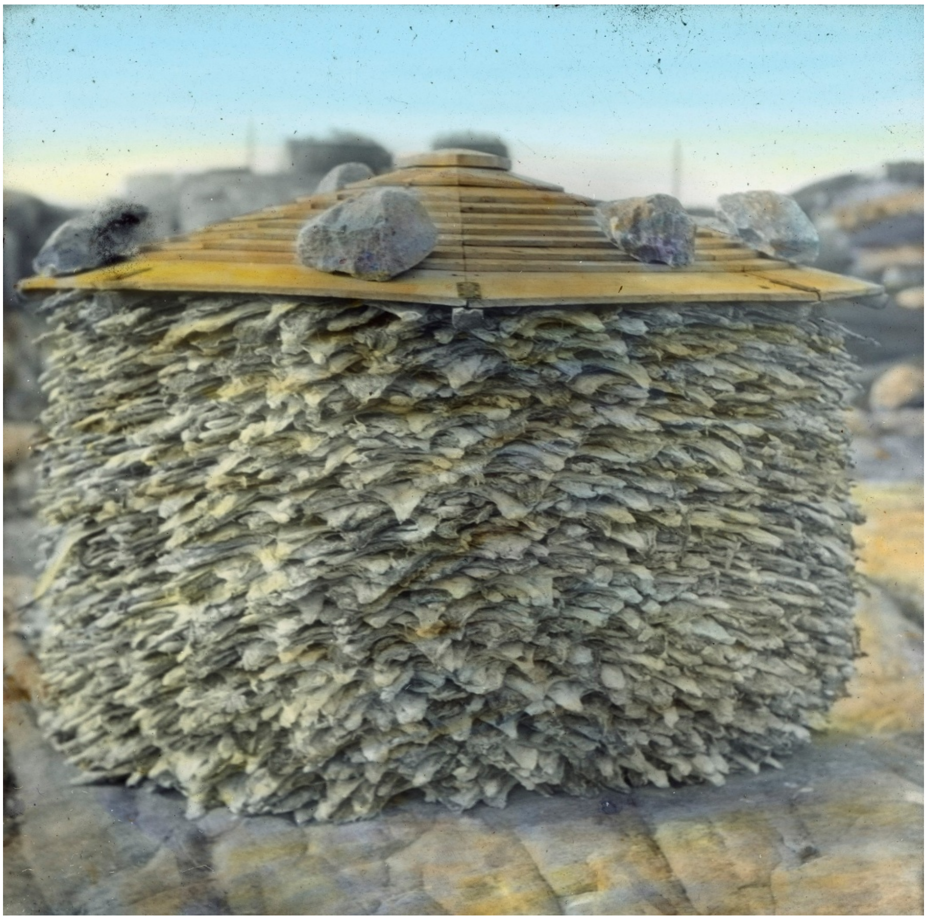
Food preservation

Dad, why don't you keep that fish in the fridge?



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Storyline

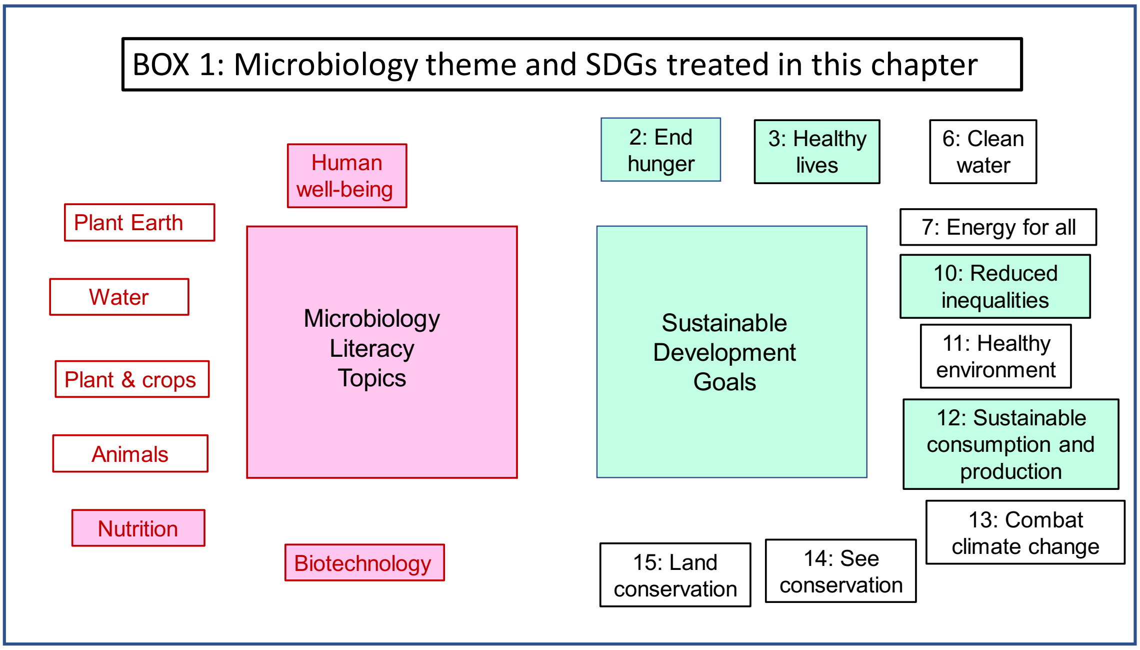
Living beings, from bacteria to humans, have learned that food must be stored under proper conditions to survive when there is a shortage of food. To achieve this, living beings have developed natural mechanisms that allow them to store nutrients within their bodies and cells -- such as sugars, lipids, and different polymeric substances – which once hydrolysed provide cells with essential nutrients and energy. But in addition to these natural mechanisms, some organisms have also learned to store food externally. Hoarding and caching food in specific locations is a common animal behaviour among many different species, including humans. In such cases, animals have to ensure that other competing organisms cannot take it, and that the food is preserved in edible conditions for long periods. For this reason, food must be properly stored and preserved in safe conditions to avoid its physicochemical damage and microbial contamination.

Few things are as important to human survival as as knowing how to store and preserve food at any time of the year. Nowadays, food preservation serves two main objectives, on the one hand, to prevent any change of its nutritional and **organoleptic** properties due to detrimental natural physicochemical processes, and on the other hand, to avoid spoilage by different contaminating microbes. Microorganisms can spoil food in different ways. They may consume it reducing its quantity, but they can also change their organoleptic properties, and make it inedible. Even worse, consuming food contaminated by microbes can cause diseases, either due to their pathogenic nature or through the production of toxic substances generated during their growth. In this chapter, we will explore how to store and preserve food to prevent microbial spoilage.

