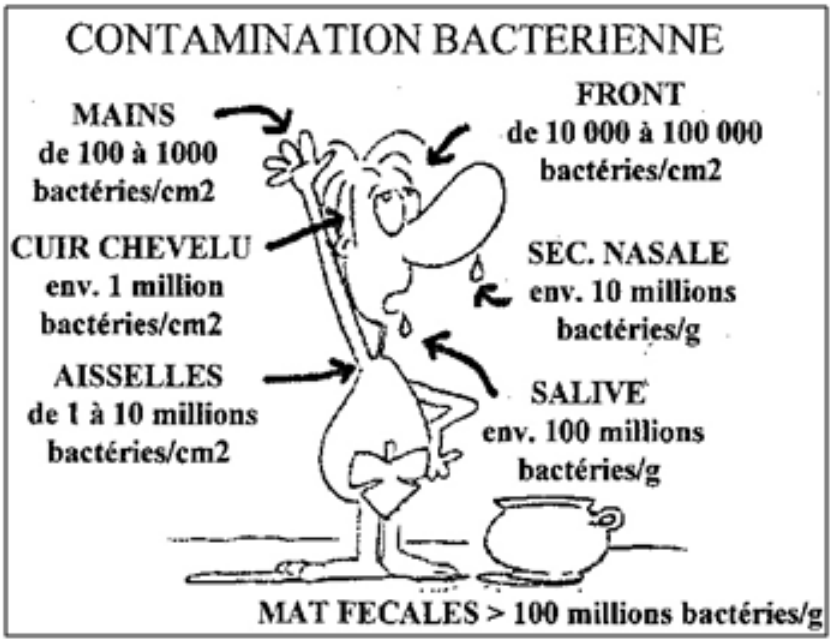


Figure 16: Personnel Contamination

- Equipment

Not all equipment used during the manufacture of a food product is sterile and therefore contributes additional flora. Proper cleaning and disinfection are necessary to prevent this introduction **(Schneider, 2013)**.

- Environment

Microorganisms in the environment can come from the air or from pests (i.e., rodents, insects, or arachnids) **(Schneider, 2013)**.

VI.2.3. Useful or technological flora

This flora is of interest to the product and is added in the "method."

- Method

Microorganisms are added during the food manufacturing process because they benefit the product, particularly microorganisms used for fermentation **(Schneider, 2013)**.

In the context of fermentation, microorganisms are called ferments: they can be molds, bacteria, or yeasts. They are necessary for the production of specific products for which a particular organoleptic aspect is desired.

Indeed, the microorganisms used to carry out fermentation metabolize, aerobically or anaerobically, substrates of different types (carbohydrates, lipids, proteins) and thus affect the properties of a food. The goal of fermentation is to modify one or more of the following characteristics **(Branger, 2004)**: aromatization, acidification, texture, external appearance, nutritional properties, stabilization and preservation.

The ferment selection process is complex and will be based on technological properties depending on the characteristics of the fermented product, and must meet preservation criteria and compatibility with other microorganisms that may be present in the product (figure 17).

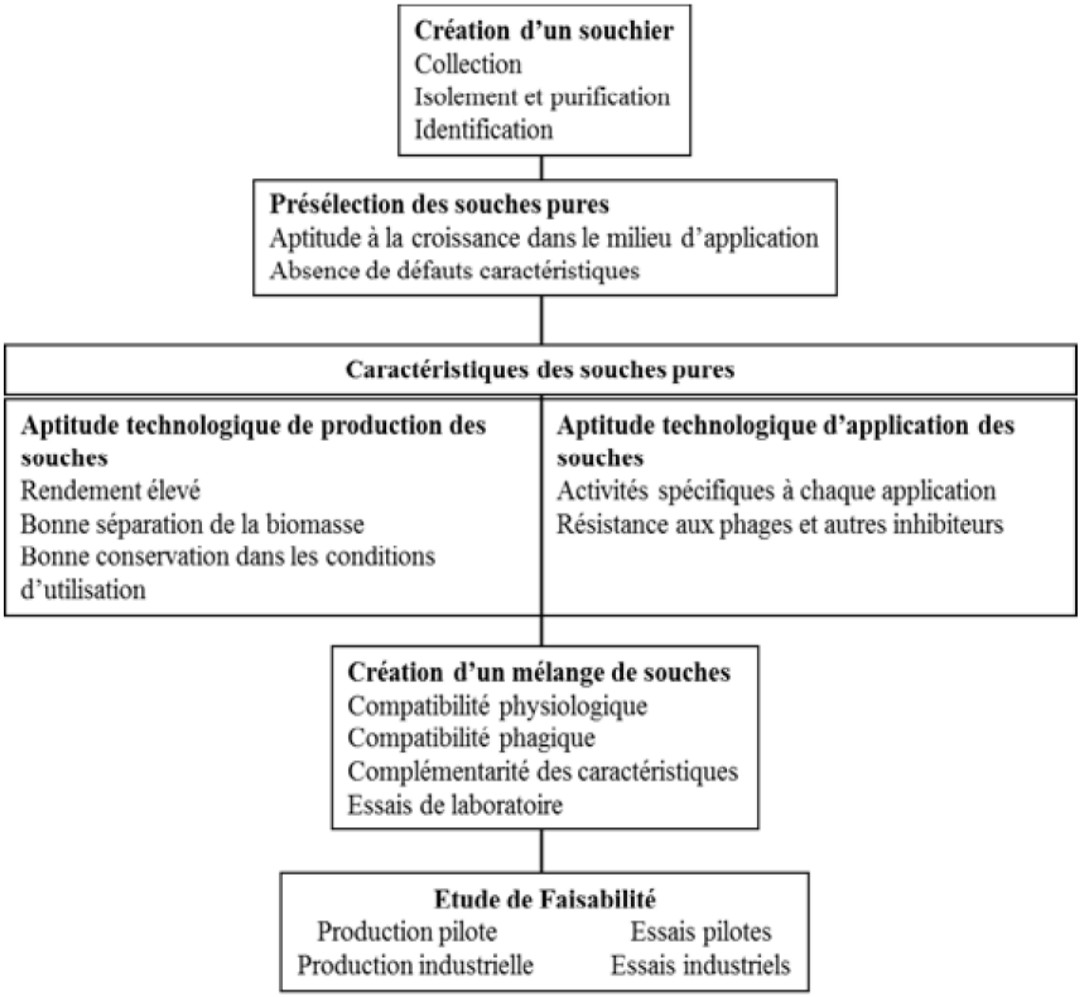


Figure 17: Strain selection phase **(Chamba *et al.*, 1994)**

VI.3. Good hygiene practices guide (GBPH)

