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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – I – Food Biotechnology – SBB2203

1. Microorganisms in food production

Nature uses microorganisms to carry out fermentation processes, and for thousands of years mankind has used **yeasts, moulds and bacteria** to make food products such as bread, beer, wine, vinegar, yoghurt and cheese, as well as fermented fish, meat and vegetables.

Fermentation is one of the **oldest transformation and preservation techniques for food**. This **biological process** allows not only the **preservation of food** but also **improves its nutritional and organoleptic qualities** (relating to the senses; taste, sight, smell, touch). A well conducted fermentation will favour useful flora, to the detriment of undesirable flora in order to prevent spoilage and promote taste and texture.

A bit of history

The first realisation that microorganisms were involved in food production processes was **in 1837**, when scientists discovered the **role of yeast in an alcoholic fermentation**.

Later, when the world renowned French chemist and biologist **Louis Pasteur** was trying to explain **what happened during the production of beer and vinegar in the 1860es**, he found that microorganisms were responsible.

However, it wasn't until **after the Second World War** that the food industry began to develop the **biotechnological techniques** we rely on today to produce a wide variety of **better, safer foods** under controlled conditions

Production of microbial cultures in the dairy industry

It would be impossible to make cheese without a starter culture. As the culture grows in the milk, it converts the sugar lactose into lactic acid, which ensures the correct level of acidity and gives the cheese its moisture. As the cheese ripens, the culture gives it a balanced aroma, taste, texture. It is also responsible for the 'holes' in cheeses such as Emmenthal. Choosing the right mixture of culture is essential for a high-quality cheese.

In yoghurt and other fermented milk products, the culture is responsible for the taste and texture of the final product. Depending on the acidity, the product will have either a mild or strong taste, and the viscosity depends on the quantity of polysaccharides – chains of sugar molecules – that are produced.

In recent years, probiotic cultures have become popular in dairy products because of their health benefits. These cultures are all very carefully selected strains, and there is good evidence that they help improve digestion, safeguard the immune system, and keep the body's intestinal flora in balance.

Production of microbial cultures in meat industry

Meat starter cultures are used to make dried, fermented products such as salami, pepperoni, chorizo and dried ham. Lactic bacteria develop the flavour and colour of the products. In addition, a wide variety of moulds are use to ripen the surface of sausages, preserving the natural quality of the product and controlling the development of flavour.

Production of microbial cultures in wine industry

Yeasts are responsible for the fermentation process which produces alcohol in wine. However, lactic bacteria also play an important role, as they convert the unstable malic acid that is naturally present in wine into the stable lactic acid. This conversion gives the stability that is characteristic of high-quality wines that improve on storage.

Production of microbial cultures in healthfood industry

Lactic bacteria are used in many different tablets and capsules sold as supplements in the healthfood industry. Our hectic modern lifestyles often lead to an imbalance in the intestinal flora; travel and medical treatment are two of the major culprits. By taking supplements containing lactic bacteria, this balance can be restored, improving the quality of life.

Reference:

https://effca.org/microbial-cultures/food-production/#:~:text=Microorganisms%20in%20food%20production,fermented%20fish%2C%20meat%20and%20vegetables.

Uses of Microorganisms in the Food Industry

Currently, more than 3500 traditionally fermented foods exist in the world. They are of animal or vegetable origin and are part of our daily life. Alcoholic drinks are not the only fermented drinks; cocoa beans, coffee grains and tea leaves are fermented after harvest in order to develop their typical flavour profiles.

Bacteria

Bacteria are the largest group of unicellular microorganisms. The shapes of medically important bacteria are classified into-cocci, or spherical cells; bacilli, or cylindrical or rod shaped cells; and spiral or curved forms. The pathogenic or disease causing bacteria are usually gram negative, however, three gram-positive rods are known to cause food intoxications : *Clostridium botulinum,C. perfringens,and Bacillus cereus*

Some of the other most common bacteria causing food spoilage, infections and disease are *Acinetobacter, Aeromonas, Escherichia, Proteus, Alcaligenes, Flavobacterium, Pseudomonas, Arcobacter, Salmonella, Lactococcus, Serratia, Campylobacter, Shigella, Citrobacter, Listeria, Staphylococcus, Micrococcus, Corynebacterium, Vibrio Enterobacter, Paenibacillus, Weissella, Enterococcus, Yersinia*

Different strains of bacteria are also used in production of various food and dairy products. Strains of Streptococcus, *Lactobacillus Bifidobacterium*, Erwiniaetc. are used in the production of fermented food and dairy products. *Streptococcus thermophilus* and *Lactobacillusbulgaricus* are used to produce yogurt.

Agriculture Food and Analytical Bacteriology

Analytical microbiology is a study, application and use of microorganisms as reagents for the quantitative determination of certain chemical compounds. These procedures are based on the reaction of a particular microorganism to its environment.

If a microorganism reacts with a measurable response to a certain chemical entity and yields a suitable result, then this analytical method for the quantitative estimation of the substance may be devised as per the requirements of food culture, fermentation or preservation.

Molds:

Molds are multicellular filamentous fungi whose growth on foods is usually readily recognized by their fuzzy or cottony appearance. They are mainly responsible for food spoilage at room temperature 25- 30oC and low pH, and have minimum moisture requirement.

Molds can rapidly grow on grains and corns when these products are stored under moist conditions. Molds require free oxygen for growth and hence grow on the surface of contaminated food.

Molds also find their use in manufacturing of different foods and food products. They are used in ripening of various types of food products as cheese (e.g. Roquefort,Camembert).

Molds are also grown as feed and food and are employed to produce ingredients such as enzymes like amylase used in making bread or citric acid used in soft drinks.

Molds are major contributors in the ripening of many oriental foods. A species of *Bothrytiscinerea*, is used in rotting of grape for production of wine. Lactic fermentations using molds results in a unique Finnish fermented milk called viili.

Yeasts:

Yeasts have the ability to ferment sugars to ethanol and carbon-dioxide and hence they are extensively in food industry. The most commonly used yeast, the baker’s yeast is grown industrially.

Saccharomyces carlsbergensis is most commonly used in fermentation of most beers. The other yeast strains of importance are *Brettanomyces, Schizosaccharomyce,, Candida, Cryptococcus, Debaryomyces, Zygosaccharomyces, Hanseniaspora, Saccharomyces*

Factors Affecting Growth of Microorganisms

To understand the uses of microorganisms in the food industry, it is imperative to understand how to use the microorganisms, as they tend to react differently in various conditions and environments.

- Removing or destroying them by trimming, washing, heating, pickling.
- Adding chemicals like acid or alcohol or by encouraging competition to form organisms.
- Minimizing contamination from raw or unprocessed food, people, equipment, and the environment.
- Minimizing microbial growth by cleaning and sanitizing the equipment (container etc).
- Adjusting storage pH, light penetration, temperature, and other environmental factors.

Although each of these factors affecting growth can happen separately, it might occur simultaneously in nature. When more than one condition is somewhat adverse to microbial growth, their inhibitory effects are cumulative.

Points to remember

- Bacteria, molds and yeast are the most important microorganisms that cause food spoilage and also find the maximum exploitation in production of food and food products.
- Different strains of bacteria and fungus are used for fermentation of dairy products for production of a wide variety of cultured milk products. Both bacteria and fungi are used in these cheese production processes.
- Lactic acid bacteria are used for coagulation of milk that can be processed to yield a wide variety of cheeses, including soft unripened, soft ripened, semisoft, hard, and very hard types.
- Microorganisms such as *Lactobacillus and Bifidobacterium* are used as in food and health industry.
- *Spirulina,* a *cyanobacterium,* also is a popular food source sold in specialty stores.

- Molds are used for rotting of grapes for production of different varieties of wines.
- Mushrooms *(Agaricusbisporus)* are one of the most important fungi used as a food source.
- Alcoholic beverages as beer are produced by fermentation of cereals and grains using different strains of yeasts.

Reference:

https://lab-training.com/2015/03/11/beneficial-role-of-microorganisms-in-food-industry/

Food Spoilage

Food is considered contaminated when unwanted microorganisms are present. Most of the time the contamination is natural, but sometimes it is artificial. **Natural contamination** occurs when microorganisms attach themselves to foods while the foods are in their growing stages. For instance, fruits are often contaminated with yeasts because yeasts ferment the carbohydrates in fruits. **Artificial contamination** occurs when food is handled or processed, such as when fecal bacteria enter food through improper handling procedures.

Food spoilage is a disagreeable change or departure from the food's normal state. Such a change can be detected with the senses of smell, taste, touch, or vision. Changes occurring in food depend upon the composition of food and the microorganisms present in it and result from chemical reactions relating to the metabolic activities of microorganisms as they grow in the food.

