**MICROBIAL ECOLOGY OF FOOD**

**Microorganisms in food**

Generally, microorganisms are of great importance in our lives. It is difficult to dissociate some daily gestures/habits to the absence of microorganisms. During our meals we ingest foods in which microorganism have or had a fundamental action – yogurt, cheese, bread, etc; beverages that without the action of microorganisms would not exist – beer, wine, cacao, etc. They are fundamental for good functioning of the human digestive system, being responsible for the fermentations that take place and responsible for producing some essential vitamins for our organism. But they don’t only exist to facilitate our lives. They are also responsible for a lot of our illnesses. Foodborne illnesses are in most cases originated by microorganisms. They are also the main cause of deterioration of food and food products.

Microorganisms in food can be classified basically as; pathogen, causing deterioration, indicator and beneficial microorganisms.

Pathogen Microorganisms: Microorganisms are ubiquitous in all terrestrial and aquatic ecosystems. Of the vast number of species, only a small subset are human pathogens, capable of causing varying degrees of illness in humans. Pathogenic microorganisms pass through food and water and cause bacterial, viral, fungal and parasitic food infections and intoxications on certain conditions. Some microorganisms reach the depth of the tissue under appropriate conditions and reproduce there, and activating the defense mechanism of the body. This is evidenced by the presence of symptoms in the affected living organism.

Microorganisms Causing Deterioration: The microorganisms in this group are not directly pathogenic, but their metabolic activation plays a role in the degradation of the food and they are called saprophytic microorganisms. Many of these microorganisms have the ability to compete well in the foods, they can multiply rapidly under appropriate conditions, leading to enzymatic reactions and deterioration.

Indicator microorganisms: Indicator microorganisms are microorganisms that provide information on the general hygienic condition of production and storage conditions as well as errors and contaminations during or after the processing of a product. These organisms often do not cause illness directly, but have demonstrated characteristics that make them good indicators of harmful pathogens in foods. Main characteristics of indicator microorganisms; able to identify easily and rapidly, easily distinguishable from other microorganisms in the microflora, indicate the presence of existing pathogens, the numbers should correlate with the relevant pathogenic microorganisms. As indicator microorganisms, coliform, fecal coliform, *E. coli, Enterobacteriaceae* and *enterococci* are used. For example, the obligatory bowel coliform bacteria present in foods indicates a fecal contamination.  Index organisms signal the increased likelihood of a pathogen originating from the same source as the index organism and thus serve a predictive function.  Higher levels of index organisms may (in certain circumstances), correlate with a greater probability of an enteric pathogen(s) being present. The presence of some other microorganisms in food affects the process applied to processed products. Heat-processed egg products must not contain species of the *Enterobacteriaceae*. It should be noted that if these bacteria are present in the egg, *Salmonella* and other enteric pathogenic bacteria may be present in this family. Therefore, enterobacteria are considered as index microorganisms in this sense.

Beneficial microorganisms: The effects of microorganisms are not always undesirable. The technological use of microorganisms constitutes an important branch of food microbiology, and starter cultures are of great importance in this context. Because some foods have specific organoleptic and technological properties that ultimately result in the development of specific bacteria and fungi and their metabolic effects. In addition, useful microorganisms suppress the growth of other microorganisms that cause pathogenesis or degradation. Much of the microorganisms used as starter cultures form yeast and molds with homofermentative lactic acid bacteria, some micrococci and staphylococci. Probiotics are live microorganisms that have positive health effects when taken in certain amounts. Probiotics have properties such as fermenting different carbohydrates, surviving at low pH values, producing bacteriocins, organic acid formation and specific enzymatic activation. Most of the probiotics used in humans are selective species of *Bifidobacterium* and *Lactobacillus*.

**Microbial Growth**

When we talk about microbial growth, we understand that it has to do with an increase of the number of cells. Growth rate is greater when all conditions are optimized and any alteration on these conditions will reflect on the growth rate. Greater growth rate on a certain food means greater nutrient intake, which in turn, implicates greater alterations.