Good hygiene practices (GHP) or prerequisite programs (PRP) cover all operations intended to ensure hygiene, i.e., food safety and wholesomeness. PRPs (or general principles of hygiene according to the Codex) include operations whose consequences for the finished product are not always measurable. PRPs provide a solid foundation for ensuring food hygiene and should be used, where necessary, in conjunction with each specific code of hygiene practice, as well as with regulations and directives governing microbiological criteria.

They apply to the food chain from primary production to final consumption, indicating the hygiene controls to be carried out at each stage. The Guide to Good Hygiene Practices (GBPH) is a reference framework based primarily on the cause-effect diagram, also known as the Ishikawa diagram (figure 18), which is a simple and effective graphical representation of all the causes and the effects they entail.

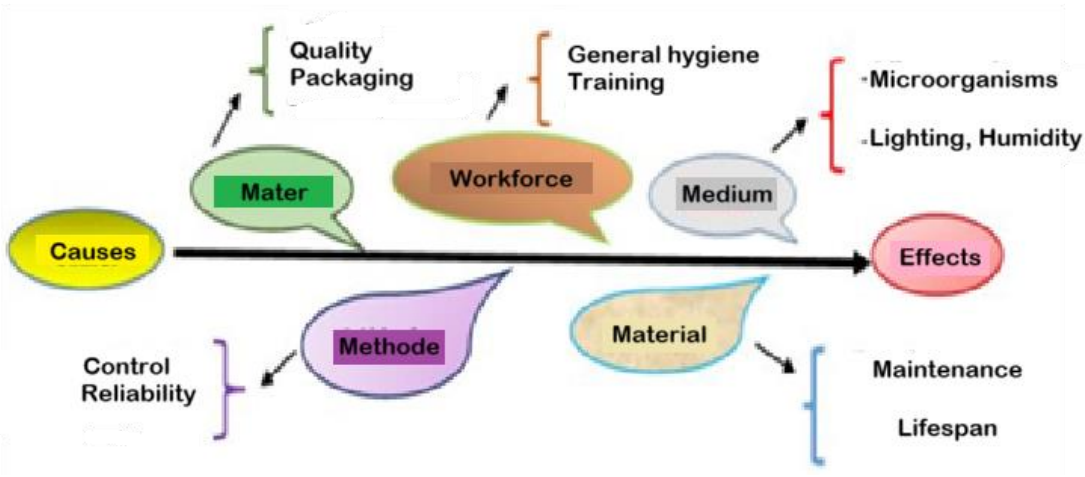


Figure 18: Cause-Effect Diagram

VI.3.1. Good hygiene practices (GHP)

The hygiene requirements applicable in the food industry are commonly referred to as prerequisite programs (PRPs), or good hygiene practices (GHP).

VI.3.1.1. Definition

Good hygiene practices cover all operations designed to ensure hygiene, i.e., food safety and wholesomeness. GHPs include operations whose consequences for the finished product are not always measurable **(Moll *et al.*, 1998)**.

Good hygiene practices (GHP) are generally grouped into seven categories:

- Personnel hygiene.
- Hygiene related to transport and storage.
- Cleaning and disinfection.
- Premises hygiene.
- Pest control.
- Waste management.

VI.3.1.2. Personnel hygiene

Upon hiring, all employees assigned to work and handle products are subject to a medical examination by the company's approved physician.

The physician issues a medical certificate to all healthy individuals and monitors them at least once a year. If necessary, particularly during follow-up visits, the physician also provides awareness-raising on personal hygiene and clothing rules. The hygiene manager is responsible for educating all newly hired employees on the hygiene rules to be observed **(Moll *et al.*, 1998)**.

VI.3.1.3. Hygiene of storage facilities

These should be isolated from production areas and must be cleaned regularly. The layout of storage racks must allow for cleaning. They must be in good condition, dry, ventilated, and weatherproof.

Toxic products essential to the company's operations must be stored in a separate room and equipped with a containment system. No contamination of storage and production premises must be possible. **(Garg *et al.*, 2021)**

VI.3.1.4. Transport and storage

Establishments must ensure that raw materials, packaged food products, and other incoming products are transported and handled in a manner that prevents any physical, chemical, and microbiological contamination **(Boutou, 2014)**.

VI.3.1.5. Cleaning and disinfection

Cleaning and disinfection must be carried out using the TACT (Temperature, Mechanical Action, Concentration, and Time) technique. **(Boyce, 2016)**

VI.3.1.6. Equipment hygiene

Establishments must use equipment designed for food production and must install and maintain it in a manner that effectively combats food contamination.

VI.3.1.7. Waste management

Food waste and other types of waste are stored outside of food storage and handling areas.

VI.3.1.8. Pest control

Raticides, insecticides, disinfectants, or any other substances that may be toxic must be stored in closed rooms and cabinets; they must be used in a manner that does not contaminate food products **(Boutou, 2014)**.

VI.3.1.9. Food hygiene, safety, and sanitation

Hygiene can be defined as the set of measures and conditions necessary to control hazards and ensure that a food product is fit for human consumption, taking into account its intended use. It also involves ensuring the safety and sanitation of food at all stages of the food chain.

It should also be noted that "food hygiene" is a medical term referring to the rational choice of foods (nutrition, dietetics) and should not be confused with "food hygiene" as defined here. We therefore note that food hygiene has two components:

VI.4. The ISO 22000 standard

The publication of the ISO 22000 standard established a model for a food safety management system that integrates both elements related to the management system itself and the fundamentals of the HACCP system and the Codex Alimentarius.