Types of spoilage. Various physical, chemical, and biological factors play contributing roles in spoilage. For instance, microorganisms that break down fats grow in **sweet butter** (unsalted butter) and cause a type of spoilage called**rancidity.** Certain types of fungi and bacteria fall into this category. Species of the Gram-negative bacterial rod *Pseudomonas* are major causes of rancidity. The microorganisms break down the fats in butter to produce glycerol and acids, both of which are responsible for the smell and taste of rancid butter.

Another example occurs in **meat**, which is primarily protein. Bacteria able to digest protein (proteolytic bacteria) break down the protein in meat and release odoriferous products such as putrescine and cadaverine. Chemical products such as these result from the incomplete utilization of the amino acids in the protein.

Food spoilage can also result in a sour taste. If **milk** is kept too long, for example, it will sour. In this case, bacteria that have survived pasteurization grow in the milk and produce acid from the carbohydrate lactose in it. The spoilage will occur more rapidly if the milk is held at room temperature than if refrigerated. The sour taste is due to the presence of lactic acid, acetic acid, butyric acid, and other food acids.

Sources of microorganisms. The general sources of food spoilage microorganisms are the air, soil, sewage, and animal wastes. Microorganisms clinging to foods grown in the ground are potential spoilers of the food. Meats and fish products are contaminated by bacteria from the animal's internal organs, skin, and feet. **Meat** is rapidly contaminated when it is ground for hamburger or sausage because the bacteria normally present on the outside of the meat move into the chopped meat where there are many air pockets and a rich supply of moisture. **Fish tissues** are contaminated more readily than meat because they are of a looser consistency and are easily penetrated.

Canned foods are sterilized before being placed on the grocery shelf, but if the sterilization has been unsuccessful, contamination or food spoilage may occur. Swollen cans usually contain **gas** produced by members of the genus *Clostridium*. Sour spoilage without gas is commonly due to members of the genus *Bacillus*. This type of spoilage is called **flat-sour spoilage.** Lactobacilli are responsible for **acid spoilage** when they break down the carbohydrates in foods and produce detectable amounts of acid.

Among the important criteria determining the type of spoilage are the nature of the food preserved, the length of time before it is consumed, and the handling methods needed to process the foods. Various criteria determine which preservation methods are used.

Applications of Enzymes in the Food Industry

Enzyme is a kind of catalytically active protein. Its catalytic efficiency is higher than inorganic catalysts. Except the general characteristics of the chemical catalyst, enzyme has the following advantages: high catalytic efficiency; high specificity; mild work condition.

Enzyme engineering is a new technology that combines enzymology theory with chemical technology. It is able to eliminate the inherent shortcomings of many chemical processes in a variety of industries, and also a driving force for the development of traditional chemical industry.

In the past, the enzyme used in food processing was mostly derived from animal offal and plant extracts. Most of the enzymes used are now from microbial fermentation. In general, the purity of the enzyme used in food processing does not need to be particularly high, mostly partially purified enzyme. Unless in the special applications, such as proteolytic enzymes used in low-calorie beer, the higher the purity, the better the effect. Most enzymes applied in the food processing are glucoamylase and then followed by protease, lipase, esterase, oxidoreductase and isomerase.

Flour product

Enzyme, from organism and made with modern biotechnology, is a pure natural biological products and green food additives. It plays a significant role in a variety of special flour production and transformation. For instance, it can improve the baking quality, nutritional quality, texture, storage resistance and other functions of flour products. The major enzymes used for flour modification are listed in Table 1 below:

Enzyme Name	Function
α-amylase	Make bread volume increased, loose texture; speed up the dough fermentation; improve the bread tissue structure, increase the softness of the internal organization; produce a good and stable bread color; improve the furnace into the swelling; anti-aging, improve bread elasticity and taste; extend the fresh storage period of bread.
Glucose oxidase	Improve the silty properties of the flour, prolong the stabilization time, reduce the softening degree, improve the tensile and gelatinization characteristics of the flour, increase the tensile resistance, enhance the elasticity, increase the mechanical impact resistance and the maximum viscosity, and reduce the damage value; improve the furnace into the swelling; increasing the bread size; improve the noodles bite taste; improve the surface condition of the noodles.
Protease	Weakening the gluten to soften the dough to improve the viscoelasticity, extensibility and fluidity of the dough, shortening the mixing time of the dough, improving the

	mechanical properties and baking quality, making the product easy to shape and improving the taste.
Lipase	Delaying the aging of starch; increasing the stability of dough fermentation, increasing the volume of bread and improving the bread quality and preservation ability; reducing the spots on the dough, increasing the bite force, making the noodles not sticking in the boiled water, not easy to break, bright; increase the tensile resistance and elongation of flour.

Table 1: Enzymes for flour modified

In addition to enzymes in Table 1, other enzymes such as phytase, hemicellulase and glutamine aminotransferase are used in the flour industry to improve the quality of wheat flour and specialty flour. In the practical application, it should be based on the characteristics of different special flour and enzymes to ensure the rational use of enzymes and the proper amount of enzymes. Besides, the rational use of a variety of enzymes (complex enzyme), can not only reduce the total amount of enzyme, but achieve a synergistic effect.

Dairy processing

The main enzymes used in dairy processing are catalase and lactase. Catalase has high enzyme activity in bovine colostrum, mainly used for the removal of excess hydrogen peroxide in dairy products thereby to kill pathogens by using H_2O_2. Lactase can reduce the content of lactose to produce low lactose milk, low lactose hydrolyzed milk can improve the milk flavor, sweetness and nutritional value. In fermented milk, the use of lactase can accelerate the reaction and improve the fermentation efficiency to make fermented milk unique frankincense flavor and relatively extend shelf life of the product. Lactase used in condensed milk not only allows lactose to avoid lactose crystallization during concentration, but also make the product tasty, increase the sweetness, reduce the amount of sucrose, thereby inhibiting bacteria. The application of lactase in ice cream can not only increase the sweetness to reduce the amount of sucrose, but also solve the sediment due to the crystallization of deep frozen lactose, reduce the freezing point and improve the anti-thawing property. The use of lactase in milk powder can improve the flavor of milk powder, its rich caramel and caramel color after hydrolysis can produce chocolate milk.

Meat products processing

Enzyme used in the meat industry is mainly used to improve product quality (color, smell, taste, etc.) and increase the added value of by-products.

Beef products, treated with papain Ca ++ intensifier, have reddish brown color, crispy taste and good flavor, which completely overcomes traditional shortcomings including the tough taste, tenderness, gray color and low yield. The use of a certain amount of bromelain combined with phosphates, calcium chloride, etc. to tender mutton can improve the taste greatly. Lamb sausage produced with raw materials, which are treated with this method, has tender meat, good elasticity, unique flavor. It also makes up for the absence of lamb sausage in ham sausage. Transglutaminase can catalyze the formation of lysine covalent bonds between molecules of proteins or within molecules to form effective protein gels that impart specific hardness and elasticity to meat products.

In the deep processing of meat products, the use of protein complex enzymes can produce protein hydrolyzate. For instance, under the process conditions of pH value of 6.5-6.8, 55℃ and six-hour reaction, utilizing papain and bacillus subtilis neutral protease to hydrolyze snake meat, after proper purification, it can produce substances with rich nutrients and bioactive characteristics, and easy-to-degested nutrition solution. Bacillus subtilis neutral protease and pepsin hydrolize Maoshi pearl meat. Most of the protein in the hydrolyzate are converted to amino acids, which makes it delicious, lighter color and easy to be absorbed. These enzymes can be applied to the production of seafood seasonings, health drinks, etc.

Common by-products of meat processing are bone, bone crackers, mechanical flesh, fat and oil residue, which can be used as raw materials to obtain new meat extracts after enzymatic treatment. Meat extracts according to their characteristics and functions are divided into two categories: one is the flavor meat extract composed of small peptides and free amino acids; the other is a functional type of meat extract, generally composed of 10 amino acid molecules and with a moderate degree of hydrolysis. Flavor meat extracts have meat extract, bone soup or bone elements, etc. Such products with the natural aroma of raw materials can be made into paste or powder added to meat products, instant noodles seasoning package, sauce or snack foods, in order to enhance food flavor and protein content; what's more, it can be precursor of flavors or meat flavor after further Maillard reaction. Functional meat extract can be treated in high temperature without protein denaturation. Due to the unique adhesive and water holding function, it can be used in sausage, ham and other products to improve the adhesive properties of the meat products, cutting, and reduce the loss of meat products during cooking.

Fruit and vegetable processing and beverage industry

Enzymes used in this area are mainly pectin, cellulase and amylase, and mostly are used alone or in combination. These enzymes are mainly used for peeling fruit, clarifying fruit juice, reducing the viscosity of fruit juice, increasing the rate of fruit juices, enhance stability, what's more, they are also applied in making vegetable juice, extending the shelf life of fruits and vegetables, reducing nutrient loss and so on.

For example, under the condition of pH value of 4.0, 60℃ and four-hour reaction, adding cellulase (600U/100g), pectinase (1000U/100g), alpha-amylase (250U/100g) and papain (10000U/100g) to the clarification lychee juice process, and good clarity, low nutritional loss of high-quality lychee juice can be achieved.