There are four phases of bacterial growth. First one called lag phase. This is the period of adjustment to new conditions. Little or no cell division occurs, population size doesn’t increase. Phase of intense metabolic activity, in which individual organisms grow in size. May last from one hour to several days. Second is log or exponential phase. Cells begin to divide and generation time reaches a constant minimum. Period of most rapid growth. Number of cells produced > Number of cells dying. Cells are at highest metabolic activity. Cells are most susceptible to adverse environmental factors at this stage. Third phase is stationary phase. Population size begins to stabilize. Number of cells produced = Number of cells dying. Overall cell number does not increase; cell division begins to slow down. Factors that slow down microbial growth are; accumulation of toxic waste materials, acidic pH of media, limited nutrients, insufficient oxygen supply. Last one is the death or decline phase. Population size begins to decrease. Number of cells dying > Number of cells produced. Cell number decreases at a logarithmic rate. Cells lose their ability to divide and a few cells may remain alive for a long period of time.

 

**Origins of microorganisms in food**

Microorganisms can have various effects on food, as mentioned before. We also talked about what main factors affected microbial growth on food, but where are they from? How do microorganisms “appear” on food? Microorganisms on food have many different origins: air, soil, water, handlers, utensils, equipment and obviously products themselves or raw material used in their fabrication.

Water, air, soil, and food themselves, contribute with their own natural microflora. Each one of these environments presents microflora of different compositions, in quantity and in diversity, but many times are interconnected with each other.

**Soil:** Soilitself is an environment with various microenvironments (sandy and dry soils have different microflora from wet and fertile soils). It’s an important source of spore forming bacteria (Bacillus, Clostridium), of fungi and yeasts. When soils are fertilized with animal manure, fecal microorganisms (present in warm blooded animal intestines) such as coliforms, salmonella, enterococci, etc. are added to the natural flora. These microorganisms are easily passed to the products cultivated there, especially roots, tubercules and ground level vegetables. On the other side, soil microorganisms can be transported in dust by wind, rain or water form watering and contaminate fruit and other vegetables. Soil microorganism dissemination can also be carried out by animals (adhered to hooves, fur, feathers, etc.), insects, etc.

**Water:** Water holds microflora whose composition reflects its origin and level of pollution. In a Public health point of view, the presence of fecal microorganisms deserves particular attention because the presence of these microorganisms may indicate the presence of pathogenic microorganisms, which through this source can easily propagate to other foods. That is why it is important to use good microbiological quality water, not only for washing or preparing foods or beverages but also for washing utensils used to prepare and/or hold foods. In fact, according to some authors, one of the main sources of cooked food held in ice (for example, seafood) contamination is the ice itself, due to the use of bad quality water for its preparation.

**Air:** On the contrary to what we think, air is not a direct source of microorganisms. It is, however, an excellent conveyer of microorganisms from other sources. The composition of air does not allow the development of microorganism because they can’t find any nutrients in it. So, what we find in air are microorganisms from the surrounding environment. It’s not difficult to foresee that microorganisms found in kitchen air are different form the ones found in the air of an office of any business. Some activities or gestures carried out by man are the main cause for the “introduction” of microorganisms in air. Simple gestures like shaking your head – especially with long free hair, sneezing or coughing, transfer to the air many microorganisms of the human flora. Activities related to production of vegetable products also transfer to the air microorganisms of different origins: tilling lifts dusts; watering – especially by aspersion – forms drops of water containing microorganisms form soil and water; etc.

**Food:** Each product, whether of animal or vegetable origin, holds characteristic flora, that essentially depends on the environment where it was brought up or produced. Generally, internal vegetable product tissues contain few microorganisms. On the contrary, external tissues carry a very extended and varied load of microorganisms, due to greater exposure to air, soil. Normally, in healthy animals, the interior of their meat is exempt form microorganisms. All animals have biological systems that limit microorganism access to the interior of the meat. During slaughter access is facilitated. Microorganisms are mainly found on the surface – skin, fur, feathers, scales, etc. and in the digestive system. Following slaughter, the removal of skin, feathers and entrails constitute the main contamination risk points. Removing the entrails requires special precaution due to the mass presence of fecal microorganisms.

**Human:** Handlers play an important role regarding the flora of food and food products. Along with air, they constitute one of the main sources of food microorganisms. Just like animals used as a food source, man also possesses a specific flora adapted to various environments that are found on the human body. Coliforms and *Staphylococcus* aureus are the main microorganisms related to food contamination by man. These microorganisms are originated from fecal matter (coliforms) and handlers´ skin. Special hygiene care is required when handling cooked food or food that is going to be consumed raw.