VI.4.1. Overview of the ISO 22000 standard

ISO 22000 is known and applied internationally. While it still coexists with other private standards, it has found its place. It should eventually establish itself as the one and only standard capable of coherently bringing together all aspects of food safety control and management:

- Regulatory monitoring and compliance.
- Prerequisite programs (PRPs).
- Internal and external communication.
- The HACCP method with control of PRPos and CCPs.
- Traceability.
- Product withdrawal and/or recall.
- Skills management.
- Emergency and crisis management.
- Continuous improvement.

VI.4.2. Advantages of ISO 22000

ISO 22000 offers two advantages:

- A comprehensive FSMS approach for effective hazard control.
- Applicable to all stakeholders in the food chain.

An international standard that has achieved broad consensus. ISO 22000 has been structured to be compatible and harmonized with other international management system standards, including ISO 9001. It can therefore be seamlessly integrated with existing management systems and processes within the company.

ISO 22000 can be applied by any entity directly or indirectly involved in the agri-food sector. It allows for the assessment and demonstration of product compliance with regard to food safety and the proof of control over food risks. The standard encompasses food safety based on four globally recognized principles:

1. Interactive communication. ISO 22000 emphasizes the importance of internal communication, on the one hand, aimed at the organization's members, and on the other, external communication, between customers and suppliers, the aim of which is to identify all relevant hazards related to food safety throughout the food chain and ensure that they can be properly controlled.
2. System management. This principle is based on the integration of the entire food safety management system into a single, structured management system that takes into account the organization's other general management activities

3. Prerequisite Programs (PRPs). PRPs, also known as general hygiene principles according to the Codex, provide a solid foundation for ensuring food hygiene and must be used, if necessary, in conjunction with each specific code of hygiene practice.

They ensure proper hygiene throughout the production chain, ensuring finished products are safe for human consumption. These PRPs must be implemented before any production activity.

The implementation of these PRPs allows for hazard analysis and control, and their definition in PRPs or CCPs. This minimizes the occurrence of hazards. PRPs are not selected to control specific, identified hazards, but rather to maintain a hygienic production, processing, and/or handling environment. **(Eltabakh *et al.*, 2023)**

VI.5. Overview of the HACCP system

VI.5.1. Definition

HACCP (Hazard Analysis Critical Control Point): risk analysis and critical control points, is an organized method, a systematic approach for building, implementing, or improving quality assurance specifically for a product-process pair **(Jouve *et al.*, 1996)**.

HACCP is a globally recognized, systematic, and preventive approach to food safety. It seeks to eliminate biological, physical, and chemical risks through anticipation and prevention rather than inspection of the finished product **(Bariller, 2007)**.

VI.5.2. History

The HACCP concept was developed as a microbiological safety system at the beginning of the US space program in the 1960s to ensure food safety for astronauts (e.g., to avoid currents in zero gravity). The original system was designed by Pillsbury Company, in cooperation with the National Aeronautics and Space Administration (NASA) in the United States and the US Army Laboratories.

In 1975, the method was recommended by the WHO (World Health Organization) and adopted by the Codex Alimentarius. Following the recommendations of the WHO and the Codex Alimentarius, the European Community introduced the use of the HACCP approach in Directive 93/43 of June 14, 1993, on the hygiene of foodstuffs. This European regulation was quickly transposed into French law for secondary and tertiary processed products.

The method was subsequently generalized to all agri-food sectors. The analysis of health hazards related to the activity according to the fundamental principles of the HACCP method has become mandatory since 1998 for all agri-food processing companies and companies wishing to conduct business internationally.

Historically, it targeted microbiological hazards and later took into account physical and chemical hazards **(Amgar, 2002)**. Among farms, only those engaged in processing are therefore subject to mandatory HACCP implementation.

HACCP is therefore a regulatory method but is not a standard in the French sense of the term. However, it is integrated into various standards such as the 2006 hygiene package, ISO 9001/9002 and ISO 22000, IFS, and BRC. HACCP guidelines were revised in 2003; the most recent document detailing HACCP at the international level is ISO 22000. **(Awuchi, 2023)**

VI.5.3. Objectives

Relying on the technical skills of professionals and their responsibilities, the HACCP method sets the following objectives: **(Owusu-apenten and Vieira 2022)**

- Consumer safety.
- Consumer information by indicating the product's origin: manufacturing date, shelf life, etc.
- Strengthening its quality assurance system while complying with regulations.
- Assist in the design of a new product with better nutritional, hygienic, and organoleptic quality (satisfaction, health, flavor) **(Larpent and Larpent-Gourgaud, 1997)**.

VI.5.4. Principles of the HACCP System

HACCP comprises seven principles that enable the establishment, implementation, and management of a HACCP plan. These seven principles are defined in the Codex Code of Practice:

VI.5.4.1. Principle 1: Hazard Analysis. Conduct a hazard analysis. Identify potential hazards associated with all stages of production, using a process flow chart. Evaluate the likelihood of each hazard occurring and the severity of its effects **(Senin *et al.,* 2014)**.

VI.5.4.2. Principle 2: Determination of Critical Control Points. Identify critical control points (CCPs). Determine the stages at which monitoring can be carried out and is essential to prevent or eliminate a food safety hazard.

VI.5.4.3. Principle 3: Establish the critical limit(s). The critical limit is the criterion that distinguishes acceptability from unacceptability. They must involve a measurable parameter and can be considered the absolute safety threshold or limit for CCPs.

VI.5.4.4. Principle 4: Establish a CCP monitoring system. Establish a monitoring system to control CCPs through planned tests or observations. The procedures applied must be capable of detecting any loss of control.

VI.5.4.5. Principle 5: Determination of corrective actions. Determine the corrective actions to be taken when monitoring indicates that a given CCP is not under control. Procedures and responsibilities for corrective actions must be specified.

VI.5.4.6. Principle 6: Implementation of HACCP system verification procedures. Apply verification procedures to confirm that the HACCP system is operating effectively.