The Microbiology and Societal Context

Food loss by either general spoilage or microbial contamination affects both the food industry and consumers, leading not only to economic and reputational damage for public administrations and companies, but also to high health costs. Food preservation is key for ensuring both food safety and food security. Extending the shelf-life of food without modifying its organoleptic qualities and preventing the growth of t pathogenic microbes are among the main challenges of the food industry, nor only in response to compliance with food safety regulations, but also because the growing interest of consumers in purchasing healthier and higher-quality gourmet foods. Even more important, proper food preservation at home helps prevent different types of food poisoning. Moreover, responsible food management – including making thoughtful purchases, placing it properly in the fridge/freezer or in the pantry, and choosing the correct packaging, is also essential to this goal. The relationship between food preservation and reducing consumer food waste is of vital for developing sustainable food options. Food preservation has an important role in facilitating this waste reducing objective, because it improves the efficient utilisation of food.

Preserving food on an industrial scale is more complex than doing so at home. For this reason, many different chemical or natural food additives have been developed over time by the food industry to meet the demands of large-scale food production. These additives are needed to ensure that processed food remains safe and maintains organoleptic qualities throughout sometimes very long transportation routes - from factories or industrial kitchens to shops, warehouses, and finally to consumers. However, there is currently a great deal of confusion among consumers regarding food additives. This confusion often stems from information gaps, or from ambiguous or misleading publications. All this favours a climate of uncertainty and concern, where many myths and fears have emerged, most of them without foundation. The preservation of food by natural additives and by artisanal methods offer enormous advantages, both for families at home and for small and medium-sized producers o artisan companies, especially when natural, simple procedures are used, with scarce resources and low inputs, and contributes to food security, as support the development of local agribusiness.



Food preservation: The Microbiology

1.- Basic methods for preservation of food from microbial contamination

There are many ways to classify the methods currently used to preserve food from microbial contamination. One of these systems classifies them in three general categories:

a) Methods that destroy germs or inactivate them: These include **sterilization**, **pasteurization**, **radiation**, high pressure treatments, etc.

b) Methods that prevent microbial growth and proliferation: refrigeration, freezing, **dehydration**, **smoking**, addition of chemical substances, fermentation, etc.

c) Methods that avoid or reduce contamination or re-contamination: aseptic processing, proper packaging, hygienic storage conditions, etc.

Alternatively, food preservation methods can also be classified according to their principle of action as: follows

a) **Cold preservation**. These methods use low temperatures to extend the freshness of the food. Cold slows down chemical and enzymatic reactions and lowers microbial. However, cold does not kill microbes, so when the food is returned to room temperature, microbes can resume growth. Cold preservation methods are divided into: i) Refrigeration; ii) Freezing; iii) Deep freezing or ultra-freezing.

b) **Heat or thermal preservation**. These practices use heat to destroy cellular structures (e.g., membranes, organelles) and to inactivate or **denaturate** proteins and enzymes. The temperature and duration of heat application play an essential role in the shelf-life of the food. The higher the temperature and longer heating, the greatest the durability of food. However, in some cases excessive heat can deteriorate some nutrients and alter organoleptic properties of food. The different methods that apply this technique are: i) Sterilization; ii) Pasteurization; iii) Cooking.

c) **Preservation by reducing humidity or moisture**. These processes are based on controlling the water content in the food. Since microorganisms need water to growth and reproduce, reducing humidity lowers the likelihood of contamination. That is, less water, less reaction capacity of enzymes and slower growth of microorganisms. The methods that apply this technique are: i) Desiccation; ii) Dehydration; iii) Evaporation; iv) **Lyophilisation (freeze-drying)**; v) Concentration.

d) **Chemical preservation**. These techniques prolong the useful shelf life of food through the addition of substances that modify the product. This method is widely used in the food industry because is cost-effective and very efficient for industrial production. In addition to preservation, additives are often use to modify its organoleptic properties. The preservation can be achieved by adding different substances: i) Alcohol; ii) Fats; iii) Sugars; iv) Salt; iv) Acids; v) Other substances of chemical or natural origin (e.g., colorants, preservatives, antioxidants, stabilizers). A special case is the addition of substances through food fermentation (e.g., alcohols, acids, antibiotics).

e) **Emerging preservation methods**. The new forms of preservation are very sophisticated, considerably increasing both the quality of the products and shelf life. The most relevant emerging methods are those that are based on high pressures, electric fields, light pulses, **ionizing radiation** (X-rays, gamma rays), **non-ionizing radiation** (UV, microwaves), edible coatings and surface treatments, encapsulation and controlled release, vacuum packaging, modified atmospheres, ultrafiltration, and sterilizing filtration.

BOX 2: Brief history of food preservation methods.

In the Palaeolithic era, the first hunters and gatherers consumed the food immediately after hunting or collect it. Aby the end of the Palaeolithic and the beginning of the Mesolithic, the first clay pot was created in Japan (Jomon period), being the first described container for food preservation. Smoking, the process of exposing food to wood smoke, which has antimicrobial effects, was probably discovered by serendipity by early cavemen. Early humans made fires in caves without chimneys, filling the cave with smoke and thus, exposing food to this chemical preservative. Over time, they learned to pre-treat food with salt before smoking, making the smoking even more effective. Today, smoking is mainly used for flavour food rather than preservation. Preservation processes involving water, such as brining and syruping also arise in ancient times. The first modernised salting and smoking techniques emerge, thanks to the Egyptians, while the Sumerians about 5,000 years ago were already skilled in salting food.

Middle East and oriental cultures dried foods as soon as 12,000 B.C. In ancient times, the sun and wind were used to create naturally dried food. Then, desiccation appears to arise as a result of human needs. In the Neolithic, human transition from nomadic life, to sedentary, developing agriculture and livestock farming. At the same time, they built the first barns, to protect their food from animals. Caves and pits dug into the ground are used for this mission. During the Iron Age, in northern Europe, dehydration techniques improved, and the first kilns emerge to dry freshly harvested wheat.

Fermentation was not invented, but rather discovered also by serendipity. It is believed that the first beer was discovered when a few grains of barley were left in the rain, allowing opportunistic microbes to ferment the sugars into alcohols. The ability of the people to observe and take advantage of these fermentations was incredible. Anthropologists believe that around 10,000 B.C., mankind went from nomads to farmers to grow barley and immediately, they began to make beer. In Mesopotamia, the oldest evidence of beer is a 6,000-year-old Sumerian tablet that depicts people drinking from a communal bowl.

Preservation using honey was a well-known method to the earliest cultures and thus, fruits kept in honey were common across many cultures. In ancient Greece quince was mixed with honey, partially dried sand packed tightly into jars. The Romans improved this method by cooking the mixture to achieve a solid texture. A new preservative, i.e., sucrose, emerged in New Guinea 6,000 years after the domestication of sugarcane. Then, it spread to India, China, other parts of the East, Persia, and finally to Europe by the 4th century.

The Greeks also preserved fruits using virgin wax or honey. The Romans kept wine for decades, bottling it in hermetically sealed amphorae. Vegetables and fruits were also dried from the earliest times. The Romans liked to prepare dried fruit. In the Middle Ages "still houses" (houses raised on stilts over the surface) were created to dry fruits, vegetables and herbs in areas that did not have enough sun to dry food. Fire-generated heat was used to dry food and, in some cases, as mentioned, for smoking. The Visigoths prepared jams with honey and apples, storing them in wineskins.