**Utensils and equipment:** Utensils and equipments don’t have their own microflora. The microflora they possess is a reflex of cleaning and maintenance care that they undergo. Considering that microorganisms easily adhere to materials, the contact of food with poorly clean surfaces – work areas, wall, etc. – substantially increases the microbial load. Poorly clean machines and accessories are inevitably contamination sources. The same happens with other utensils, knives, cutting boards and recipients. It is fundamental that the same utensils are not used to manipulate or store different foods in order to avoid cross contaminations.

**FACTORS THAT INFLUENCE THE GROWTH OF MICROORGANISMS IN FOOD**

Just like all living beings, all microorganisms require a set of factors that allow them to grow/live in certain environments. These factors are obviously different from microorganism to microorganism. Food is a chemically complex matrix, and predicting whether, or how fast, microorganisms will grow in any given food is difficult. Most foods contain sufficient nutrients to support microbial growth. Several factors encourage, prevent, or limit the growth of microorganisms in foods.

The most important factors that affect microbial growth in foods can be summarized in the following categories:

* factors related to the food itself, the “intrinsic factors,” which include nutrient content, water activity, pH value, redox potential, and the presence of antimicrobial substances and mechanical barriers to microbial invasion.
* factors related to the environment in which the food is stored, the “extrinsic factors,” including the temperature of storage, and the composition of gases and relative humidity in the atmosphere surrounding the food.
* factors related to the microorganisms themselves, the “implicit factors,” including interactions between the microorganisms contaminating the food and between these microorganisms and the food, e.g., their abilities to utilize different nutrient sources, tolerate stresses, and produce promoters or inhibitors of growth of other microorganisms.
* processing factors, which include treatments such as heating, cooling, and drying that affect the composition of the food and also affect the types and numbers of microorganisms that remain in the food after treatment

**Intrinsic Factors**

**Water Activity (a*w*):** Microorganisms need water in an available form to grow in food products. The control of the moisture content in foods is one of the oldest exploited preservation strategies. Water activity is a measure of the amount of freely available water within a food. It is expressed as the ratio of water vapor pressure of the food substrate to the vapor pressure of pure water at the same temperature.

a*w* = p/po

p: vapor pressure of the solution

po: vapor pressure of the solvent

The a*w* of pure water is 1.00 and the a*w* of a completely dehydrated food is 0.00. Most fresh foods, such as fresh meat, vegetables, and fruits, have a*w* values that are close to the optimum growth level of most microorganisms (0.97 to 0.99). Foods with low water activities cannot support the growth of microorganisms. Pathogenic and spoilage bacteria do not grow in food with a water activity of less than 0.85. Many yeasts and moulds however are capable of growth at much lower water activities than this; some can even grow at a*w* 0.60. Microorganisms generally have optimum and minimum levels of a*w* for growth depending on other growth factors in their environments. For example, Gram (–) bacteria are generally more sensitive to low aw than Gram (+) bacteria. It should be noted that many bacterial pathogens are controlled at water activities well above 0.86 and only *S. aureus* can grow and produce toxin below a*w* 0.90.

The a*w* can be manipulated in foods by a number of means, including addition of solutes such as salt or sugar, physical removal of water through drying or baking, or binding of water to various macromolecular components in the food. Weight for weight, these food components will decrease a*w* in the following order: ionic compounds > sugars, polyhydric alcohols, amino acids and other low-molecular-weight compounds > high-molecular-weight compounds such as cellulose, protein or starch.

It is as a result of water activity that dry foods such as crackers or dried pasta can have a shelf life of many months and not be spoilt by microorganisms. Foods such as jams and parmesan cheese (a*w* 0.60 - 0.85) will show signs of mold growth over time but no bacterial growth, and foods such as meat and milk (a*w* 0.98 - 0.99) are associated with food poisoning causing bacteria.

Microorganisms that require minimum concentrations of salt (sodium chloride [NaCl] and other cations and anions) are called halophiles, whereas organisms that can grow in high concentrations of organic solute, particularly sugars, have been called osmophiles.