VI.5.4.7. Principle 7: Establish a Documentation and Recording System. Create a file containing all procedures and records related to these principles and their implementation **(Jund, 2010)**. These principles are invariable; however, the manner in which they are applied varies depending on the nature, size, level of development, and specific characteristics of the company (figure 19) **(Senin *et al.*, 2014)**.

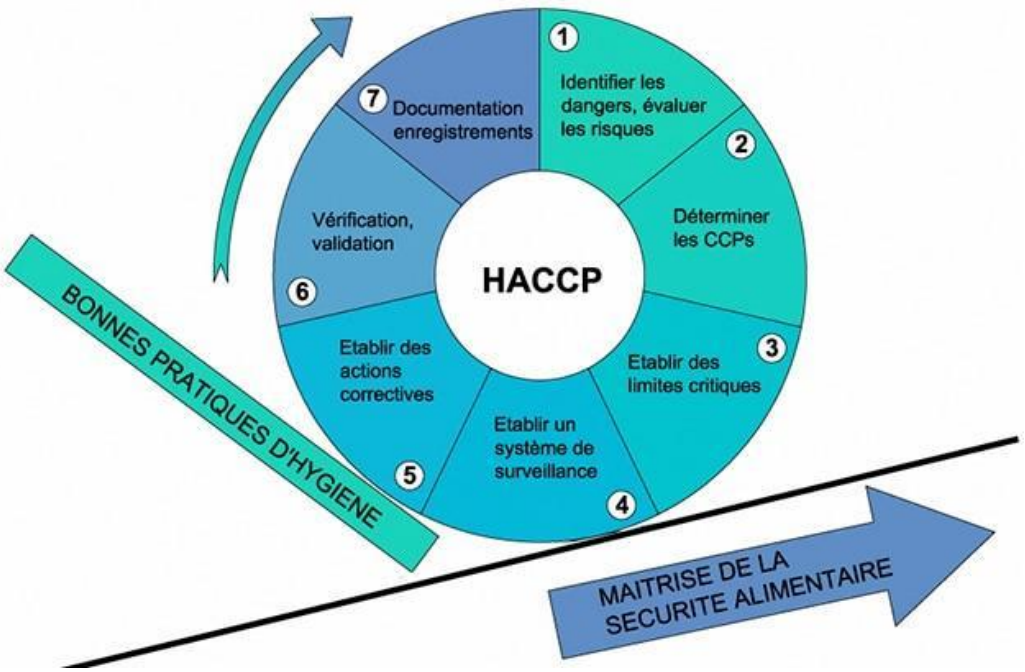


Figure 19: Principles of the HACCP system **(Jund, 2010)**.

VI.5.5. The stages of the HACCP System

The implementation of the HACCP method can be broken down chronologically, ensuring nothing is overlooked and maintaining methodological precision throughout the study. HACCP therefore represents a systematic approach focused on preventing problems rather than revealing them through the analysis of finished products. The application of HACCP principles can be summarized by following these 12 steps.

VI.5.5.1. Step 1: Establishing the HACCP team: the HACCP team must be multidisciplinary and composed of representatives from the production, quality control, and food microbiology departments.

Management must provide full support to this team. If the necessary skills are not available within the company, the company may seek the assistance of a consultant.

VI.5.5.2. Step 2: Product description. A complete description of the product, including information on its composition and distribution methods, must be provided.

- The Product

This phase is a prerequisite for the hazard assessment and requires:

- Grouping products into homogeneous families.
- Listing the raw materials and packaging used.
- Describing the product's physicochemical characteristics.

- Packaging and conditioning

Conditioning and packaging methods must be taken into account (e.g., preservatives).

- Storage conditions (theoretical methods)

- Total shelf life

This must be specified at this stage if it is a commercial imperative. "The organization must identify the legal and regulatory requirements for food safety associated with the elements mentioned above." Descriptions must be updated, particularly when required" (**ISO 22000/2005)**.

VI.5.5.3. Step 3: Description of the intended use of the product. The use of the product should be defined based on the end user or consumer. It is important to identify whether the product will be used in a way that increases the risk to consumers or whether it will be used primarily by consumers who are particularly sensitive to a particular hazard.

VI.5.5.4. Step 4: Establish a process flow diagram. The HACCP team is responsible for establishing this flow diagram. It lists the main steps of the manufacturing process.

VI.5.5.5. Step 5: On-site verification/confirmation of the process flowchart. The HACCP team should verify on-site the progress of the various production operations against the flowchart at all stages and at all times during the process and, if necessary, modify it by adopting correct times and appropriate temperatures.

VI.5.5.6. Step 6: Analyze hazards and study control measures for identified hazards

VI.5.5.6.1. Hazard listing (principle 1)

According to Jouve et al. **(1996)**, hazard analysis is a key step in HACCP. It consists of collecting and interpreting all available information on hazards and the conditions under which they occur. The term hazard should include:

- Biological hazards: microorganisms, viruses, etc.
- Chemical hazards: pesticide residues, excess additives, etc.
- Physical hazards: plant materials, metal, cardboard, glass, nails, etc.

Likely to represent a potential hazard and/or a significant hazard to public health. The term "Condition" refers to any situation where there is a risk of:

- The presence of biological, chemical, or physical contaminants at unacceptable levels in the raw material, intermediate product, or finished product.
- The production or persistence of toxins or other undesirable products of microbial metabolism at unacceptable levels.
- Recontamination of biological, chemical, or physical contaminants at unacceptable levels in the intermediate product or finished product **(Jouve *et al.*, 1996)**.

VI.5.5.6.2. The hazard identification phase

The hazards to be considered as a priority are those related to product safety. The analysis must begin with a general study aimed at defining all biological, physical, and chemical hazards consistent with the characteristics of the finished product and its manufacturing process.

For each hazard, repeat the step-by-step analysis of the raw material to identify the conditions under which it is present. For each hazard identified in the previous analysis, this involves identifying "what can go wrong" in the production system. **(Owusu-apenten and Vieira 2022)**

VI.5.5.6.3. The risk assessment phase

This phase allows for a qualitative or, preferably, quantitative assessment, for each hazard and for each identified condition:

- Severity (S): the impact of the risk on the product, customer, or company.
- Frequency (F): the probability that the risk will occur.
- Detection or non-detection of the risk.