Additionally, enzymes are also widely used in deep processing of tea. Tannase can improve the tea cold-soluble, prevent tea cloudy, and can improve the strength of the strength of tea. And now it is used in black tea, green tea and oolong tea. Cellulase and pectinase can break down the cell wall of tea, making the active ingredients of tea more easily to dissolve, improving the rate of instant tea products and, product clarity and the aroma of tea. Protease can improve tea extract rate and clarity, enhance the taste and improve the separation performance of tea.

Reference:

https://www.creative-enzymes.com/blog/applications-of-enzymes-in-the-food-industry/

Fruit and Vegetable Processing Enzymes

- Increase juicing yields
- Protect original color
- Improve filtration efficiency

Categories	Product Name
Pectinases Pectinases are the most commonly used enzyme in the fruit juice industry because they increase juice yields and accelerate juice clarification.	Pectinase
	Pectinase for Fruit Juice
	Pectinase blend to for vegetable juice
Cellulases	Cellulase

	Cellulase-AN
Tannases	Tannase (Food grade)
Xylanases	Xylanase
	Xylanase for Mash Viscosity Reduction and Xylan hydrolysis
Esterase	Pectin Methylesterase

Baking Enzymes

Baking enzymes have become an essential part to the industry. By extending shelf-life of breads, improving dough handling, providing anti-staling properties, and increasing manufacturer's control over crumb texture, color, taste, moisture, and volume, these enzymes are revolutionizing the baking industry.

- Dough conditioning
- Larger volume and longer shelf-life
- Improved softness

Categories	Product Name
Xylanases	Xylanase

	Xylanase based enzyme blend for baking
	Xylanase for Mash Viscosity Reduction and Xylan hydrolysis
Amylases	α-amylase for baking
	Fungal amylase for baking
	Maltogenic amylase for baking
Glucose Oxidase	Glucose Oxidase (Food Grade)
Lipases	Lipase
Proteases	Protease for special flours
	Protease for baking
Cellulases	Cellulase for baking
	Fungal hemicellulase enzyme for flour
Glucoamylase	Glucoamylase for baking

Brewing Enzymes

Brewing enzymes increase starch liquefaction and saccharification, which in turn increase the production of fermentable sugars. The enzymes work to simplify filtration, reduce the presence of viscous polysaccharides like glucans, and increase free amino nitrogen production.

- Reduce viscosity
- Increase fermentable sugars

- Chill-proof & excellent clarification

Categories	Product Name
α-Amylases	High-temperature α-amylase for Alcohol Industry
	Mid-temperature Refining α -amylase for beer
Glucoamylase	Glucoamylase for Alcohol Industry
	Glucoamylase for light beer
Peptidase	Peptidase
β-Glucanases	β-Glucanase
Proteases	Neutral Protease for Beer Brewing (Food Grade)
	Acid Protease for beer
Xylanase	Xylanase
Decarboxylase	α-acetolactate decarboxylase
Custom blends	Beer blend enzyme

Starch & Sweeteners Enzymes

- Better tastes of healthy choices
- Improved texture

Categories	Product Name
Pullulanases	Pullulanase for Saccharification
	Pullulanase (Food Grade)
Glucoamylases	Glucoamylase for Saccharification in Starch Industry
	Glucoamylase enzyme for crust
α-Amylases	Thermostable α-amylase for Liquefaction in Starch Industry
	α-Amylase enzyme for liquefaction
Carbohydrases	Carbohydrase blend for cereal grain
Custom blends	Corn Starch Processing Complex Enzyme (Food Grade)
	Enzyme blend for ereal grain starch processing

Except for these enzymes, **Creative Enzymes** also provides multiple enzyme blends used in various applications.

Reference:

https://www.creative-enzymes.com/cate/Food-And-Beverage-Applications_108.html

Enzymes for Health and Nutrition Selection Guide

There are four basic types of enzymes: **proteases** for protein digestion, **amylases** for carbohydrate digestion, **lipases** for fat digestion and **cellulase** for fiber digestion. In addition, metabolic enzymes are specialized to handle the exact needs of each individual organ, the bones and blood, as well as each individual cell within the body. These enzymes control new growth of all body cells and maintain all tissue.

Categories	Product Name
Proteases	Neutral/alkaline protease for meat proteins
	Neutral/alkaline protease for vegetable proteins
	Acid Stable Protease
	Endo-protease
Amylases	Heat Stable α Amylase (High Temperature) (Food Grade)
	Fungal α Amylase
Lipases	Triacylglycerol lipase enzyme for fat and dairy
	Lipase (Yeast)
	Lipase-AN
Cellulase	Cellulase
Lactase	Fungal Lactase

Digestive Blends	Native Bovine Pancreatin
	Native Porcine Pancreatin

Reference:

https://www.creative-enzymes.com/cate/Health-Diet-And-Nutrition_109.html

Cancer causing foods

1. Processed meat

According to the World Health Organization (WHO), there is "convincing evidence" that processed meat causes cancer. Classified as a Group 1 carcinogen, it is connected specifically to colorectal and stomach cancer.

Examples of processed meats that have carcinogenic properties include: Frankfurter hotdogs, ham, sausages, corned beef, beef jerky and canned or lunch meat.

- Alternatives: White fish, white meat such as chicken or turkey, or meat-substitutes such as Quorn, Tofu or Seitan.

2. Red meat

Only marginally better for us than processed meat, red meat is classified as Group 2A, "probably carcinogenic to humans". The strongest link between eating red meat and cancer is colorectal cancer, however, there is also evidence of links to both pancreatic and prostate cancer.

Cancer Council recommend that, to reduce your risk of cancer, you should eat no more than 65-100g of cooked red meat per week.

- Alternatives: Swap red meat for beans, pulses, white meat or fish.

Barbecues and charred meat

"Some research suggests that burnt or charred meat may increase the risk of cancer. Substances called heterocyclic amines are formed in foods that are cooked at high temperatures and blackened or charred. In animal studies, heterocyclic amines are proven to cause cancer However, the evidence in human studies is not clear."

Cancer Council

3. Alcohol

Many of us enjoy the occasional drink, some of us more than others. However, the medical advice is to reduce your alcohol intake to as little as possible. Alcohol is classified as a Group 1 carcinogen, which means there is sufficient evidence of carcinogenicity in humans.

The forms of cancer that are particularly linked to alcohol consumption are cancer of the mouth, throat, oesophagus, breast, liver, stomach and bowel.

The cancer risk associated with alcohol is thought to be dose dependent in some forms of cancer. That is, consuming one glass of wine with dinner every now and then does not have as much of a negative effect as binge-consuming several units of alcohol in one sitting. In fact, one study suggests that moderate consumption of red wine can be linked to a lower overall mortality and reduced risk of coronary heart disease and stroke.

4. Salted fish (Chinese style)

Salting is a traditional method of preserving food — especially fish — frequently used in South-East Asia and China. This method of preserving unfortunately results in the production of carcinogenic by-products, meaning it can cause cancer in humans. Chinese-style salted fish is a Group 1 carcinogen, like processed meat.

- Alternatives: Fresh fish or seafood such as prawns, mussels or squid.

5. Sugary drinks or non-diet soda

Obesity is a major risk factor for several cancers, and as such it is important to maintain a healthy weight. This can be achieved through a balanced diet that incorporates all food groups. If consumed regularly, sugary drinks can lead to weight gain, and in excessive amounts, obesity.

6. Fast food or processed foods

Greater body fatness is a cause of many cancers. The World Cancer Research Fund recommends limiting 'fast foods' and other processed foods high in fat, starches or sugars, as this helps control calorie intake and maintain a healthy weight.

- Alternatives: Homemade sandwiches on wholegrain bread, sushi or salads.

Reference:

https://www.aetnainternational.com/en/about-us/explore/fit-for-duty-corporate-wellness/cancer-causing-foods-cancer-fighting-foods.html

Food Contaminants Which Pose A Carcinogenic Threat To Humans

Section 1: Those agents with the highest level of evidence

Aflatoxin

NTP - Known to be a human carcinogen

IARC - Carcinogenic to humans

Aflatoxins are a class of toxic metabolites produced by certain species of fungi. A number of different specific chemical entities make up the class aflatoxins and they are generally present in food as mixtures. The most famous source of aflatoxin is *Aspergillus flavus* that can infect peanuts, but also infects tree nuts and grains. Animals eating infected foodstuffs can produce animal food-products that contain aflatoxins. Women consuming infected foodstuffs can pass on aflatoxin to infants through breast milk.

Laboratory studies have demonstrated the carcinogenicity of aflatoxins in rodents, primates, and fish [2]. Hepatocellular carcinoma has been seen in numerous species indicating that the liver is an important target organ. Nevertheless, tumors in the colon and kidney are also induced in some species. Initially, ecologic [3] and occupational [4] studies suggested an association between excess aflatoxin exposure and the incidence of cancer. Further evidence from a case-control study [5] and cohort study [6] have confirmed the association and the later sought to disentangle the interaction between aflatoxin and hepatitis on the risk of liver cancer. Recently, chemopreventive trials in endemic liver cancer areas of the People's Republic of China have suggested that interventions can reduce the carcinogenic impact of aflatoxin [7].