Pickling is the way of preserving food in vinegar or other organic acids. Vinegar is mainly produced from sugars or starches that are first fermented to alcohol and then by oxidation transformed by certain bacteria to acetic acid. Alcoholic beverages such as wines, beer, and cider can be transformed into vinegars. The arrival of new food in the sixteenth century in Europe sparkled a remarkable increase in the interest of food preservation. The interest of trade with India and the Orient that brought pickles to Europe also brought sugarcane. In northern countries with little sunlight to dry food, homemakers learned to preserve fruits by heating them with sugars – a process that evolved into make canning.

In 1658, Athanasius Kircher, a German priest, when examining decomposing food, was perhaps the first to notice the role that microorganisms played in spoiling food. He suggested that the deterioration of food was due to the presence of small animals, invisible to the naked eye. In 1765, Lazzaro Spallanzani, an Italian priest, demonstrated that heating can prevent the appearance of "***animalcules***" in infusions, although the duration of heating necessary to make an infusion sterile varied. He concluded that "*animalcules*" can be introduced into infusions through the air, disproving the theory of spontaneous generation in well-heated infusions.

Freezing has always been an obvious preservation method in regions with cold climates. The humans from any geographic area that had freezing temperatures for at least part of the year used the low temperatures to preserve food. Temperatures below 0 °C were used to store time in cellars or caves. For extended periods. In the United States, farmers built icehouses to store ice and food which eventually evolved to the household "icebox". Mechanical refrigeration, invented in the 1800s, soon became practical and widespread. Clarence Birdseye, an American inventor, in the late 19th century, discovered that rapid freezing at very low temperatures preserved the flavour and texture of vegetables and meats far better than slow freezing.

In the 19th century, it was discovered that certain sources of salt gave meat a red colour instead of the usual unpleasant grey of aged meat. Consumers overwhelmingly preferred the red-coloured meat. This mixture of salts contained nitrites (saltpetre). As the microbiology of *Clostridium botulinum* was elucidated in the 1920's it was realized that nitrites also inhibited the growth of this foodborne human pathogen.

Another fascinating development was the discovery of canning (bottling). Napoleon III, during his military campaigns, faced an important challenge: food spoiling was decimating his troops, these foodborne deaths being more numerous than those that from fighting. For this reason, he offered a prize of 12,000 francs to whoever managed to preserve food in a practical way to be easily transported and suitable for the troops. **Canning** emerged as one of the newest of the food preservations methods being pioneered in the 1790s when a French confectioner, Nicolas Appert, won the Napoleon Award by discovering that heating food inside sealed glass bottles prevented food spoilage. He reasoned, if it works for wine, why not for foods? Around 1806 Appert's principles were successfully tested by the French Navy on a variety of foods including meat, vegetables, fruits and even milk. In 1810, the English merchant Peter Durand used tin cans instead of bottles based on Appert's methods.

2. The role of temperature in food preservation

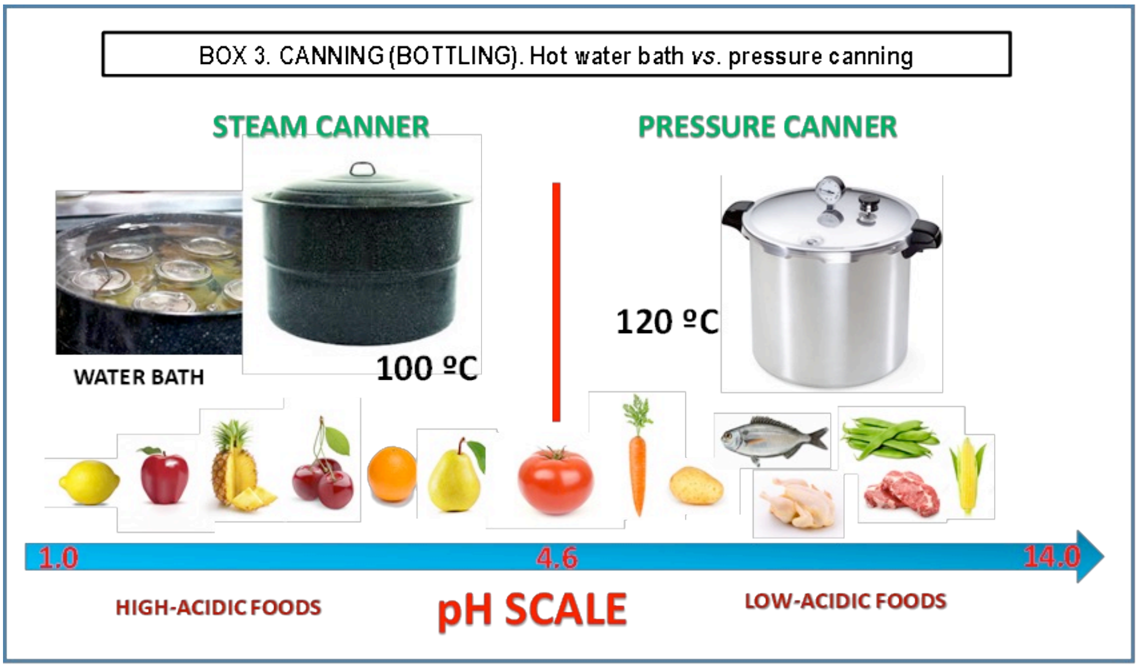
Temperature plays a fundamental role in the preservation of food. The vast majority of microorganisms normally grow within a temperature range of 5-60 ºC, although there are some microorganisms that can grow outside this range. Thus, the role played by low temperatures and high temperatures outside of this range is very different. Temperatures below 5 ºC slow down the growth of microorganisms, whereas temperatures above 60ºC usually kill them. Nevertheless, there are exceptions: some microorganisms can be killed at freezing temperatures, others can grow very efficiently below 5 ºC (**psychrophilic**) and others can survive or even grow better above 60 ºC (**thermophilic**). In the special case of spores, temperatures of at least at least 130 ºC are required to kill them.

A fridge maintains temperatures between 2- and 8 ºC, whereas a typical home freezer usually works at about -20 ºC. However, there are special freezers that can reach -40 ºC or -80 ºC or even ultracryogenic freezers that reach -temperatures as low as -150 ºC. At temperatures of 2-8 ºC some microorganisms can still grow, but most will do so very slowly and will require many hours to generate a significant progeny. Thus, it is possible to preserve foods from microbe spoilage at least for several days in a fridge or under any kind of refrigeration (e.g., in ice). In the case of freezers, at temperatures well below 0 ºC, microorganisms cannot grow, allowing food to be preserved from microbial spoilage for an indefinite time. It is important to know that freezing in general does not kill microorganisms and therefore, when food is thawed, microbes can grow again. Moreover, we know that at low temperature food can be deteriorated due to various physicochemical processes even without the intervention of microbes.