Therefore, the risk assessment is performed by calculating the criticality:

Criticality = Severity rating (G) * Frequency Rating (F)* Detectability Rating (D)*

G, F, D, are rated from 1 to 5

VI.5.5.6.4. The preventive measures establishment phase

A preventive measure is any practice, factor, or situation that can be used to address the occurrence of an identified product. The objective of this third phase is to determine the preventive measures already in place and those that can be considered to reduce or eliminate the hazard(s) that may arise by adjusting their frequency, severity, and detection **(Jaudon, 2008)**.

It should be noted that for low-impact hazards, preventive measures are simple and consist of the application of good manufacturing and hygiene practices. This results in a relatively limited or even restricted HACCP. On the other hand, for high-impact hazards and/or conditions, beyond the implementation of good manufacturing and hygiene practices, which are always essential, it is necessary

to specifically identify the activities, techniques, and means most likely to determine control. This results in a detailed HACCP plan. **(Owusu-apenten and Vieira 2022)**

VI.5.5.7. Step 7: Identify critical control points (principle 2)

Critical control points (CCPs) correspond to the points, steps, factors, or procedures where a control measure can be applied to prevent, eliminate, or reduce a hazard to an acceptable level **(Jouve *et al.*, 1996)**.

Indeed, failure to control a CCP results in an unacceptable risk with no possibility of subsequent correction. Each CCP thus has a dual requirement:

- Specific intervention requirement (locations, means appropriate for control and the severity of the hazard and/or condition).
- Requirement to ensure (and to ensure) that the selected interventions are carried out at all times under controlled conditions **(Huss *et al.*, 1996)**.

When a serious hazard and/or condition has been identified and there is no intervention means in place or possible in relation to this hazard and/or condition, the approach must lead to adjusting or even modifying the process or product in order to introduce a specific intervention that both controls the corresponding hazard and/or condition and provides proof of this control **(Jouve *et al.*, 1996)**. The CCPs to consider are:

- Any raw material or ingredient.
- Any characteristic of the intermediate or finished product.
-Any step in the process, and within it, any point, factor, or procedure whose control is critical.

It should be noted that CCPs are often specific to a particular product, process, or operation, and their identification is intended to guide operators in developing and formalizing preventive measures on the one hand, and monitoring measures on the other **(Bourgeois, 1980)**. CCPs can be identified by using a decision framework proposed as an example by the Codex Alimentarius, which should be used with flexibility and common sense. **(Rosak-szyrocka and Abbase, 2020)**

Answer each question in turn in the order indicated, at each step, and for each hazard identified. This tool is used, but it can also be used in conjunction with another system, called the Rating System, which takes into account three criteria: 1, 3, and 5 (table 8).

Table 8: Rating system

	Gravity	Frequency	Detection
1	May be serious	Uncommon	Always detected
3	Quite serious	Common	Rarely detected
5	Very serious	Very common	Never detected

These coefficients are then multiplied together, giving us a maximum score of 125. We then determine the score from which a hazard is significant enough to be considered a critical point (15 in this case). The rating is performed by the members of the HACCP team. Generally, if the criticality is less than 15, the hazard is

not retained, unless the team deems it necessary to retain it, which provides a more accurate idea of the CCP determination. It should be noted that this method has the advantage, over the previous one, of being able to quantify the severity of the hazard **(Amgar, 2002)**.

VI.5.5.8. Step 8: Establish critical limits for each CCP and the control elements of the PRPo

Critical limits must be specified for each critical point in order to control hazards. Identifying these critical points requires the creation of a decision tree.

-Establish measures: these are the measures on which the controls for each CCP will be based. Define the critical limits and target levels for the controls:

1) Critical limits: critical limits are the values that separate a "safe" product from a "possibly unsafe" product. They are often defined by regulations or by GBPHs.

2) Target levels: the team can choose a guideline value to which it must constantly strive. For a control, we will therefore have three statuses: "compliant," "acceptable," and "noncompliant."

- Include critical limits in HACCP plans: critical limits should be included in HACCP plans.

Frequently used criteria include measurements of temperature, time, humidity, pH, available chlorine levels, and sensory parameters such as visual appearance and texture. Critical limits must be:

- Determined for monitoring purposes established for each CCP.
- Established to ensure that the identified acceptable level of the food safety hazard in the finished product is not exceeded.
- Measurable.

The reasons for selecting the critical limits must be documented. Critical limits based on subjective data (such as visual inspection of the product, process, handling, etc.) must be supported by instructions or specifications and/or initial and professional training.

VI.5.5.9. Step 9: Establish a monitoring system (principle 4)

For each CCP, a monitoring system must be established to demonstrate that the CCP is under control. This system must include all scheduled measurements or observations related to the following critical limits:

- The monitoring system must consist of relevant operating procedures, instructions, and records covering the following: measurements or observations providing results within an appropriate timeframe.

- The monitoring devices used.
- The applicable calibration methods.
- The monitoring frequency.
- The responsibility and authority associated with monitoring and evaluating monitoring results.
- Recording requirements and methods.

Monitoring methods and frequency must allow for timely determination of critical limit exceedances, so that the product can be isolated before use or consumption. The controls performed must be recorded in the HACCP plan to provide evidence internally and for external service providers. These controls must take place:

Online: i.e., at the trial and during the trial.

Offline: samples are taken for analysis, generally in a separate laboratory or even externally.

VI.5.5.10. Step 10: Establish Corrections and Corrective Actions (Principle 5)

In the context of the HACCP system, specific corrective actions must be planned for each CCP to respond to deviations as they occur. The actions taken must verify that the CCP has been brought back under control. They must also include the disposition of the affected product (recycle the product, modify the product, or destroy it). Deviations and procedures for the disposition of products must be documented in the HACCP records.

 Correction: Action to eliminate a detected nonconformity.

 Corrective action: Action to eliminate the cause of a detected nonconformity or other undesirable situation.