Alcoholic beverages

NTP - Known to be a human carcinogen

IARC - Carcinogenic to humans

Alcoholic beverages of all types (fermented and those further distilled) can cause cancer in humans. Animal studies have not convincingly demonstrated that ethanol itself is carcinogenic leading to the hypothesis that other contaminants in alcoholic beverages or ethanol metabolites (see acetaldehyde below) are responsible for these effects. The solvent action of ethanol may be relevant for co-carcinogens either in the beverages or in other dietary components [8].

Numerous epidemiologic studies have demonstrated an association between alcoholic beverages and cancers of the mouth, pharynx, larynx, and esophagus [9], and possibly the breast [10] and liver [11]. The cancer risk appears to be dose-dependent [12]. Many of these studies suggest that the cancer risk from

alcoholic beverages is synergistically increased among tobacco users [12] and confounding by smoking status does not explain the associations.

Alcoholic beverages have been widely studied for health effects other than cancer and important detrimental and beneficial associations have been reported. Alcoholic beverages are the primary cause of liver cirrhosis in the US [13] and have been linked to a large proportion of fatal and non-fatal motor vehicle accidents. Conversely, moderate consumption of alcoholic beverages has been linked to a lower overall mortality and reduced risk of coronary heart disease and stroke [14]. Some hypotheses state that the effects may be peculiar to wine and be due to resveratrol or other non-ethanol components of wine [15], alternatively a host of ethanol effects may be the beneficial agent with regard to heart disease [14].

2,3,7,8-Tetracholordibenzo-p-dioxin

NTP - Known to be a human carcinogen

IARC - Carcinogenic to humans

Also known as TCDD or more simply Dioxin, 2,3,7,8-tetracholordibenzo-*p*-dioxin is the prototypical compound of a class of agents (e.g. other dioxins, some halogenated dibenzofurans, some halogenated biphenyls, and some non-halogenated polycyclic aromatic hydrocarbons (see PAHs below)) thought to act through a similar mechanism. These agents serve as ligands of the aryl hydrocarbon receptor (Ahr) [16]. 2,3,7,8-Tetracholordibenzo-p-dioxin serves as the prototype because of its high potency, high Ahr binding affinity, and because it is more resistant to either environmental or biological degradation than most of the other agents in this class. Both the NTP and IARC name this agent specifically as a human carcinogen, but discuss the use of the Toxic Equivalency Factor to estimate the potency of the other less potent and less well studied members of the class. 2,3,7,8-Tetracholordibenzo-*p*-dioxin was never produced intentionally, except for research purposes, but was created as a by-product in the manufacture of PCBs, chlorinated herbicides (e.g. Agent Orange) and in minute amounts in any process where there is incomplete combustion in the presence of chlorine. Minute amounts are also produced naturally (e.g. by forest fires).

Studies in multiple animal species have demonstrated the carcinogenic potential of 2,3,7,8-tetracholordibenzo-*p*-dioxin in producing tumors of the liver [17], thyroid, the upper aerodigestive tract, and skin [18].

Associations with cancer in human studies have focused on highly exposed subjects in occupational settings. Associations with total cancer, lung cancer, and non-Hodgkin's lymphoma have been noted [19].

2,3,7,8-tetracholordibenzo-*p*-dioxin was originally listed by the NTP as reasonably anticipated to b e a human carcinogen in its second annual report. The elevation to known human carcinogen in the Ninth annual report was contested in US Federal court system, but was completed in 2001.

Salted fish

NTP - Not listed

IARC - Carcinogenic to humans

Salted fish are produced in several parts of Asia using a method that appears to result in the production of carcinogenic by products. Several potential carcinogens have been identified including *N*-nitrosodimethylamine (see separate listing below), other *N*-nitroso compounds [20].

Minimal work has studied the carcinogenicity of Chinese-style salted fish in animals [21], but this product does contain agents that are mutagenic [22].

Ecologic [23] and case-control studies [24,25] have demonstrated an increased risk of nasopharyngeal carcinoma in subjects consuming larger amounts of Chinese-style salted fish. The consistency of the association led IARC to classify these preserved fish as a Group 1 carcinogen. Much more limited evidence has linked this product to increased risk of stomach and esophageal cancer as well.

Section 2: Those agents with a moderate level of evidence

Acetaldehyde

NTP - Reasonably anticipated to be a human carcinogen

IARC - Possible carcinogenic to humans

Acetaldehyde is produced in many different geological, industrial, and biological processes and is the first metabolite produced in humans from ethanol after ingestion[26]. Humans are exposed to naturally occurring acetaldehyde in the air and from numerous foods. Humans are also exposed to it from automobile exhaust, cigarette smoke, fireplaces, and in occupational settings,.

Acetaldehyde has been demonstrated to cause cancer in animals, mainly in the upper respiratory tract.

The evidence of human carcinogenicity is more indirect and has generally been linked to heavy alcohol consumption. Heavy drinkers experience higher rates of oral, pharyngeal, and esophageal cancers [8] and one hypothesized mechanism is that ethanol is converted to acetaldehyde. Several studies of genetic polymorphisms in the genes which metabolize ethanol and acetaldehyde have shown that subjects who carry one inactive acetaldehyde dehydrogenase 2 allele are at higher risk of alcohol related cancers [27]. Subjects with two inactive alleles generally cannot tolerate alcohol.

Acetaldehyde may underlie the increase in certain alcohol related cancers (see above). The widespread exposure to acetaldehyde is of concern, but the relevance to cancer of other natural and anthropogenic sources is still unclear.

Polycyclic aromatic hydrocarbons including Benzo[a]pyrene

NTP - Reasonably anticipated to be a human carcinogen

IARC - Probably carcinogenic to humans

Benzo[a]pyrene is a prototypical member of the polycyclic aromatic hydrocarbon family, which also includes benz[*a*]anthracene, benzo[*b*}fluoranthene and other similar compounds that show similar toxic profiles. In total, the Report on Carcinogens includes 15 different polycyclic aromatic hydrocarbons. They are produced inadvertently during the incomplete combustion of organic material.

Human exposure occurs through many routes including food. Intentional exposure comes through the therapeutic use of coal tar for certain skin conditions. Many people are exposed to PAHs occupationally and through exposure to smoke from tobacco, automobile exhaust, forest fires, and other sources. Food is contaminated with PAHs during smoking, barbequing, grilling, and is also present at low concentrations in oils, coffee, sausages, etc. In areas of the world that cook their food using coal, the uncooked food can be contaminated from the cooking smoke [27,28].

In experimental animals, different specific PAHs and exposures routes lead to different tumors in different species. Gavage leads to forestomach, lung, and liver tumors, intraperitoneal injection to mammary and uterine tumors, and topical application to skin tumors. The site of exposure is often the site of tumor development.

PAHs are present in tobacco smoke, but evidence for PAH carcinogenicity in other exposure routes is less well developed in humans. Occupational exposed cohorts have shown elevations in lung, skin,

and bladder tumors [29]. Epidemiologic studies are hampered by the difficulty in assessing individual PAH exposure.

N-Nitrosodimethylamine and some other nitrosamines

NTP - Reasonably anticipated to be a human carcinogen

IARC - Probably carcinogenic to humans

N-Nitrosodimethylamine and some related nitrosamines were only briefly produced intentionally for purposes beyond research. These compounds are formed during rubber processing, are present in metal working fluids and are inadvertently present in foodstuffs including smoked foods, preserved meats, and some alcoholic beverages [30]. This agent may also be present in some pharmaceuticals and in tobacco smoke.

A significant source of exposure to N-nitroso compounds, especially in non-occupationally exposed non-smokers, is internal formation in the gut [31-33]. About 25% of dietary nitrate is recycled to the salivary glands. Oral and gut bacteria can reduce dietary nitrate to nitrite and this nitrite can react with amines to form nitrosamines spontaneously.

N-Nitrosodimethylamine produces tumors in experimental animals through many different exposure routes. Most studies have shown that this agent produces liver tumors of several different histologies and sometime tumors in the bile duct, kidney, lung, and nasal cavity.

No adequate human studies have demonstrated that this agent is a human carcinogen. As for many other agents the conduct of epidemiologic studies of this agent are hampered by the inability to assess exposure. Despite this short coming, the overwhelming carcinogenicity in animals suggests that this agent is carcinogenic in humans.

Hot Maté

NTP - Not listed

IARC - Probably carcinogenic to humans

Maté is an herbal infusion of the plant *Ilex paraguariensis* drunk in Argentina, Uruguay, and southern Brazil. Minimal use outside this geographic area precludes its evaluation by the US NTP. The infusion is typically drunk, often at very high temperatures, through a metal straw in a special apparatus.