When we talk about the use of high temperatures to preserve food, of course we are not referring to storing food in ovens, as it is the case of the freezers, but rather are applying high temperatures to kill microbes. In most cases, the application of high temperatures to food plays a double purpose. On the one hand, it helps us to cook or process the food to make it edible; and on the other hand, it allows us to kill c microbes that can spoilage the food. For instance, boiling food exposes microorganisms at a temperature of nearly 100 ºC, while baking exposes microbes at temperatures above 150 ºC. A special case is when we cook food in a pressure cooker where water can reach temperatures of 130ºC. We can also apply heat to the food using other kitchen instruments as a microwave oven, toaster oven, griddle, deep fryer or even directly over the fire in a grill.

Sometimes, high temperatures are applied solely to food we want only to preserve by killing the microbes. In general, we call this process food sterilization. The sterilization process is generally carried out in an equipment called autoclave at a temperature of 115-130 ºC, maintaining a pressure of 200 kPa for several minutes. The joint action of temperature and water steam will cause the proteins essential for life of microorganisms to coagulate and therefore, they are destroyed. The problem with food sterilization is that it can alter organoleptic properties and destroy essential nutrients such as some vitamins. Therefore, the heating and cooling times of the sterilization processes are essential to maintaining food quality.

To avoid the loss of quality, pasteurization can be used instead of Pasteurization is a process in which packaged and non-packaged foods - mainly liquids such as milk or fruit juice - are treated with mild heat, usually below 100 °C, to eliminate most of pathogens, while preserving nutritional properties. Since pasteurization is not a complete sterilization, it does not kill spores. There are three distinct types of pasteurization processes: i) VAT or slow pasteurization; ii) pasteurization at high temperatures for a short period (HTST, High Temperature and Short Time), 72 ºC for 15 s; iii) Ultra-High temperature (UHT, **Uperization**) processing, at about 138 ºC for 2 seconds. In some cases, we can apply a second pasteurization process some hours after the first process to allow spores to germinate and thus, kill them during the second heating.



3. The role of water in food preservation.

As mentioned in the short history of food preservation presented above (BOX 2), the presence of water (**water activity**) in food has been controlled throughout human history by different methods, i.e., such as drying, and the addition of sugar or salt, to prevent spoilage and maintain food quality. It should be noted that freezing also reduces water activity.

We call "water activity" to the ratio of the partial vapour pressure of water in equilibrium with a food to the partial saturation vapour pressure of water vapour in air at the same temperature.in other words, it represents the relative humidity of air in equilibrium with the food. The water activity of a food describes its capacity to solubilize hydrophilic molecules, to participate in chemical or biochemical reactions and to facilitate the growth of microorganisms. Thus, this property is used to predict the stability and safety of food with respect to microbial growth, rates of deteriorative reactions, and chemical or physical properties.

Reducing water activity in foods prevents the growth of vegetative microbial cells, the germination of spores, and toxin production by fungi and bacteria. A decrease in water activity increases the lag phase of microbial growth and decreases the growth rate. Nevertheless, this does not mean that all microorganisms necessarily die under these conditions. Some microbes can survive these treatments, and when returned to adequate environmental conditions, they can grow again.

We know that microorganisms cannot grow under water activity below 0.60. At around of 0.65 we can find dried fruits containing about 15-20% of moisture. Around 0.65-0.75 we can find molasses, marmalade, jelly, raw cane sugar, nuts, or jam. Concentrated fruit juices, syrup, or condensed milk are around 0.80. Food such as Salami, dry cheese and margarine have values above 0.80. Around 0.90 we find some cheeses and cured meat (e.g. ham). Below 0.80, only some fungi and halophilic bacteria are able to grow. Between 0.80 and 0.90 many yeasts and moulds as well as some bacteria as *Staphylococcus aureus* can grow. Above 0.90 many fungi and bacteria are able to grow without restrictions.

a) <u>Drying food</u>

Desiccation by heating, sun drying, air drying, spray drying, freeze drying, freeze concentration, and osmotic concentration methods are used to reduce water activity of food. Water activity can also be reduced by using other basic methods, such as dehydration, crystallization, and addition of solutes like salts or sugars.

Desiccation, drying and dehydration are very similar words. However, desiccation and drying are mainly used to refer to processes that drastically eliminated the water from food. Dehydration is used when the process leaves some residual water.

A special case of drying food is the process that we call freeze drying, also known as lyophilisation or cryodesiccation. This is a low temperature dehydration process that involves freezing the product, lowering the pressure through vacuum, and then removing the ice water by **sublimation**. This process is very different from conventional dehydration or drying methods that evaporate liquid water using heat or air. Although lyophilisation is a very expensive method that consume time and energy, it yields a high-quality product., that usually retain the original shape of the product and can be recovered after rehydration. This process is used for high added value foods. It is also used to preserve food for astronaut and military purposes. Curiously, freeze drying is one of the best methods to preserve many microorganisms, making them almost immortal. This process does not usually kill microbes, but prevents their growth as long as the product remains dry and non-rehydrated.

<u>Adding salt</u>

Salting is one of the oldest methods used by humans to preserve food. Typical salt-cured foods include salted fish (e.g., dried and salted cod, salted herring), salt-cured meat (e.g., bacon) and salted vegetables (e.g., runner beans, cabbage). This method consists in the preservation of food with dry edible salt (sodium chloride) or previously dissolved in water. Most bacteria and fungi, and in particular most human pathogens, cannot survive in a highly salt environment. The hypertonic environment caused by salt draw water out of living cells through osmosis dying or becoming temporarily inactivated.

There are two types of salting, dry and wet. Dry salting consists of applying salt (with or without other seasonings) to cover and surround the food. This method is used for larger and fattier pieces. Although this method works more slowly and the food is saltier, it is a safer curing process and preserves the products for longer than the liquid one. Wet salting or brining consists of treating food with saline solutions (brine) of varying concentration generally between 3% and 10%. Salt can be applied by immersion or by injecting the brine into the food. This method is better suited for lean meats or for irregularly shaped pieces with bones, as the salt solution reaches all the cavities in the piece between the bone and the meat, which is impossible in salting. Another advantage of this wet form of curing is that we obtain results much faster than by dry salting.

By adding salt, we can preserve different foods: i) Meats (e.g., jerky, ham, pork shoulder, lard, salted bacon, salty veal bones); ii) Fish (e.g., tuna, cod); iii) Vegetables (e.g., dried tomatoes, dried seaweed, olives, pickles); iv) Roe (e.g., tuna, anchovy, herring, kusaya); v) Cheese, where its function is to create a skin-like barrier in the cured cheese.

b) <u>Adding sugar</u>

As mentioned above, sugars have a very long history in food preparation and preservation. Sugar helps preserve food by binding water and therefore, reducing the amount of water available for microbial growth. Like salt sugar creates an osmotic effect, this is, in a concentrated sugar solution water is drawn out of the cells so that microorganisms can no longer be active or even survive. Sucrose from cane or beet is the most common source of sugar used for preservation, but corn syrup and honey can be also used to replace at least part of sucrose.