VI.5.5.11. Step 11: Establish verification procedures (principle 6)

This involves establishing procedures to ensure that the HACCP system is functioning properly. Monitoring and verification methods (confirmation, through objective evidence, that specified requirements have been met) and procedures and tests, including random sampling and analysis, can be used to verify that the HACCP system is functioning properly. The frequency of verifications must be sufficient to validate the HACCP system (figure 20). Verification activities include, for example:

- Review of the HACCP system and its documents;
- Review of deviations and product destination;
- Confirmation that CCPs are under control;
- Revalidation of established critical limits.

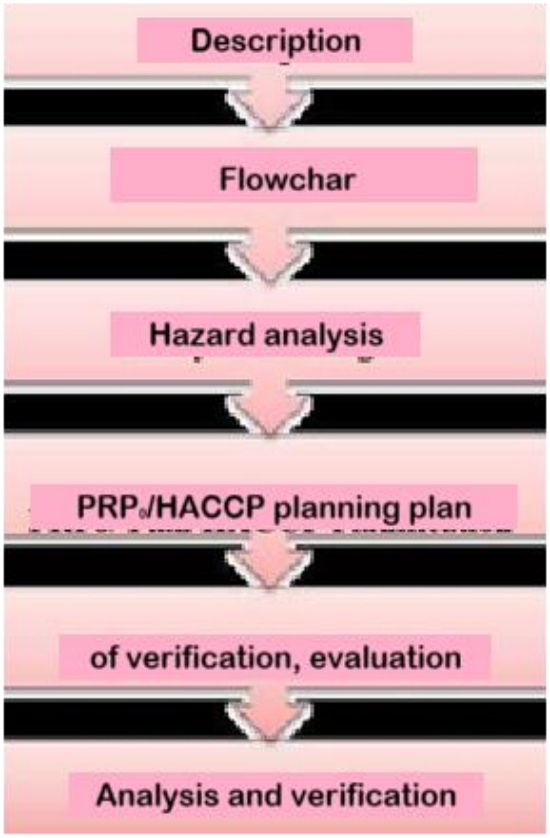


Figure 20: Place of verification in the system **(Boutou, 2014)**

VI.5.5.12. Step 12: Establish documentation and record keeping (principle 7)

- HACCP plans: the HACCP plan describes the operation of the method; it is documented and contains information relating to the control of each critical control point.

- Records: these are records of inspections, audits, corrective actions, and validation, which attest to the proper functioning of the HACCP system and food safety. The HACCP procedures relating to each step must be documented and these documents must be compiled in a manual. Records include, for example: product safety, processing, packaging, storage and distribution, records relating to deviations, and changes made to the HACCP system.

VI.5.6. The benefits of HACCP

The benefits of HACCP for those who produce, process, market, or transport food include reduced complaints, returns, and rejections for official inspection, and resource savings, and for the consumer, the ability to have safe food.

HACCP is compatible with a comprehensive quality control system, meaning that safety, quality, and productivity go hand in hand with the benefits of greater consumer confidence, greater profits for the industry, and better relationships among all those working towards the common goal of improving food safety and quality; all of this implies

a clear benefit for the health and economy of countries.

These considerations explain the importance of the HACCP method in international food trade. Its invaluable value for the prevention of foodborne diseases must also be recognized, an aspect of the utmost importance for developing countries, which must bear the brunt of these diseases and the increasingly pressing limitations of resources allocated to food safety control. **(Peristeropoulou *et al.*, 2015)**

VI.5.7. The relationship between ISO 22000 and HACCP

HACCP is a method for analyzing food safety hazards and identifying critical control points. This method was described and published by the Codex Alimentarius Commission (in 1995). Since then, it has been adopted by most food legislation. ISO 22000 fully integrates the HACCP method as described by Codex, complementing it in two areas:

- Amendments and additions to the method based on experience.
- Development of all "system" elements, entitled "Food safety management systems", ISO 22.000 is rectifiable.

VII. Main technological operations in the food industry

In recent years, numerous technological innovations have been made to make food industry processes more efficient, safer, more space-saving, less energy-intensive, and more environmentally friendly.

VII.1. General principles of food hygiene

VII.1.1. Primary production

The objective is to ensure that food remains safe and suitable for its intended use. This involves ensuring:

- Environmental hygiene
- Hygiene in food production areas:
    - by controlling contamination of air, soil, water, livestock feed, fertilizers, pesticides, and veterinary drugs;
    - by controlling the health status of plants and animals so that they do not pose a risk to human health through food consumption or negatively affect product acceptability;
    - by protecting primary production sources from fecal or other contamination.
    - In particular, waste should be treated and harmful substances stored appropriately.
    - handling, storage, and transport:
        - by sorting food unfit for consumption;
        -by protecting food and ingredients from pests, chemical, physical, or microbiological agents;
        - by implementing appropriate measures, which may include temperature, humidity, and/or other controls.
        - cleaning, maintenance, and personal hygiene operations at the primary production level. **(WHO, 2023)**

VII.1.2. Establishments: design and facilities

The objective is to ensure effective hazard control through compliance with good hygiene practices in the design and construction of buildings, in an appropriate location. This must be achieved by ensuring:

- **location**: by deciding on the location of buildings;

- **hygiene** of premises and rooms: by appropriate design and layout (control of cross-contamination); by internal structures and accessories (walls, partitions, floors, doors) that are easy to clean and made of suitable materials.

- equipment:

- by their ease of cleaning, disinfection, and maintenance; the surface in contact with food must be inert, smooth, visible or easily removable for inspection, accessible, and must be designed for complex and easy filling and emptying;

- by the choice of equipment material. The main surface materials fall into two categories: stainless steels (ferritic X6Cr17, martensitic X10Cr13, etc.), and polymers (polyethylene, polypropylene, etc.). Surfaces in contact with food must naturally be non-toxic, smooth, non-porous, and free of cracks, crevices, and holes.