The carcinogenicity of maté has not been studied in animals but multiple case-control studies have demonstrated an association between hot maté and oral, oropharyngeal, head and neck, and esophageal cancer [34-37]. Most studies have found a dose dependent increase in cancer risk with increased consumption of maté. Quantities greater then 1 liter/day are not uncommon and some people report >3 liters/day. Furthermore, the typical temperature of consumption may also effect the association with subjects reporting the highest temperature at the greatest increased risk in some studies.

The interaction between cancer risk and the quantity consumed and the temperature of consumption suggests that thermal injury and not the maté are responsible for some or all of the increased risk of cancer. A similar hypothesis has been studied in a population in Northern Iran at very high risk for esophageal cancer. These subjects consume large quantities of tea, some preferring very high temperatures, sometimes in excess of 65 °C. Further research will be required to disentangle the effects of maté and the temperature at which it is consumed. Alternatively, contaminants such PAHs, which are introduced during preparation of the leaves, may be the carcinogenic agent [38].

Section 3: Those agents with a lower level of evidence

Bracken Fern

NTP - Not listed

IARC - Possibly carcinogenic to humans

Bracken fern are common, grow worldwide, and were first identified as a hazard to grazing animals. For humans, the greatest concern is the direct consumption of bracken fern in Japan and a few other areas Studies in mouse, rat, guinea pig, and quail have examined the fresh and processed bracken fern products and sufficient evidence has been developed to demonstrate that this agent can be carcinogenic in animals.

Several studies have suggested that subjects who live in areas where bracken ferns are very common have higher risk of gastric and/or esophageal cancer [39-41]. One study of bladder cancer found no increased risk associated with fern consumption [42] and a recent review suggests that bracken fern is unlikely to be a serious health hazard [43].

Dichlorodiphenyltrichloroethane (DDT)

NTP - Reasonably anticipated to be carcinogenic to humans

IARC - Possibly carcinogenic to humans

DDT is the nickname for the pesticide dichlorodiphenyltrichloroethane that was used in the United States from 1939 until in it was banned for general use, but not manufacture, in 1972. Debate about its use was heightened by the findings of its affect on raptor populations. Because DDT is highly lipophilic it concentrates in fat and bioaccumulates in higher consumers in the food web. In addition to direct exposure to pesticide applicators, all people are exposed to DDT through food, especially but not exclusively through animal products, but also some vegetables.

There is ample evidence that DDT is carcinogenic in animals. The liver appears to be the primary target organ, but lung tumors and lymphomas have also been noted.

The evidence for carcinogenicity in humans remains mixed. No prospective epidemiologic studies have been reported which examine the association between DDT/DDE exposure and incident cancers at any site. Some ecologic and case-control studies have suggested an association with human cancer, especially liver and breast[44,45], but no clear associations have been uncovered[46-49]. In addition, concerns about co-exposure to other organochlorines make the interpretation less clear [50].

Although banned in most developed countries DDT is still used in some parts of the world. DDT could appear in the food of countries with bans through food imports. Aerial deposition is also a concern as has been demonstrated in the findings of DDT in animals in the arctic and other areas that have little direct use of the pesticides [51,52].

Toxins from Fusarium moniliforme, Fumonisins, Fumonsin B_1

NTP - Not listed

IARC - Possibly carcinogenic to humans

*Fusarium moniliforme (*now known as *Fusarium verticilliodes)* is a species of fungus that, among others, produces a series of toxic metabolites known as fumonisins, the best studied being fumonisin B_1. This fungus is most closely associated with maize, but can infect other food stuffs as well. Fumonisins produce a broad spectrum of toxic effects in animals including equine leukoencephalomalakia and porcine pulmonary edema [53]. Occasional inadvertent farm animal poisonings have occurred from moldy grain.

The carcinogenicity of fumonsin B_1 in animals was demonstrated by the NTP after completion of a two-year bioassay in rats and mice[54]. These studies showed an increase in renal tubule adenomas in male rats and hepatocellular tumors in female mice.

Evidence for the carcinogenicity of fumonisins in humans is limited to ecologic associations in the People's Republic of China [55-58], in the Republic of South Africa[59,60], and in Iran [61]. Each of these countries have sub-populations at high risk for esophageal squamous cell carcinoma and these sub-populations appear to have greater exposure to fumonisin then sub-populations at lower risk for esophageal squamous cell carcinoma. Only a single case-control study has examined the association between fumonisin exposure and esophageal squamous cell carcinoma and it reported no apparent association, but this study used an exposure biomarker of uncertain value [62].

Ochratoxin A

NTP - Reasonably anticipated to be carcinogenic to humans

IARC - Possibly carcinogenic to humans

Ochratoxin A can be produced by *Aspergillus*, *Penicillium*, and other molds. Food is the primary route of human exposure, especially from grain but also through animal products from animals consuming contaminated feeds and possibly from processed grain products including beer.

The liver and possibly the kidney are target organs for the carcinogenic effects of Ochratoxin A in laboratory animals.

Little evidence of carcinogenicity in humans exists because few epidemiologic studies of Ochratoxin A have been conducted. An ecologic association between high ochratoxin A contamination and nephropathy has been noted in southeastern Europe[63,64].

2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and other heterocyclic amines

NTP – Reasonably anticipated to be a human carcinogen

IARC - Possibly carcinogenic to humans

PhIP is an aryl amine that forms during the cooking of meat, especially at high temperatures. The temperature and form of cooking can significantly alter the production of PhIP and two related heterocyclic amines, namely 2-amino-3,4-dimethylimidazo[4,5-*f*]quinolone (MeIQ) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxalone (MeIQx) [65].

All three compounds are considered to be mutagenic and cause tumors in animals. The site of tumor depends on the route of exposure include the intestine (small and large), prostate, lymphomas and other sites.

Human data that has specifically estimated heterocyclic amine intake are limited and since the primary route of exposure, cooked meat, is also likely to contain PAHs (and will also be confounded by fat intake, iron, etc.) [66]. Some recent studies have demonstrated an association between estimated heterocyclic amine intake and cancer risk [67].

One method which might help separate the effects of PAHs and heterocyclic amines is the study of genetic variation in the cytochromes P450 which metabolize these agents. Distinct profiles might be drawn with different enzymes being more active towards PAHs then heterocyclic amines and vise-versa. The association of polymorphisms in these genes with the cancers of interest may suggest the importance of particular agents.

Coffee

NTP – Not listed

IARC - Possibly carcinogenic to humans

Coffee is grown and consumed the world over and was listed in 1991 by IARC as possibly carcinogenic to humans. Some evidence had linked coffee to an increase risk of bladder cancer. Subsequent studies suggest that this association is unlikely or very weak [68,69]. The association is biologically plausible, but the lack of a dose-response in most studies suggests that residual confounding could be to blame. An association with pancreatic cancer has been suggested but remains unconvincing [70]. As later noted by IARC and others, coffee may have an inverse association with the risk of colon cancer [68].

Pickled vegetables

NTP – Not listed

IARC - Possibly carcinogenic to humans

Pickled vegetables have been study for their association with cancer mainly in Asia and especially in the People's Republic of China. The pickling process is different from that used in many parts of the world and uses no salt or vinegar. Instead it relies on natural fermentation and can lead to contamination with mold.

A small amount of laboratory evidence suggests that these vegetables may contain mutagens [71,72]. Epidemiologic studies have suggested an increased risk of esophageal cancer in pickled vegetable consumers [73,74]. In the highest esophageal cancer risk area of north central China no association between pickled consumption and cancer has been noted in multiple studies [75-77]. This population

was subject to a public health campaign against the consumption of these vegetables prior to the baseline interview. This may have led to exposure misclassification if subjects recently discontinued consumption or prevarication due to the repeated warnings to stop consuming the pickles.

Acrylamide

NTP – Reasonably anticipated to be a human carcinogen

IARC - Probably carcinogenic to humans

In 2002 Professor Margareta Törnqvist at the University of Stockholm reported that acrylamide is created in the process of high temperature cooking of certain foods such as potatoes and cereals. Acrylamide was previously listed by the NTP as *reasonably anticipated to be a human carcinogen* and by IARC as *Probably carcinogenic to humans*. But, when reviewed neither of the agencies had data on the presence of acrylamide in food due to high temperature cooking and the primary routes of exposure were occupational and possibly in drinking water due to residues from polyacrylamide flocculants.

Since the high profile reporting on this potential new source of contamination, several studies have examined the association between foods that may contain acrylamide (e.g. fried potatoes, coffee) and cancer risk [78-81]. These studies used exposure estimates derived from dietary data on acrylamide content of different foods and failed to find significant associations with cancer sites studied.