There are different ways to use sugars to preserve foods.

One method is to desiccate food, mainly fruits, by drying them and thereafter, packing the desiccated fruits together with pure sugar (sugaring). Examples include ginger, cherries and the peel of citrus fruits. Foods can be also stored in a sugar syrup or cooked with o sugar until crystallised. On the other hand, to produce jams and marmalades, fruits are initially boiled to reduce the water content and the contaminating microorganisms and thereafter, sugar is added to prevent the growth of microbes. In some cases, other sugar preservation techniques involve combining sugar and alcohol to enable the preservation of fruits in alcoholic spirits. Sugar is also used in combination with salt to preserve fish and meat. Sugar can be added to salt to create a dry mixture to cover food or can be dissolved in water to make a brine to surround the food. Adding sugar to a brine helps to confer a sweetness to meat and fish, but also helps to reduce the harsh taste of salt.

Although sugar can be used as a preservative in a controlled environment (such as a sealed jar), it also attracts moisture, so where and when water is available in the air, sugar will absorb water creating an environment attractive for some microorganisms that can tolerate high sugar concentrations.

It is important to note that the preservative effect of sugar is dependent on its final concentration in food. Due to the health concerns associated with sugar, you may consider reducing the amount of sugar used in food preservation; however, adding too little sugar to food can allow microbial growth. This is often seen in the production of low sugar jams and jellies, where insufficient sugar promotes the growth of mould and yeasts.

In this sense, it is also important to know that with the use of sugar, even if we use the correct concentration (about 60% of sugar), water activity cannot be reduced below 0.845. This level of water activity is sufficient to inhibit many bacteria and yeasts, but it does not complete prevent mould attack. For example, moulds can grow well in the surface of a well-prepared jam inside a jar that has been opened several times or kept for some time out of the fridge. In these cases, because the contamination usually appears only in the surface, some people simply remove the mould and continue eating the jam. However, this can be very dangerous, as the moulds might have produced toxins that spread into the jam. In such cases, it is recommended to discard the entire jam.

Box 3. Pathogens in food

We have learned that it is necessary to preserve food to prevent it from microbial spoilage, but we also have to bear in mind that preservation prevents the proliferation of pathogenic microbes that can cause human diseases, either by themselves or through their toxins.

The number of illnesses, hospitalizations, and deaths caused by known **foodborne** pathogens is very high, even in well-developed countries. The CDC (Centers for Disease Control and Prevention) estimates that in USA there are about 10 million cases per year of diseases caused by well-known foodborne pathogens. The number is even higher when we considered the people that gets sick by others non-so well characterized food derived agents.

The main pathogens that can be transmitted by food are of different types:

a) Bacteria like *Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens, Escherichia coli O157:H7, Listeria monocytogenes, Salmonella enteritidis, Salmonella typhimurium, Shigella, Staphylococcus aureus, Streptococcus* sp., *Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificus, Yersinia enterocolitica*, *Plesiomonas shigelloides, Aeromonas hydrophila, Enterobacter sakazakii, Francisella tularensis.*

b) Viruses like norovirus, rotavirus, hepatitis A, astrovirus, and sapovirus.

c) Protists, eukaryotic unicellular parasites, like *Cryptosporidium parvum*, *Entamoeba histolytica Giardia intestinalis*, *Toxoplasma gondii*, and *Cyclospora cayetanensis.*

We also have to consider the intoxications caused by bacterial toxins present in food, produced by *Staphylococcus aureus*, *Clostridium botulinum, Salmonella*, *Escherichia coli* O157:H7 (shiga toxin), *Bacillus cereus* and *Clostridium perfringens*, or by mycotoxins produced by fungi (e.g., *Aspergillus, Penicillium, Claviceps, Fusarium*), such as aflatoxin or ergot alkaloids, such as ergotamine.

There are some well-known important **zoonoses**, such as bovine tuberculosis, brucellosis and Q-fever, associated with livestock and agricultural farming. These can be acquired by occupational exposure of workers, but also by foodborne bacteria. Bovine tuberculosis is caused by *Mycobacterium bovis* through the consumption of unpasteurized, contaminated dairy products. Brucellosis, also known as "Malta fever," is a zoonotic disease caused by an intracellular gram-negative cocco-bacilli bacterium named *Brucella melitensis*. The pathogen can be acquired orally since bacteria pass to the digestive system by the ingestion of non-pasteurized milk or dairy products (soft cheeses), especially those from sheep and goats. *Coxiella burnetii*, which causes Q-fever, can be also transmitted via ingestion of contaminated unpasteurized milk or dairy products.

It is common to find food poisoning or infections caused by the consumption of raw bivalve molluscs (e.g., clams, mussels, oysters and scallops). These animals are filter feeders and can harbour numerous microbial pathogens (mainly bacteria, viruses and protozoa), as well as toxins from cyanobacteria and algae (e.g., dinoflagellates or diatoms). These toxins are quite stable and cannot be eliminated by cooking. They can be only eliminated by a proper cleaning/depuration.

Other typical and important foodborne diseases caused by the ingestion of macroorganisms like the worms *Trichinella, Taenia* or *Anisakis* are not considered here. Moreover, another different issue are also the foodborne prion diseases that are not considered here either.

4. Other antimicrobial food additives.

A food additive is a substance added to food for a technological purpose. It is added during the manufacturing, transformation, preparation, treatment or packaging phases, to avoid spoilage, risks and other alterations caused by microorganisms, to maintain the organoleptic qualities (e.g., colour, smell, taste, texture) of food or even to provide new properties (e.g., colour, flavour, sweetness).

Additives can be synthetic (also called artificial) or natural, -- that is substances derived from plants, animals or minerals. They can be single molecules or complex mixtures of compounds, as in the case of some natural additives. There is a large number of currently authorized food additives, but we will study in this section only those used as antimicrobials.

In this sense, salt and sugar presented above are examples of simple, naturally occurring antimicrobial additives of mineral and plant origin, respectively. But, there are many others natural and synthetic additives.

Organic acids and their salts - such as formic, acetic, propionic, lactic, citric, oxalic, malic, tartaric, sorbic, and benzoic acids - are currently used as food preservatives. Acids reduce the pH of the medium. The external pH of the surrounding environment of food is important to allow or hinder the growth of microbes. Moreover, upon passive diffusion of organic acids into the microbial cell, the acids dissociate and lower the internal cellular pH, leading to situations that will impair or stop the growth.

Sulphites and nitrites are inexpensive compounds used as meat additives, because they have not only antioxidant properties, but also are able to inhibit the growth of many human foodborne pathogens, such as *Salmonella enterica* and *Listeria monocytogenes*. However, these additives have created many concerns due to the health problems that their use can cause in humans. Sulphites may trigger allergic and respiratory reactions in sensitive people and chronic skin symptoms. Nitrites are toxic at high concentrations and can form nitrosamines when exposed to certain heating or acid conditions during food processing. Natural equivalent additives have been studied as an alternative to sulphites and nitrites, but most of them have hardly been applied in the food industry for technological and economic reasons.