- through control of food product control and monitoring equipment;
- through identification of waste containers;
- through control of the water supply;
- through drainage and waste disposal;
- through effective cleaning;
- through sanitary facilities and toilets;
- through temperature control;
- through air quality and ventilation;
- through sufficient lighting;
- through controlled storage. **(WHO, 2023)**

VII.1.3. Operational control

The objective is to reduce the risk of unsafe food by taking measures to ensure food safety. The following must be controlled:

- Food hazards:
- By identifying them at all stages;
- By implementing procedures;
- By monitoring and periodically reviewing procedures.
- Key aspects of control systems include:
- Temperature and time control;

- Specific processing steps (cooling, heat treatment, ionization, drying, chemical prevention, vacuum or modified atmosphere packaging);
- Knowledge of microbiological criteria and other specifications;
- Control of microbiological cross-contamination;
- Control of physical and chemical contamination;
- Requirements for raw materials and packaging. **(WHO, 2023)**

VII.1.4. Establishment: maintenance and sanitation

The objective is to facilitate the effective and continuous control of health hazards, pests, and other agents that may contaminate food. This is achieved by controlling:

- maintenance and cleaning operations through appropriate procedures;
- the pest control system:
- by eradicating any pests;
- by treating waste;
- by monitoring the pest control plan.

VII.1.5. Establishment: personal hygiene

The objective is to prevent people from contaminating products. This is achieved by controlling:

- their health, illnesses, and injuries;
- personal cleanliness;
- behavior through adequate training in good hygiene practices;
- visitor access.

VII.1.6. Transportation

The objective is to protect food until it is delivered to the customer. To achieve this, it is necessary to define:

- the specifications of vehicles and other containers;
- their use and maintenance.

VII.1.7. Product information and consumer awareness

The objective is to have clearly identified products to ensure traceability and inform consumers. To achieve this, it is necessary to:

- identify product batches;
- accompany products with adequate information through appropriate labeling;
- educate consumers through repeated communication!

VII.1.8. Training

The objective is to have operators aware of the harmful impacts on human health in the event of non-compliance with hygiene rules. To achieve this, it is necessary to:

- To ensure awareness and define responsibilities;
- To define a training program;
- To supervise the effectiveness of training programs;
- To ensure refresher training as needed.

These prerequisites (cleaning plan, pest control plan, personnel hygiene, forward movement, etc.) must be recognized as being intended to control hazards that are virtually common to every agri-food organization.

VII.2. Main technological operations

VII.2.1. Mechanical operations

VII.2.1.1. Decantation and centrifugation processes

a. Decantation

Decantation is a process used to separate:

- Either a solid phase of suspended matter in a liquid of lower density;

- Or two immiscible liquid phases of different densities. In both cases, the action consists of letting the phases rest in contact and waiting a sufficient time for them to separate under the action of gravity. It is a simple but lengthy operation, requiring little equipment, and therefore inexpensive, but not very selective. It only involves a constant external force, gravity, and only requires avoiding any agitation or remixing action, once the separation is done.

Applications: decantation is widely used in the oil industry to separate certain unwanted components, and in the fruit and vegetable juice industry during fining and enzymation operations to remove pectins responsible for cloudy juices.

b. Centrifugation

Centrifugation is a technique that separates the components of a mixture based on their density under the action of centrifugal force. It allows the recovery of a precipitate (pellet) and a supernatant. The mixture to be separated can consist of two liquid phases or solid particles suspended in a liquid. Ultracentrifugation uses even higher rotation speeds (up to 75,000 rpm) and allows the sedimentation of ultramicroscopic particles.

Applications: centrifugation plays an important role in the dairy industry for the separation of milk fat or the elimination of microbial spores. It also finds applications in biotechnology (microbial cultures, vaccine and enzyme production, etc.) for the separation of biotic and abiotic phases. **(Mzoughi and Firatligil, 2025)**

VII.2.1.2. Pressure filtration and extraction processes

a. Filtration

Filtration is a mechanical method for separating a continuous phase (liquid or gas) and a dispersed phase (solid or liquid) initially mixed. Separation is achieved by passing the mixture through a filter medium, a porous medium adapted to the characteristics of the suspension to be filtered, under the action of a pressure force providing the suspension with the necessary energy to pass through the porous medium. It therefore requires defining the appropriate filter medium, as well as its implementation conditions, i.e., the filter and its environment.

In practice, the application of filtration to analytical methods most often concerns suspensions (solids dispersed in a liquid) or fumes (solids dispersed in a gas), which use the same filtration media. The case of mists (liquid dispersed in a gas) or emulsions (dispersion of a liquid in another immiscible liquid) will not be discussed, only solid-liquid separation being considered.

Applications: bacterial purification of skimmed milk; casein standardization; fractionation of globular fat from whole or enriched milk; production of clarified apple juice; demineralization of fruit and vegetable juices (stabilization of red beet concentrate); gelatin clarification; biological water purification; separation of amino acids and peptides from milk; effluent treatment. **(Shirato, 2017)**

VII.2.1.3. Grinding

Grinding consists of reducing the particle size of a divided solid. The goal may be to increase the exchange surface area between the powdered solid and the external medium, liquid (dissolution) or gas, or to facilitate mixing with another solid, or to increase transfers in drying, cooling, heating, extraction, etc. operations. This operation is therefore very common in the food industry. Grinders can be classified into three main families of functions:

- Coarse grinding: in this category, large solids are used to obtain elements with dimensions of around cm. This category of grinders operates by "cutting": knife grinders, used for frozen meats; beet graters, for making cossettes before sugar extraction, potato graters before starch extraction, etc.; Alfalfa grinders before drying or sugar cane grinders before pressing.

- Fine grinding: This category produces particles with dimensions between 1 cm and 0.1 mm.
This is the area where the most food applications are found:
- Disc grinders, used in particular for continuously producing coarse flours from cereals, oilcakes, etc.
- Roller grinders, machines extremely popular in the food industry, especially in the grain industry, derived from stone mills (still used in special cases such as for mustard grains).

- Ultra-fine grinding: these grinders are used for small product tonnages, but have been developing in recent years in the food industry for high-value products: spices, pigments, active ingredients, yeast cells, sugars, etc. The average particle diameter is 100 to 10 µm. The grinding method here is primarily by attrition, i.e., abrasion of the grains against themselves and/or against the metal components of the mill.