Reference:

Christian C. Abnet, Carcinogenic Food Contaminants. Cancer Invest. 2007 Apr–May; 25(3): 189–196. doi: 10.1080/07357900701208733

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – II – Food Biotechnology – SBB2203

1. ANTIOXIDANTS

Antioxidants are compounds that inhibit oxidation, a chemical reaction that can produce free radicals and chain reactions that may damage the cells of organisms. Antioxidants such as thiols or ascorbic acid (vitamin C) may act to inhibit these reactions. To balance oxidative stress, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione.

The only dietary antioxidants are vitamins A, C, and E. The term *antioxidant* is also used for industrial chemicals added during manufacturing to prevent oxidation in synthetic rubber, plastics, and fuels, or as preservatives in food and cosmetics.[1]

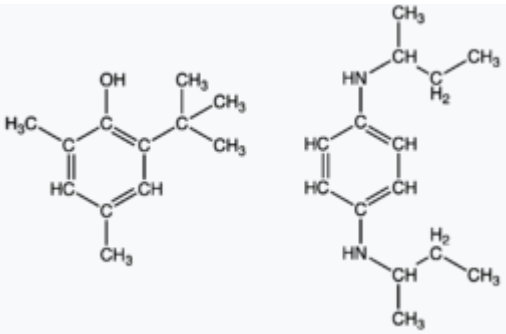
Dietary supplements marketed as antioxidants have not been shown to improve health or prevent disease in humans.[2] Supplements of beta-carotene, vitamin A, and vitamin E have no positive effect on mortality rate[3][4] or cancer risk.[5][*needs update*][6] Additionally, supplementation with selenium or vitamin E does not reduce the risk of cardiovascular disease.[7][8]

Antioxidants are used as food additives to help guard against food deterioration. Exposure to oxygen and sunlight are the two main factors in the oxidation of food, so food is preserved by keeping in the dark and sealing it in containers or even coating it in wax, as with cucumbers. However, as oxygen is also important for plant respiration, storing plant materials in anaerobic conditions produces unpleasant flavors and unappealing colors.[138] Consequently, packaging of fresh fruits and vegetables contains an ~8% oxygen atmosphere. Antioxidants are an especially important class of preservatives as, unlike bacterial or fungal spoilage, oxidation reactions still occur relatively rapidly in frozen or refrigerated food.[139] These preservatives include natural antioxidants such as ascorbic acid (AA, E300) and tocopherols (E306), as well as synthetic antioxidants such as propyl gallate (PG, E310), tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA, E320) and butylated hydroxytoluene (BHT, E321).[140][141]

The most common molecules attacked by oxidation are unsaturated fats; oxidation causes them to turn rancid.[142] Since oxidized lipids are often discolored and usually have unpleasant tastes such as metallic or sulfurous flavors, it is important to avoid oxidation in fat-rich foods. Thus, these foods are rarely preserved by drying; instead, they are preserved by smoking, salting or fermenting. Even less fatty foods such as fruits are sprayed with sulfurous antioxidants prior to air drying. Oxidation is often catalyzed by metals, which is why fats such as butter should never be wrapped in aluminium foil or kept in metal containers. Some fatty foods such as olive oil are partially protected from oxidation by their natural content of antioxidants, but remain sensitive to

photooxidation.[143] Antioxidant preservatives are also added to fat based cosmetics such as lipstick and moisturizers to prevent rancidity.[*citation needed*]

Industrial uses[edit]



Substituted phenols and derivatives of phenylenediamine are common antioxidants used to inhibit gum formation in gasoline (petrol).

Antioxidants are frequently added to industrial products. A common use is as stabilizers in fuels and lubricants to prevent oxidation, and in gasolines to prevent the polymerization that leads to the formation of engine-fouling residues.[144] In 2014, the worldwide market for natural and synthetic antioxidants was US$2.25 billion with a forecast of growth to $3.25 billion by 2020.[145]

Antioxidant polymer stabilizers are widely used to prevent the degradation of polymers such as rubbers, plastics and adhesives that causes a loss of strength and flexibility in these materials.[146] Polymers containing double bonds in their main chains, such as natural rubber and polybutadiene, are especially susceptible to oxidation and ozonolysis. They can be protected by antiozonants. Solid polymer products start to crack on exposed surfaces as the material degrades and the chains break. The mode of cracking varies between oxygen and ozone attack, the former causing a "crazy paving" effect, while ozone attack produces deeper cracks aligned at right angles to the tensile strain in the product. Oxidation and UV degradation are also frequently linked, mainly because UV radiation creates free radicals by bond breakage. The free radicals then react with oxygen to produce peroxy radicals which cause yet further damage, often in a chain reaction. Other polymers susceptible to oxidation include polypropylene and polyethylene. The former is more sensitive owing to the presence of secondary carbon atoms present in every repeat unit. Attack occurs at this point because the free radical formed is more stable than one formed on a primary carbon atom. Oxidation of polyethylene tends to occur at weak links in the chain, such as branch points in low-density polyethylene.[*citation needed*]

Fuel additive	Components[147]	Applications[147]
AO-22	N,N'-di-2-butyl-1,4-phenylenediamine	Turbine oils, transformer oils, hydraulic fluids, waxes, and greases
AO-24	N,N'-di-2-butyl-1,4-phenylenediamine	Low-temperature oils
AO-29	2,6-di-tert-butyl-4-methylphenol	Turbine oils, transformer oils, hydraulic fluids, waxes, greases, and gasolines
AO-30	2,4-dimethyl-6-tert-butylphenol	Jet fuels and gasolines, including aviation gasolines
AO-31	2,4-dimethyl-6-tert-butylphenol	Jet fuels and gasolines, including aviation gasolines
AO-32	2,4-dimethyl-6-tert-butylphenol and 2,6-di-tert-butyl-4-methylphenol	Jet fuels and gasolines, including aviation gasolines
AO-37	2,6-di-tert-butylphenol	Jet fuels and gasolines, widely approved for aviation fuels

Levels in food[edit]

Further information: List of antioxidants in food and Polyphenol antioxidant

Fruits and vegetables are good sources of antioxidant vitamins C and E.

Antioxidant vitamins are found in vegetables, fruits, eggs, legumes and nuts. Vitamins A, C, and E can be destroyed by long-term storage or prolonged cooking.[148] The effects of cooking and food processing are complex, as these processes can also increase the bioavailability of antioxidants, such as some carotenoids in vegetables.[149] Processed food contains fewer antioxidant vitamins than fresh and uncooked foods, as preparation exposes food to heat and oxygen.[150]

Antioxidant vitamins	**Foods containing high levels of antioxidant vitamins**[21][151][152]
Vitamin C (ascorbic acid)	Fresh or frozen fruits and vegetables
Vitamin E (tocopherols, tocotrienols)	Vegetable oils, nuts, and seeds
Carotenoids (carotenes as provitamin A)	Fruit, vegetables and eggs

Other antioxidants are not obtained from the diet, but instead are made in the body. For example, ubiquinol (coenzyme Q) is poorly absorbed from the gut and is made through the mevalonate pathway.[63] Another example is glutathione, which is made from amino acids. As any glutathione in the gut is broken down to free cysteine, glycine and glutamic acid before being absorbed, even large oral intake has little effect on the concentration of glutathione in the

body.[153][154] Although large amounts of sulfur-containing amino acids such as acetylcysteine can increase glutathione,[155] no evidence exists that eating high levels of these glutathione precursors is beneficial for healthy adults.[156]

Measurement and invalidation of ORAC[edit]

Measurement of polyphenol and carotenoid content in food is not a straightforward process, as antioxidants collectively are a diverse group of compounds with different reactivities to various reactive oxygen species. In food science analyses in vitro, the oxygen radical absorbance capacity (ORAC) was once an industry standard for estimating antioxidant strength of whole foods, juices and food additives, mainly from the presence of polyphenols.[157][158] Earlier measurements and ratings by the United States Department of Agriculture were withdrawn in 2012 as biologically irrelevant to human health, referring to an absence of physiological evidence for polyphenols having antioxidant properties *in vivo*.[159] Consequently, the ORAC method, derived only from *in vitro* experiments, is no longer considered relevant to human diets or biology, as of 2010.[159]

Alternative in vitro measurements of antioxidant content in foods – also based on the presence of polyphenols – include the Folin-Ciocalteu reagent, and the Trolox equivalent antioxidant capacity assay.[160]

Reference:

https://en.wikipedia.org/wiki/Antioxidant

COLOURING AGENTS USED IN FOOD PRODUCTS

A color additive is any dye, pigment or substance which when added or applied to a food, drug or cosmetic, or to the human body, is capable (alone or through reactions with other substances) of imparting color. FDA is responsible for regulating all color additives to ensure that foods containing color additives are safe to eat, contain only approved ingredients and are accurately labeled. Certified colors are synthetically produced (or human made) and used widely because they impart an intense, uniform color, are less expensive, and blend more easily to create a variety of hues. Color is added to food for one or more of the following reasons:

(1) to replace color lost during processing,

(2) to enhance color already present,

(3) to minimize batch-tobatch variations, and

(4) to color otherwise uncolored food.