**pH and acidity:** pH is a term used to describe the acidity or alkalinity of a solution. At pH 7, there is an equal amount of acid (hydrogen ion: H +) and alkali (hydroxyl ion: OH-), so the solution is “neutral”. pH values below 7 are acidic, while those above 7 are alkaline. The pH is a function of the hydrogen ion concentration in the food

pH = –log10 [H+]

pH has a profound effect on the growth of microorganisms. The control of intracellular pH is required in order to prevent the denaturation of intracellular proteins. Each organism has a specific requirement and pH tolerance range; some are capable of growth in more acid conditions than others. It is well known that groups of microorganisms have pH optimum, minimum, and maximum for growth in foods. Most bacteria grow best at about pH 7 and grow poorly or not at all below pH 4. Yeasts and molds, therefore, predominate in low pH foods where bacteria cannot compete. The lactic acid bacteria are exceptions; they can grow in high acid foods and actually produce acid to give us sour milk, pickles, fermented meats, and similar products.

Fruits are naturally acidic, which inhibits the growth of many bacteria, therefore spoilage of these products is usually with yeasts and molds. Meat and fish however have a natural pH much nearer neutral and they are therefore susceptible to the growth of pathogenic bacteria. Another important characteristic of a food to consider when using acidity as a control mechanism is its buffering capacity. The buffering capacity of a food is its ability to resist changes in pH. Foods with a low buffering capacity will change pH quickly in response to acidic or alkaline compounds produced by microorganisms as they grow. Meats, in general, are more buffered than vegetables by virtue of their various proteins.

**Redox potential (Eh):** Also known as the oxidation-reduction potential. Is defined in terms of the ratio of the total oxidizing (electron accepting) power to the total reducing (electron donating) power of the substance. In effect, redox potential is a measurement of the ease by which a substance gains or loses electrons. The redox potential (Eh) is measured in terms of millivolts. A fully oxidized standard oxygen electrode will have an Eh of +810 mV at pH 7.0, 30 °C, and under the same conditions, a completely reduced standard hydrogen electrode will have an Eh of –420 mV.

The major groups of microorganisms based on their relationship to Eh for growth are aerobes, anaerobes, facultative aerobes, and microaerophiles. Aerobic organisms require a food to have a positive redox potential (an oxidised state) whereas anaerobes require a negative potential (a reduced state) for growth. It should be noted that the presence of oxygen is not an absolute requirement for oxidation reduction reactions as other compounds can accept electrons.

Different foods have distinct redox potentials and these influence the type of microbial growth typically seen in that food. These values can be highly variable depending on changes in the pH of the food, microbial growth, packaging, the partial pressure of oxygen in the storage environment, and ingredients and composition (protein, ascorbic acid, reducing sugars, oxidation level of cations, and so on).

**Nutrient content:** Microorganisms require certain basic nutrients for growth and maintenance of metabolic functions. The amount and type of nutrients required range widely depending on the microorganism. These nutrients include water, a source of energy, nitrogen, vitamins, and minerals.

The carbohydrate (simple sugar) are most commonly utilized as an energy source. Protein rich food like meat, egg, fish etc. are always spoiled by proteolytic organism because they can utilize protein as a source of energy if sugar is not available. In fact, protein rich food promotes more growth of bacteria then yeast and mold. Similarly, in the general mold can grow in the higher concentration of sugar, yeast in fairly high concentration but most bacteria grow best in the low concentration of sugar. In the same way, some microorganisms are unable to manufacture or synthesis some of their requirement(like amino acid, vitamins etc) and most depends on the food for their supply. In general gram-positive bacteria are least synthetic, gram-negative bacteria and mold are able to synthesize most of their requirement.

Foodborne microorganisms can derive energy from carbohydrates, alcohols, and amino acids. Most microorganisms will metabolize simple sugars such as glucose. Others can metabolize more complex carbohydrates, such as starch or cellulose found in plant foods, or glycogen found in muscle foods. Some microorganisms can use fats as an energy source.

Amino acids serve as a source of nitrogen and energy and are utilized by most microorganisms. Some microorganisms are able to metabolize peptides and more complex proteins. Other sources of nitrogen include, for example, urea, ammonia, creatinine, and methylamines.

Examples of minerals required for microbial growth include phosphorus, iron, magnesium, sulfur, manganese, calcium, and potassium. In general, small amounts of these minerals are required; thus a wide range of foods can serve as good sources of minerals.