Antibiotics and antifungal compounds are not yet universally accepted as food preservatives, however some of them are used legally or illegally. Nisin is currently the only antibiotic legally used as food preservative. Its safety and efficacy as a food preservative have resulted in its widespread use throughout the world. Nisin is a fermentation product of *Lactococcus lactis*, a food-grade bacterium. It is a ribosomally synthesized peptide that has broad-spectrum antibacterial activity against many bacteria that are food-spoilage pathogens. Natamycin, also known as piramicin, is a polyketide produced by *Streptomyces chattanoogensis,* used as fungicide for food and beverages since 1967. Hen egg-white-lysozyme, an enzyme extracted from eggs that degrade the bacterial cell walls, has been used to preserve many different types of foods (e.g., fresh fruits and vegetables, seafood, meat, sausage, cheese). Moreover, lysozyme added to infant feeding formulae makes them more closely similar to human milk. Lactoferrin is a protein present in milk that has an antibacterial activity mainly associated with its ability to bind iron, although it appears to have other antimicrobial properties. It is used as a food additive in infant dairy formulas, but also in viniculture, and vegetable oil, poultry, dairy and red meat industries.

Postharvest technologies have allowed horticultural industries to meet the global demands of local and large-scale production and intercontinental distribution of fresh produce that have high nutritional and sensory quality. Chlorine-based solutions, peroxyacetic acid (PAA) and hydrogen peroxide (H_2O_2) has long been used as disinfectants by food distributors to reduce microbial spoilage during shipment.

Box 4. Biopreservation: Preservation of food by fermentation.

The term **biopreservation** refers to the use of natural or controlled microbiota, or their antimicrobials as a method for preserving food. The biopreservation of food using lactic acid bacteria has been practiced since early ages. Some bacteria or their fermentation products are used to control microbial spoilage and inactivate pathogens in food. Bacteria and yeasts can inhibit the growth of other microbes by producing organic acids that lower the pH of the food, or by secreting different antimicrobial molecules. Nevertheless, biopreservation is now a broad concept that also includes the use of bacteriophages (**phages**) that are viruses which infect bacteria. Phage preparations specific against *L. monocytogenes*, *E. coli* O157:H7, and *S. enterica* have been commercialized for food applications.

Fermented foods have been part of the human diet for centuries. They are foods that promote digestion and provide beneficial microorganisms for the gut microbiota (probiotics). Food fermentation serves to produce new and desirable flavours, and to change its texture as well as to enhance preservation. Moreover, fermentation can increase the presence of vitamins in food.

Speaking in biochemically terms, fermentation is a metabolic process different from anaerobic and aerobic respiration, and should be strictly applied only to some anaerobic metabolic processes. However, routinely, when we talk about food fermentation, and in general when we talk about fermentative biotechnological processes, we mean both aerobic and anaerobic processes (sometimes a mixture of both) carried out by microbes,

Fermented foods are derived from meat, fish, milk and vegetables. Yogurt, cheese, tofu, kefir, bread, miso, tempeh, kimchi, pickles sauerkraut, sausages, wine, beer or vinegar, among many others are examples of fermented foods. Kombucha tea, a very old fermented beverage that has become recently fashionable among young people, has recovered the benefits that fermentation has on our health.

Food fermentation involves transformation by the action of a single bacterium or fungus or by complex consortia of bacteria or fungi, or even by consortia of both types of microbes (e.g., wine, kombucha tea). The microbes used for food fermentation include bacteria (e.g., lactic acid bacteria (LAB) such as *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Lactococcus* and *Bifidobacterium*), molds (e.g., *Aspergillus oryzae, Aspergillus sojae, Penicillium roqueforti* and *Penicillium chrysogenum*), and yeasts (e.g., *Saccharomyces cerevisiae, Kluyveromyces marxianus* and *Candida humilis*). During fermentation sugars in food are converted into acids or alcohol (ethanol), which act as natural food preservatives. Thus, preservation by fermentation depends on the production of acids or alcohol that impair the growth of other undesired microbes.

Pickling combines salting and fermentation. It is used to preserve cucumbers, cabbages, olives, some vegetables and fruits. In this process, some carbohydrates are transformed into acids through controlled bacterial fermentation.

Successful food fermentation must inhibit the development of microorganisms capable of causing putrefaction or infections, but in some cases, a decomposition of fermented products can occur due to: i) Poor conditions during fermentation; ii) Oxidation of lactic acid and other acids in the fermented product caused by yeasts and moulds that allow the growth of other microbes and affect the appearance, taste, texture, and colour of the product. Therefore, cold storage of fermented and pickled products ensures better stability of food for several months.

In some cases, such as occurs in the preparation of certain yogurts, the final fermented product is pasteurized to increase shelf life, but this process kills the probiotic bacteria of the yogurt.

5.- Other physical ways to sterilize food

In addition the methods discussed above, there are many other alternative physical procedures used to preserve food, that are nowadays applied industrially and domestically. . The most important ones are:

a) High pressure

Exposure to high pressure kills many microbes. High-pressure processing (pascalization) is used in the food industry to kill viruses, bacteria, yeast, moulds, and parasites, maintaining food quality. High pressure ranging between 100-8 and 00 MPa, that is, many times above atmospheric pressure, is sufficient to denature proteins and kill microbes. Nevertheless, endospores can survive such pressures.

b) Ionizing radiation

Ionizing radiation, such as X-rays, gamma rays, and high-energy electron beams, can penetrate cells, altering molecular structures and damaging cell components. Ionizing radiation causes double-strand breaks in DNA molecules producing DNA mutations either directly or indirectly, as a consequence of cell attempts to repair the DNA damage. When these mutations accumulate, they eventually lead to cell death. Both X-rays and gamma rays easily can easily penetrate paper and plastic packaged foods. In Europe, gamma irradiation for food preservation is widely used, where packaged dried spices are often treated this way, whereas it is not the case in the United States.

c) Nonionizing radiation

Nonionizing radiation, such as ultraviolet (UV) light, is commonly used for sterilization and uses less energy than ionizing radiation. Germicidal lamps typically emit UV light at a wavelength of 260 nm. Microbial cells must be exposed directly to the light source, since UV light does not penetrate surfaces and cannot pass through normal glass or plastics. UV exposure causes thymine dimers to form between adjacent thymines within a single strand of DNA. Then, when DNA polymerase meets the thymine dimer, it may fail to incorporate the correct complementary nucleotides (i.e., two adenines), and this causes mutations that can kill microorganisms. Consumers can also use at home UV light to control microbial growth. UV lamps are now commonly incorporated into water purification systems used at home. Campers can use small portable UV lights to purify water obtained from natural environments before drinking.