VII.2.1.4. Agglomeration and coating

Agglomeration refers to techniques for increasing the size of divided solids by "gluing" n grains. Coating solid particles consists of covering their surface with substances that solidify in their final state.

The main objectives of agglomeration are:

- Modifying the powder's density for technological or commercial purposes, as well as its compressibility and suitability for compaction and bulk storage;
- Improving flowability and mixing ability in the solid phase;
- Increasing wettability, improving the dispersibility and dissolution of agglomerates in a solvent (instant beverages).

The main objectives of particle coating are:
- Protection of sensitive substances from the environment such as oxygen, humidity, and light;
- Separation of incompatible active substances within the coated entity;
-Improvement of hardness, appearance, particle shaping, or surface texturization (smoothing);
- Concealment of an odor or taste, flavoring, or coloring of a solid; controlled release of an active ingredient through the coating (disintegration, dissolution, diffusion depending on temperature, pH, etc.)

b. Pressure extraction

From a product composed of solids and liquids, pressure extraction (or pressing) separates the liquids by applying external pressure. The product is supported by a wall or a cloth that allows the liquid to pass through.

Applications: In the food industry, this process is relatively widespread and is used to: extract juice (grapes, fruits, tomatoes); extract oil (olives, oilseeds); remove whey from cheese curds; and concentrate dry matter (sugar beet pulp, various wastes, and sludge).

VII.2.2. Thermal operations

VII.2.2.1. Heating by electric and electromagnetic fields

These heating techniques generate heat within the material and are therefore not strictly surface-based; the field is established or the wave penetrates more or less deeply into the material depending on the field used and the product to which it is applied. **(Lewis, 2006)**

a. Poorly penetrating wave: thermal radiation

Transfers by thermal radiation can therefore be defined as an exchange of energy between a material system and the radiation field with which it interacts. This mode of energy transfer is commonly encountered in the food industry.

Applications: unpackaged solid products (cookie baking with a browning effect on the surface); packaged solid products (baked goods, pastries, or viennese pastries wrapped in plastic to prevent mold growth). It should also be noted that IR is commonly used for space heating.

b. Penetrating wave: ohmic heating, microwaves, and high frequencies (HF)

These fields are implemented in two ways. The field can either be established between two electrodes (HF and ohmic) or originate from radiation emitted by a source or antenna and propagate according to the laws of optics. In the second case, the "electrical mass" is formed by the metal walls that confine the field (microwave). In ohmic heating, there must be intimate contact between the product to be heated and the electrodes, whereas the presence of air between the electrodes and the product is not very problematic in HF.

c. Ohmic heating

The passage of an electric current through a material is accompanied by the production of heat per unit volume and time (W.m-3).

Applications: Ohmic heating is suitable for solids that require core heating, immersed in a liquid that ensures continuity (such as in sauce or fruit in syrup).

e. Microwaves and high frequencies (HF)

These correspond to radiation with frequencies between:

- 3 and 30 MHz (wavelength of 10 to 100 m in air) for HF;

- 0.3 and 3 GHz (wavelength of 0.1 to 11 m in air) for microwaves.

Microwaves and high frequencies act by polarizing an insulating material (dielectric). Dipoles (molecules with an asymmetrical electrical charge, for example) subjected to an electromagnetic field will tend to orient themselves in this field like a compass.

If the field is alternating, these dipoles oscillate from one direction to the other, and their movement is accompanied by friction that produces heat. For this friction to occur, these molecules must be close to each other and the material must be dense.

Applications:

The classic application of HF is the thawing of large pieces of meat that are too thick to be treated by surface heating, that microwaves cannot penetrate, and whose shape is too irregular for good contact with ohmic heating electrodes. There are also applications in the final phase of biscuit baking or, to standardize the water content, take advantage of core heating to reverse the thermal profile.

Microwaves will be used for thin products, in cooking, pasteurizing packaged food, or thawing. They are used in two types of metal-walled enclosures called waveguides or cavities (these guides are rectilinear channels whose rectangular cross-section has perfectly defined dimensions based on the wavelength).

VII.2.2.2. Heat exchangers

Extremely widespread in all material processing industries, these devices allow:

- to bring a fluid to the temperature required for a reaction (e.g., pasteurization, sterilization, fermentation) or other operation (evaporation, drying, etc.) using another hot fluid, a heat transfer liquid, or condensing water vapor;

- to then cool this fluid using a fluid to be heated, thereby recovering heat, and/or using a refrigerant;

- to condense a vapor to recover the product in its liquid state, which also allows for heat recovery.

VII.2.2.3. Evaporation

Evaporation is the process of concentrating a solution or suspension of essentially non-volatile compounds, here called "dry matter," by boiling the solvent, which is itself volatile (usually water).

It is therefore a thermal separation process, which involves providing the solvent's latent heat of vaporization ΔHv, as is the case with drying. But here, unlike drying, the initial product is necessarily liquid, and the final product, called "concentrate," must remain a "pumpable" liquid, despite its high viscosity after partial evaporation of the solvent. In the food industry, the solvent is most often water, but other volatile substances may also vaporize at the same time: flavors, alcohol, ammonia, dissolved gases, etc.

The purpose of evaporation is often multiple:

- to reduce volume and weight, in order to lower transportation, packaging, and storage costs (musts, fruit juice concentrates, milk, meat extracts, etc.);

- to improve preservation by reducing the product's water activity: sugar syrup, jam, cheese whey, etc. However, concentration alone is rarely sufficient to prevent microbial growth, and additional measures (pH, preservatives, aseptic techniques, etc.) are often required, and concentrates may be more vulnerable to certain chemical deterioration.

- Most often, evaporation is an intermediate step before other operations such as crystallization, precipitation, coagulation, or final drying, knowing that it can be much more energy-efficient.

When considering concentration by evaporation in the food industry (IAA), it is essential to consider the thermal effects on the product, whether desired or not: coloring for sweet juices and fruit juices, insolubilization of proteins and "cooked" taste for milk, thermal and/or oxidative destruction of pigments for tomatoes, vitamins for citrus fruits, microorganisms, etc. **(Kerr, 2019)**

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