**Antimicrobial substances and mechanical barriers:** Certain foods naturally contain antimicrobial substances that will exhibit an inhibitory action on the growth of micro-organisms. There are a number of plant-based antimicrobial constituents, including many essential oils, tannins, glycosides, and resins, which can be found in certain foods. Specific examples include eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allyl isothiocyanate in mustard, eugenol and thymol in sage, and carvacrol (isothymol) and thymol in oregano.

Some animal-based foods also contain antimicrobial constituents. Examples include lactoferrin, conglutinin and the lactoperoxidase system in cow’s milk, lysozyme in eggs and milk, and other factors in fresh meat, poultry, and seafood.

It is also known that some types of food processing result in the formation of antimicrobial compounds in the food. The smoking of fish and meat can result in the deposition of antimicrobial substances onto the product surface. Some types of fermentations can result in the natural production of antimicrobial substances, including bacteriocins, antibiotics, and other related inhibitors. Bacteriocins are proteins or peptides that are produced by certain strains of bacteria that inactivate other, usually closely related, bacteria. The most commonly characterized bacteriocins are those produced by the lactic acid bacteria. The antibiotic nisin produced by certain strains of *Lactococcus lactis* is one of the best characterized of the bacteriocins. Nisin is a polypeptide that is effective against most Gram (+) bacteria but is ineffective against Gram (–) organisms and fungi and it is approved for food applications in over 50 countries around the world.

In addition to naturally occurring antimicrobial compounds in foods, a variety of chemical preservatives and additives can extend the shelf life of food and/or inhibit pathogens, either singly or in combination.

Plant- and animal-derived foods, especially in the raw state, have biological structures that may prevent the entry and growth of pathogenic microorganisms. Examples of such physical barriers include testa of seeds, skin of fruits and vegetables, shell of nuts, animal hide, egg cuticle, shell, and membranes.

Plant and animal foods may have pathogenic microorganisms attached to the surface or trapped within surface folds or crevices. Intact biological structures thus can be important in preventing entry and subsequent growth of microorganisms. Several factors may influence penetration of these barriers. The maturity of plant foods will influence the effectiveness of the protective barriers. Physical damage due to handling during harvest, transport, or storage, as well as invasion of insects can allow the penetration of microorganisms. During the preparation of foods, processes such as slicing, chopping, grinding, and shucking will destroy the physical barriers. Thus, the interior of the food can become contaminated and growth can occur depending on the intrinsic properties of the food.

**Extrinsic Factors**

**Temperature:** As temperature influences enzymatic reactions it has an important role in promoting or preventing microbial growth. The relationship between temperature and growth rate constant varies significantly across groups of microorganisms. Four major groups of microorganisms have been described based on their temperature ranges for growth: thermophiles, mesophiles, psychrophiles, and psychrotrophs.



At low temperatures, two factors govern the point at which growth stops; reaction rates for the individual enzymes in the organism become much slower, and low temperatures reduce the fluidity of the cytoplasmic membrane, thus interfering with transport mechanisms. At high temperatures, structural cell components become denatured and inactivation of heat-sensitive enzymes occurs. While the growth rate increases with increasing temperature, the rate tends to decline rapidly thereafter, until the temperature maximum is reached.

Mesophilic and psychrotrophic microorganisms are of great importance for food safety. The optimal temperature for mesophilic growth is around 37 ° C. This group includes pathogens such as *Salmonella*, *S. aureus* and *C. perfringens*, which are originated from human and animals and frequently cause the formation of food infections and intoxications. Some pathogenic microorganisms, such as *Aeromonas hydroproilia*, *Clostridium botulinum* type E, *Listeria monocytogenes, Yersinia enterocolitica*, from psychrophile-psychrotrophic microorganisms-even if they are slow- can grow at the temperature of the refrigerator, resulting in public health hazards. Psychrotrophs are most commonly found in foods and are belong to the *Pseudomonas* and *Enterococcus* lines. These bacteria cause deterioration in the temperature of the refrigerator and in foods normally stored in the cold, such as meat, fish, poultry, eggs.

When considering growth rate of microbial pathogens, time and temperature are integral and must be considered together. Increases in storage and/or display temperature will decrease the shelf life of refrigerated foods since the higher the temperature, the more permissive conditions are for growth.