On the other hand, the typical microwave ovens, that you can find at home, use electromagnetic nonionizing radiation to heat food. Food absorbs microwaves and produces the heat that cooks it. Microwaves cause water molecules in food to vibrate, producing the heat that at the end cooks the food. Then, all foods that are higher in water content (e.g., fresh vegetables), can be cooked more quickly than those with lower water content.

d) Filtration

Microorganisms can be removed from liquid food (e.g., water, wine, beer and beverages) by filtration because filtration preserve food flavours. Membrane filters that are currently used to remove bacteria and fungi have an effective pore size of 0.2 μm., small enough to retain bacteria (average size 1 μm), and of course smaller than size of fungi. Membrane filtration is useful for removing bacteria from heat-sensitive solutions such as antibiotic and vitamin solutions. Large volumes of culture media used in fermentations may also be sterilized by filtration to protect heat-sensitive components.

e) Vacuum. Modified atmospheres.

Vacuum packaging is a procedure designed to preserve solid or liquid food by removing air from the package prior to sealing. After removing the air, the amount of oxygen inside the bag diminishes proportionally to the vacuum generated. This greatly reduces the risk of alteration due to food oxidation and the proliferation of aerobic microbes that develop in the presence of oxygen. If the amount of oxygen is reduced, their growth is slowed down. Frozen food that is vacuum sealed lasts an average of 2-3 years. The vacuum technique has given rise to a new way of cooking, named vacuum cooking.

After extracting the air from the bag containing the food, we can introduce a certain volume of an inert gas blend and then we can hermetically seal the bag. The main gases used to make modified atmospheres are nitrogen (N_2) and carbon dioxide (CO_2). But the choice of a particular packaging atmosphere depends on many considerations such as effect on microorganisms, retaining food stability, prevention of oxidative deterioration and inhibition of ripening. The gas can also prevent the food from being crushed. Storage on controlled atmospheres is not new, since it was used from the 1930s when ships transported fresh apples and pears in rooms with a high level of CO_2.

However, microorganisms that can survive under low-oxygen environments, such as *C. jejuni*, *C. botulinum*, *E. coli*, *Salmonella*, *Listeria* and *A. hydophila*, remain of major concern for packaging products using these technologies.

Box 5. The dark side of smoked and fire-grilled food

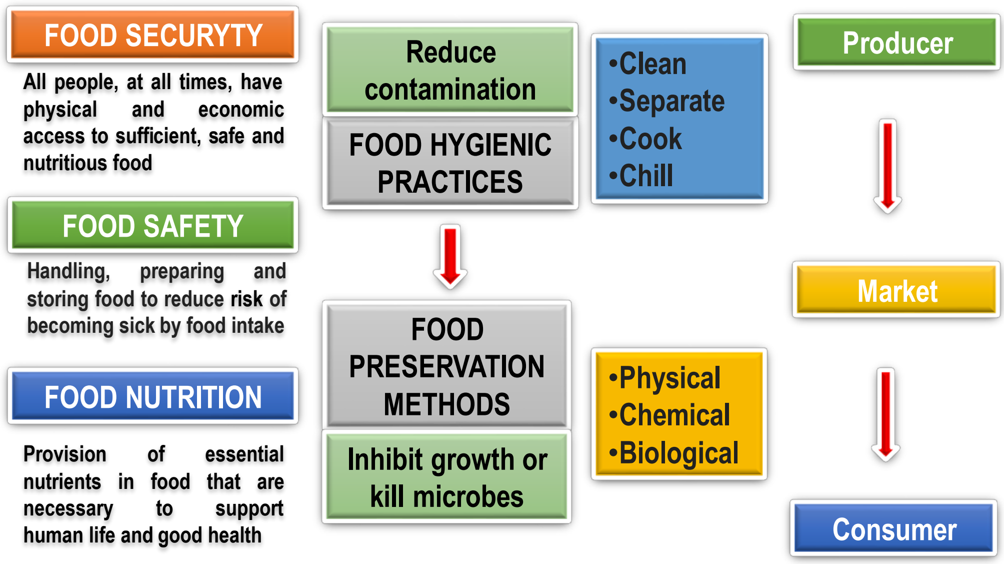
The processes of smoking and grilling foods by burning of coal, oil, gas, wood, garbage or other fuels are culinary practises followed by many cultures across the globe to cook and preserve food.

The smoking of food likely dates back to the Palaeolithic era. It is assumed that after the discovery of fire, humans hung meat to dry it and keep it away from pests, so most likely by chance they learned that meat that was kept in smoked areas was not only better preserved, but also acquired a different flavour. Although smoke acts both as antimicrobial and antioxidant, it is not sufficient to preserve food, because it does not penetrate far into meat or fish. Therefore, to preserve food, smoke was further combined with salt-curing or drying. Nowadays, smoking is a process mainly used for flavouring food.

Many carcinogenic chemical compounds are formed during the combustion of fuels. They include polycyclic aromatic hydrocarbons (**PHAs**) (e.g., benzopyrenes), dioxins, formaldehyde, and sulphur and nitrogen oxides (related to the formation of nitrosamines). Furthermore, combustion gases can also contain heavy metals. The different kinds of contaminants and their concentrations depend on the fuel used, as well as the temperature and many other parameters. Different epidemiological studies have found a statistical correlation between the occurrence of gut and other cancers, and a frequent intake of smoked foods.

Cooking over fire is an ancient practice contemporary to the emergence of our species. The smell and taste of meat charred by fire most probably evokes an ancient instinct. Today, grilling meats is an extended tradition in many countries, though it is not the healthiest practice. We know that cooking food over a flame is linked to cancer, since grilling or charring raises the levels of PAHs in food.

BOX 6. Relationships within food security, safety, nutrition and preservation



Relevance for Sustainable Development Goals and Grand Challenges

Gola 2: End hunger

One of the main contributors to world hunger is food loss and waste. To combat global hunger, the preservation of food after the harvest must play an important role. It is necessary to minimize losses due to spoilage of post-harvested food.

Currently, more than one-third of the food produced globally is lost to waste or spoilage. This is, approximately $1 trillion worth of food every year, accounting for roughly one-third of the world's food. Some studies conclude that there is potentially already enough food produced in the world to feed a growing population, if we could properly store and preserve it instead of letting it go to waste. In many parts of sub-Saharan Africa, farmers can lose almost half of their harvest before it leaves the farm.

Goal 3. Ensure healthy lives and promote well-being for all at all ages

According to WHO "access to sufficient amounts of safe and nutritious food is key to sustaining life and promoting good health". Food safety problems lead to quality losses and can have devastating impacts on nutrition and health. WHO aims "to achieve a world capable of preventing, detecting and responding to public health threats associated with unsafe food" and tell us that" food safety provides leadership in global efforts to lower the burden of diseases, thereby assisting Member States to develop food safety policies and successfully implement risk-based foodborne disease surveillance, prevention and control programmes".

WHO data shows that about 600 million people, this is, almost 1 in 10globally, fall ill after eating contaminated food and about 420000 die every year. About $110 billion is lost each year in productivity and medical expenses resulting from unsafe food in low- and middle-income countries. Foodborne diseases can hamper socioeconomic development of the poorest countries by overloading health care systems and damaging national economies, especially tourism and trade. As mentioned above (see Box 6) food safety, nutrition and food security are indivisibly linked. Unsafe food creates a malicious cycle of disease and malnutrition, particularly affecting young and elderly people.

Goal 10. Reduce inequality within and among countries

People in developing countries, where food insecurity risks are the highest, face the greatest negative impact from the loss of food, as well as in many cases the greatest hurdles to overcoming the challenge of preserving food. Although food losses occur along the entire food supply chain, regions with the greatest need for food, probably suffer the hardest hit. In addition, to the threat it poses to food security, post-harvest losses negatively affect farmers and consumers in the lowest income groups. On the other hand, in developing countries, food losses have also significant implications on the income of smallholder farmers, who dominate food production and account for a large proportion of the poor and undernourished populations. On-farm losses reduce the number of crops to be sold, thereby reducing the income of farmers, especially smallholders.

Goal 12. Ensure sustainable consumption and production patterns

According to WHO "a profound change in the global agri-food system is necessary if we have to feed more than 820 million hungry people and the 2 billion more people who will live in the world in 2050". Increasing agriculture productivity and ensuring sustainable food production are crucial to help alleviate hunger. But in addition, we have to ensure that the food we produce is used efficiently in such a way that food waste is minimal. Globally, as already mentioned, subsistence farmers can lose almost half of their harvest before it leaves the farm, simply because they do not have access to modern storage equipment. It is estimated that about one- third of world agricultural production never reaches the consumer or arrives in poor condition. Interestingly, in middle- and high-income countries, the greatest waste occurs in the consumption phase, because many consumers discard food that may still be fit for consumption. It is clear that more developed countries waste more food per capita than developing countries. However, in low-income countries, food is lost mainly at the beginning or the middle of the food chain, due to a number of financial, technical and other constraints. Techniques to improve harvesting, storage, refrigeration, and packaging are lacking in less developed countries, and such shortages contribute to food losses.

Potential Implications for Decisions

1. Individual

Individuals play an important role in the preservation and proper use of food in the supply chain. A portion of food losses result from our consumer behaviour. Therefore, we generate as consumers a great impact on the entire agri-food supply chain. Interaction between consumers and retailers is critical and enables a better understanding of food safety and food waste throughout the supply chain.

2. Community policies

In medium- and high-income countries the highest levels of food waste occur at the consumption stage when consumers discard food still edible. Industrialized countries waste more food per capita than developing countries. It is necessary to make a clear call to the general public, the private sectors and the national or local authorities to increase efforts to reduce food loss and waste toward ensuring food security for all, but particularly for the most vulnerable persons.

3. National policies related to food preservation

At a national level, it is necessary to continue public awareness campaigns through the media to educate people about food waste. It is also important to consider that food supply chains now cross multiple national borders and thus, collaboration between governments, producers and consumers helps ensure food safety. It could be helpful if developed countries could implement programs in developing countries aimed towards substantially reducing post-harvest food losses. It could be useful to provide developing countries methods to improve food security on their own. Some solutions could be as simple and inexpensive, as for instance, developing solar food driers to preserve food, solar refrigeration systems, improved biological control of pests, and smart packaging for food.

Pupil Participation

1. Class discussion of the issues associated with food preservation

- Distinguish which preservation methods kill microbes and which o only inhibit their growth.

- Discuss whether broken eggs are safe to eat

2. Student awareness activities

- Describe the main requirements for safe food preparation and preservation. Food storage, preparation and serving areas should be clean.

- Outline personal hygiene requirements when handling food. Your hands, as well as dishes, glasses and utensils must be kept clean by regular washing in clean water.

- use a household scale you to estimate how much food you throw away every day.

3. Exercises

- Take a lettuce leaf and cut it into three pieces: place one piece in the fridge, another piece in the freezer and leave one at room temperature in the kitchen. Observe what happens after a week. The same can be repeated with other types of food.

- Keep a strawberry several days at room temperature in different containers, such as a zippered plastic bag, a Tupperware, plastic wrap or simply on the kitchen without covering it.

- Prepare 3 different honey dilutions, e.g., at 1%, 5% and 10%, using honey and sterile water. Spray with the different dilutions three strawberries and made another one for control sprayed only with water. Let the berries dry overnight, placed them together in a bowl, and left at room temperature. During the next days, check for signs of soft spots, dark spots, or mould. The experiment can be also carried out using sucrose or salt instead of honey.

References. The evidence base, further reading and teaching aids

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Glossary

Animalcules. An old term used by early scientists to describe tiny living organisms such as bacteria and protozoa, invisible to the naked eye.

Biopreservation. Using natural or controlled microorganisms and their products to keep food safe and extend its shelf life.

Bottling. Preserving food or drinks by sealing them in sterilized bottles and heating them to prevent spoilage.

Canning. Preserving food in airtight containers by heating to kill microbes and sealing to keep them out.

Dehydration. Removing water from food to stop microbes and enzymes from causing spoilage.

Denaturate. Changing the natural structure of a protein or molecule (by heat, acid, or pressure) so it loses its normal function.

Foodborne. Refers to illnesses caused by eating or drinking contaminated food or beverages.

Ionizing radiation High-energy radiation (like gamma rays or X-rays) that kills microbes by damaging their DNA.

Lyophilization (Freeze-drying). Preserving food by freezing and then removing water through sublimation, keeping flavor and nutrients.

Nonionizing radiation. Low-energy radiation (like UV light) that kills microbes but doesn't penetrate deeply into materials.

Organoleptic. Describes food characteristics like taste, smell, look, and texture that affect how we sense and enjoy it.

Pasteurization. Heating food or drinks (like milk or juice) to kill harmful microbes without changing flavor or quality much.

Phages. Short for bacteriophages — viruses that infect and kill bacteria, used to improve food safety.

PHAs (Polycyclic Aromatic Hydrocarbons). Chemicals formed during incomplete burning of fuels; some, like benzo[a]pyrene, are cancer-causing and found in smoked or grilled foods.

Psychrophilic. Describes microbes that grow best at cold temperatures (0–10 °C), often spoiling refrigerated food.

Smoking. Preserving and flavoring food by exposing it to smoke from burning wood or other materials.

Sublimation. When a solid is transformed directly into a gas without becoming liquid first — used in freeze-drying.

Sterilization. Completely removing or destroying all microbes, including spores, using heat, chemicals, or radiation.

Thermophilic. Microbes that grow best at high temperatures (45–80°C), common in some fermentations.

Uperization (UHT processing). Heating food, especially milk, to very high temperatures (135–150°C) for a few seconds to sterilize it.

Water activity (a_x). A measure of how much water in food is available for microbes to grow, from 0 (dry) to 1 (pure water).

Zoonoses. Diseases that can pass between animals and humans, either directly or through contaminated food or water.