**Gas/Atmosphere:** Many scientific studies have demonstrated the antimicrobial activity of gases at ambient and sub ambient pressures on microorganisms important in foods. Gases inhibit microorganisms by two mechanisms. First, they can have a direct toxic effect that can inhibit growth and proliferation. Carbon dioxide, ozone, and oxygen are gases that are directly toxic to certain microorganisms. This inhibitory mechanism is dependent upon the chemical and physical properties of the gas and its interaction with the aqueous and lipid phases of the food. Oxidizing radicals generated by ozone and oxygen are highly toxic to anaerobic bacteria and can have an inhibitory effect on aerobes depending on their concentration. Carbon dioxide is effective against obligate aerobes and at high levels can deter other microorganisms. A second inhibitory mechanism is achieved by modifying the gas composition, which has indirect inhibitory effects by altering the ecology of the microbial environment. When the atmosphere is altered, the competitive environment is also altered.

A variety of common technologies are used to inhibit the growth of microorganisms, and a majority of these methods rely upon temperature to augment the inhibitory effects. Technologies include modified atmosphere packing (MAP), controlled atmosphere packaging (CAP), controlled atmosphere storage (CAS), direct addition of carbon dioxide (DAC), and hypobaric storage.

The preservation principle of antimicrobial atmospheres has been applied to fruits and vegetables, raw beef, chicken and fish, dairy foods including milk and cottage cheese, eggs, and a variety of prepared, ready-to-eat foods.

**Relative Humidity:** The relative humidity in which a food is stored can have an influence on the water activity of that product and an influence on the growth of microorganisms on the surface of a product. If the growth of micro-organisms in a food is controlled by the water activity of a product then it is very important that the food be stored under relative humidity conditions which will not allow the uptake of moisture from the air, and therefore an increase in water activity. Packaging can be used to limit the migration of moisture into the product.

A very high relative humidity level favors microbial growth, especially those that are found on surfaces. For some time now, dehydration and drying techniques have been used to maintain foods for longer periods of time. However, foods must be stored in low relative humidity conditions; otherwise, the humidity that is present in the surrounding environment will eventually end up increasing the amount of water in foods, and consequently, increase the risk of microbial proliferation.

**Implicit Factors**

**Synergism and Antagonism:** Microorganisms are found in food as mixed cultures rather than pure cultures, and there is a mutual interaction between them, expressed as synergism and antagonism. Microorganisms may compete with another microorganism for nutrients and these competitions often result to inhibition of one of the microorganisms. This is termed as antagonism which is described as the inhibition of a bacterium by the products of another. Some examples of it are;

* *E. coli*cannot grow in the rumen due to presence of high amounts of lactic acid produced by rumen anaerobes.
* High concentrations of ethanol (eg: wine production) precludes most microbes other than the yeasts. However, *Acetobacter* converts ethanol to acetate if O2 is present.
* Lactate and propionate production in cheese manufacturing and acetic acid in vinegar inhibit spoilage causing microbes.

However, microorganisms may also rely on other species for nutrient supply. At some instances, both bacteria and/or fungi may benefit from one another. This is termed synergism. An example of which is when one species produces a substance not synthesized by another species but beneficial to it.

* A population of *Streptococcus faecalis* produces ornithine which *Escherichia coli* can not synthesize. *E. coli* may then take up ornithine in order to synthesize protein. *E. coli*, however, produces putresine which is not synthesized by *S. faecalis*, and this nutrient may be beneficial to *S. faecalis*.
* B vitamins synthesized by yeast may enhance the growth of lactic acid bacteria.
* Molds increase the aw value in relative dry foods, making the substrate suitable for the development of bacteria.

**Processing Factors**

Processing factors are processes that are consciously applied during the preparation or storage of food. Depending on these factors, changes in the composition of the foods and the microflora and the number of microorganisms occur.

Food processing dates back to prehistoric ages, with crude processing methods that included slaughtering, fermenting, sun drying, preserving with salt, and various means of cooking (such as roasting, smoking, steaming, and oven baking). Salt-preservation was especially common for foods that constituted the diets of warriors and sailors, up until the introduction of canning methods.

Thermal processing is one of the most widely used unit operations employed in the food industry. There are two main temperature categories employed in thermal processing: Pasteurization and Sterilization. Other examples of processing techniques used in foods are, irradiation, packaging, adding chemical preservatives, fermentation etc.