Introduction

A colouring is any substance that is added to change formulation colour. One of the most obvious ways to influence the way a product looks is to add colouring agents. These range from “natural” and artificial colours to washes that enhance browning.
Food colours fall into two main categories: artificial and natural, respectively. From a regulatory standpoint, natural colours cannot legally be termed natural colours on a food label unless they are used to colour the same type of product. For example, beet juice is really only a natural colour if it is used to colour beets. If it colours cherry juice, it is technically considered to be artificially coloured and the beet juice is deemed a colour additive.

Colourings

A growing number of natural food colourings are being commercially produced, partly due to consumer concerns surrounding synthetic colourings. Some examples include:

- Caramel colouring, made from caramelized sugar, used in cola products and also in cosmetics.
- Annatto, a reddish-orange dye made from the seed of the Achiote.
- A green dye made from chlorella algae.
- Cochineal, a red dye derived from the cochineal insect, Dactylopius coccus.
- Betanin extracted from beets.
- Turmeric
- Saffron
- Paprika
- Elderberry

To ensure reproducibility, the coloured components of these substances are often provided in highly purified form, and for increased stability and convenience, they can be formulated in suitable carrier materials (solid and liquid).

Artificial colouring

The colours below are known as “Primary Colours”, when they are mixed to produce other colours, those colours are then known as “Secondary Colours”.

*Brilliant Blue, E133 (Blue shade)

* Indigotine, E132 (Dark Blue shade)

* Fast Green, E143 (Bluish green shade)

* Allura Red AC, E129 (Red shade)

* Erythrosine, E127 (Pink shade)
* Tartrazine, E102 (Yellow shade)
* Sunset Yellow, E110 (Orange shade)

Dyes and Lakes

Colour additives are available for use in food as either “dyes” or “lakes”. Dyes dissolve in water, but are not soluble in oil. Dyes are manufactured as powders, granules, liquids or other special purpose forms. They can be used in beverages, dry mixes, baked Goods, confectionry, dairy products, pet foods and a variety of other products. Lakes are the combination of dyes and insoluble material. Lakes tint by dispersion. Lakes are not oil soluble, but are oil dispersible. Lakes are more stable than dyes and are ideal for colouring products containing fats and oils or items lacking sufficient moisture to dissolve dyes. Typical uses include coated tablets, cake and donut mixes, hard candies and chewing gum.

Artificial colours consist of water soluble synthetic dyes or the aluminum salts of these dyes, called “Lakes.” These seven synthetic dyes and their salts are deemed acceptable by most Food authorities for use in food. Blending the seven produces a wide spectrum of colour, including purple, black, brown, and variations of the primary colours.

Dyes display colours when dissolved in the aqueous phase of a food product. However, their solubility varies with temperature and often with the solute. Although at typical usage levels this will not ordinarily affect the finished product, it could make a difference when colour solutions are prepared. If the temperature of the solute changes, all the dye may not be in solution, and this could affect the colour of the finished product.

The Lakes are insoluble in most solvents and instead colour by dispersion. They are used in low moisture, often high-fat applications, but are not fat soluble. They may bleed color slightly in water, but in most applications they minimize colour bleed into adjoining areas. The shades produced depend on the method of production and to some extent on particle size. Lakes are more resistant than dyes to fading when exposed to high heat and light.Dyes go into solution and function on a molecular level. Lakes are different because the particles are dispersed. Particle size, particle shape and how well the particles are dispersed in the finished product all influence the outcome. In some cases, simply increasing the shear during dispersion may intensify the colour.

ARTIFICIAL COLOURANTS-

Colouring agent	**description**	**Colour**	**odour**	**Stability to light**	**oxidation**	**pH**	**density**	**Solubility**
Erythrosine	Solid,fine powder	Red	Odorless	Poor	Fair	8-10	0.80	Propylene glycol>water>glycerine>alcohol
Sunset Yellow	Solid,fine powder	Reddish Orange	Odorless	Good	Fair	6-8	0.80	Glycerine>water>propylene glycol>alcohol
tartrazine	Solid,fine powder	Light orange	Odorless	Good	Fair	6-8	0.70	Glycerine>water>propylene glycol>alcohol
Ponceau	Solid,fine powder	Red	Odorless	Good	Fair	6-8	0.80	Water>propylene glycol>glycerine>alcohol
Allura red	Solid,fine powder	Deep red	Odorless	Very Good	Fair	6-8	0.80	Water>glycerine>propylene glycol>alcohol
amaranth	Solid,fine powder	Rediish brown	Odorless	Good	Poor	6-8	0.80	Water=glyceine>propylene glycol>alcohol

Indigo carmine	Solid,fine powder	Bluish brown	Odorless	Very poor	poor	6-7	0.80	Water>glycerine>propylene glycol>alcohol
Brilliant blue	Solid,fine powder	Bluish violet	Odorless	Good	poor	6-7	0.80	Propyleneglycol>water>glycerine>alcohol
Quinoline yellow	Obtained from coal tar	Yellow to orange powder	Tar like odor	Fair	Forms quinolinic acid	8-9	0.20 - 0.70	water>glycerol>methoxy ethanol

NATURAL COLOURANTS-

Colouring agent	**Description**	**Colour**	**Odour**	**Stability to light**	**Oxidation**	**pH**	**Density**	**Solubility**
Paprika	Clear dark red oily liquid,	Dark red	Odorless	stable	poor	4.6	0.935 - 0.945	Soluble in fats and oil
Annatto	Crystalline powder	Bright Yellow To Orange red	Peculiar odour	Fades in Strong& Direct light	Degrades By oxidation	5.5	0.6	Soluble in alcohol, ether,oils

Caramel	Thick liquid	Dark brown	Pleasent	stable	stable	2-10	0.6-0.7	Soluble in water ,dilute alcohol. Insoluble in benzene, chloroform, ether
Carotene	Powder, crystals	Red to Brown		stable	stable	2-14		Soluble in carbon disulphide, benzene, chloroform. Sparingly soluble in methanol and ethanol. Insoluble in water,acid,alkali.
Curcumin	Crystal form	Orange yellow	Odorless	Degradation	Occurs with ferrous oxide	8-9	Low density	Alcohol>Acetic acid
Riboflavin	Fine powder in needle form	Whitish yellow	Slightly Odorless	Light resistant	Dark at 240 degree	4.5-7	high	Ethanol>water>cyclohexane
Carmine	Powder form	Bright red	Odorless	Stable	Oxidation		True bulk	Soluble in ethanol.

					changes blue to purple colour	11.4-13	density	Insoluble in water.
Curcumin	Crystal form	Orange yellow	Odorless	Degradation	Occurs with ferrous oxide	8-9	Low density	Alcohol>Acetic acid

PERMITTED ARTIFICIALLY PREPARED COLOURING AGENTS:

Application (permitted mg/kg)	E-102 Tartrazine	E-104 Quinoline Yellow	E-110 Sunset Yellow	E-122 Carmoisine	E-123 Amaranth	E-124 Penceau 4R	E-127 Erythrosine	E-128 Red 2G	E-129 Allure Red	E-131 Patent Blue	E-132 Indigo Carmine	E-133 Brilliant Blue	E-142 Green S	E-151 Brilliant Black
Confectionery	300	300	50	300	NP	50	NP	NP	300	300	300	300	300	qs
Soft Drinks	100	100	50	100	NP	50	NP	NP	100	100	100	100	100	qs
Decorations & Coatings	500	500	500	500	NP	500	NP	NP	500	500	500	500	500	qs
Snack Foods	100-200	100-200	100-200	100-200	NP	100-200	NP	NP	100-200	100-200	100-200	100-200	100-200	qs
Desserts	150	150	50	150	NP	50	NP	NP	150	150	150	150	150	qs
Edible ices	150	150	50	150	NP	50	NP	NP	150	150	150	150	150	qs
A. Beverages	200	200	200	200	30-100	200	NP	NP	200	200	200	200	200	qs
Sauces & Seasonings	500	500	500	500	NP	500	NP	NP	500	500	500	500	500	qs
Jams, Jellies & Marmalades	NP	100	100	NP	NP	100	NP	NP	NP	NP	NP	NP	100	qs
Baked Goods	200	200	50	50	NP	50	NP	NP	200	200	200	200	200	qs
Meat & Fish	100-500	100-500	100-500	100-500	NP	100-500	NP	20	100-500	100-500	100-500	100-500	100-500	qs
Soups	50	50	50	50	NP	50	NP	NP	50	50	50	50	50	qs
Processed Cheese	100	100	100	100	NP	100	NP	NP	100	100	100	100	100	qs
Mustard	300	300	300	300	NP	300	NP	NP	300	300	300	300	300	qs
Fruit Wines	200	200	200	200	NP	200	NP	NP	200	200	200	200	200	qs
Pet Food	qs	qs	qs	qs	NP	qs	qs	qs	qs	qs	qs	qs	qs	qs

PERMITTED NATURALLY PREPARED COLOURING AGENTS:

Application (permitted mg/kg)	E-110 Curcumin	E-101 Riboflavin	E-120 Carmine	E-140 Chlorophyll	E-141 Cu-Chlorophyll	E-150 (a-d) Caramel	E-153 Carbon Black	E-160a Carotene	E-160c Annatto	E-160c Paprika	E-161b Lutein	E-162 Beetroot	E-163 Anthocyanins	E-171 Titanium Dioxide
Confectionery	300	qs	300	qs	qs	qs	qs	qs	NP	qs	300	qs	qs	qs
Soft Drinks	100	qs	100	qs	qs	qs	qs	qs	NP	qs	100	qs	qs	qs
Decorations & Coatings	500	qs	500	qs	qs	qs	qs	qs	20	qs	500	qs	qs	qs
Snack Foods	100-200	qs	100-200	qs	qs	qs	qs	qs	10-20	qs	100-200	qs	qs	qs
Desserts	150	qs	150	qs	qs	qs	qs	qs	10	qs	150	qs	qs	qs
Edible ices	150	qs	150	qs	qs	qs	qs	qs	20	qs	150	qs	qs	qs
A. Beverages	200	qs	200	qs	qs	qs	qs	qs	10	qs	200	qs	qs	qs
Sauces & Seasonings	500	qs	500	qs	qs	qs	qs	qs	NP	qs	500	qs	qs	qs
Jams, Jellies & Marmalades	Qs	qs	100	qs	qs	qs	qs	qs	NP	qs	100	qs	qs	qs
Baked Goods	200	qs	200	qs	qs	qs	qs	qs	10	qs	200	qs	qs	qs
Meat & Fish	100-500	qs	100-500	qs	qs	qs	qs	qs	0-10	qs	100-500	qs	qs	qs
Soups	50	qs	50	qs	qs	qs	qs	qs	NP	qs	50	qs	qs	qs
Processed Cheese	100	qs	100	qs	qs	qs	qs	qs	15	qs	100	qs	qs	qs
Mustard	300	qs	300	qs	qs	qs	qs	qs	NP	qs	300	qs	qs	qs
Fruit Wines	200	qs	200	qs	qs	qs	qs	qs	NP	qs	200	qs	qs	qs
Pet Food	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs

ARTICLE BY,

Ass. Prof. MS Rathore, Richa Sood CT Institute Of Pharmaceutical Sciences, Jalandhar.

Reference:
https://www.pharmatutor.org/articles/colouring-agents-used-in-food-products

What are Flavouring Agents? What do FSSAI Regulations say?

What are Flavouring agents? What do FSSAI Regulations say?

Flavouring agents are key food additives with hundreds of varieties like fruit, nut, seafood, spice blends, vegetables and wine which are natural flavouring agents. Besides natural flavours there are chemical flavours that imitate natural flavours. Some examples of chemical flavouring agents are alcohols that have a bitter and medicinal taste, esters are fruity, ketones and pyrazines provide flavours to caramel, phenolics have a smokey flavour and terpenoids have citrus or pine flavour.

According to Codex Alimentarius "*flavourings or flavouring substances are added to food to impart aroma or taste. Like other food additives their use should not present an unacceptable risk to human health and should not mislead consumers. The quantity added to foods should be at the lowest level necessary to achieve the intended flavouring effect. Flavours and flavouring substances should also be of appropriate food grade quality; and be prepared and handled in the same way as a food ingredient."*

Flavours are used as additives to enhance, modify the taste and the aroma in natural food products which could have got lost due to food processing. Flavours are also used to create flavours in foods like candies and snacks that do not have likeable flavours of their own. Flavours are normally classified into three categories natural flavouring and artificial flavourings and nature-identical flavourings.

Natural flavouring substances are extracted from plants, herbs and spices, animals, or microbial fermentations. Essential oils and oleoresins that are created by solvent extract with the solvent removed, herbs, spices and sweetness are all natural flavourings. Natural flavourings can be either used in their natural form or processed form for human consumption and they cannot contain any nature-identical or artificial flavouring substances.

The U.S. Code of Federal Regulations defines natural flavourings as *"the essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, or any product of roasting, heating or enzymolysis, which contains the flavoring constituents derived from a spice, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf or any other edible portions of a plant, meat, seafood, poultry, eggs, dairy products, or fermentation products thereof, whose primary function in food is flavoring rather than nutritional."*

Artificial flavouring agents are chemically similar to natural flavourings but are more easily available and less expensive. However, one drawback is that they may not be an exact copy of the natural flavourings they are imitating like amyl acetate which is used as banana flavouring or ethyl butyrate for pineapple.

Nature-identical flavouring agents are the flavouring substances that are obtained by synthesis or are isolated through chemical processes. There chemical make-up of artificial flavourings is identical to their natural counterparts. These flavouring agents cannot contain any artificial flavouring substances.

Besides this category there are also natural flavour enhancers like monosodium glutamate (MSG) which bring out the flavours of foods. They have a taste that is different and cannot be called any of the known flavours like sweet, sour, salty or bitter. In fact the taste of MSG is called 'umami' and is known as the fifth taste also found in high protein foods like meat. Monosodium glutamate was once derived from seaweed but now it is manufactured commercially by the fermentation of starch, molasses, or sugar.

What FSSAI says

Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011 have described flavouring agents under the head 'Flavouring Agents and Related Substances' in the Regulations.

Flavouring agents include flavour substances, flavour extracts or flavour preparations, which are capable of imparting flavouring properties, namely taste or odour or both to food. Flavouring agents may be of following three types:

- Natural Flavours and Natural Flavouring substances means flavour preparations and single substance respectively, acceptable for human consumption, obtained exclusively by physical processes from vegetables, for human consumption
- Nature-Identical Flavouring Substances means substances chemically isolated from aromatic raw materials or obtained synthetically; they are chemically identical to substances present in natural products intended for human consumption, either processed or not.

- Artificial Flavouring Substances means those substances which have not been identified in natural products intended for human consumption either processed or not.

Use of anti-oxidants, emulsifying and stabilising agents and food preservatives in flavour

The flavouring agents may contain permitted anti-oxidants, emulsifying and stabilising agents and food preservatives.

Use of **Anticaking agent in flavours** – Synthetic Amorphous Silicon Dioxide may be used in powder flavouring substances to a maximum level of 2 per cent.

Restriction on use of flavouring agents the flavouring agents named below are not permitted for use in any article of food

- Coumarin and dihydrocoumarin;
- Tonkabean (Dipteryl adorat);
- β-asarone and cinamyl anthracilate.
- Estragole
- Ethyl Methyl Ketone
- Ethyl-3-Phenylglycidate
- Eugenyl methyl ether
- Methyl β napthyl Ketone
- Propylanisole
- Saffrole and Isosaffrole
- Thujone and Isothujone α & β thujone

Solvent in flavour

Diethylene Glycol and Monoethyl ether, shall not be used as solvent in flavours.

Use of Flavour Enhancers

Monosodium Glutamate may be added to foods as per the provisions contained in the Regulations subject to Good Manufacturing Practices (GMP) level and under proper label declaration as provided

in Regulation of Food Safety and Standards (Packaging and Labelling) Regulations, 2011. It shall not be added to any food for use by infant below twelve months and in the following foods.

List of foods where **Monosodium Glutamate is not allowed**

- Milk and Milk Products including Buttermilk, Fermented and renneted milk products (plain) excluding dairy based drink.
- Pasteurized cream, Sterilised, UHT, whipping or whipped and reduced fat creams.
- Fats and Oils, Pulses, Oil seeds and grounded/ powdered food grains, Food grains, Sago,
- Butter and concentrated butter, Margarine, Fat Spread
- Fresh fruit, Surface treated fruit, Peeled or cut fruit.
- Fresh vegetables, Frozen vegetables.
- Pastas and noodles (only dried products).
- Fresh meat, poultry and game, whole pieces or cuts or comminuted. Fresh fish and fish products, including mollusks, crustaceans and echinoderms. Processed fish and fish products, including mollusks, crustaceans and echinoderms.
- Fresh eggs, Liquid egg products, Frozen egg products.
- White and semi-white sugar (sucrose and saccharose, fructose, glucose (dextrose), xylose, sugar solutions and syrups, also (partially) inverted sugars, including molasses, treacle and sugar toppings. Other sugars and syrups (e.g. brown sugar and maple syrup),
- Honey, Saccharine
- Salt, Herbs, spices and condiments, seasoning (including salt substitutes) except seasoning for Noodles and Pastas, meat tenderizers, onion salt, garlic salt, oriental seasoning mix, topping to sprinkle on rice, fermented soya bean paste, Yeast.
- Infant food and Infant milk substitute including infant formulae and follow-on formulate, Foods for young children (weaning foods).
- Natural Minerals water and Packaged Drinking water, Carbonated Water
- Concentrates (liquid and solid) for fruit juices.
- Canned or bottled (pasteurized) fruit nectar.
- Coffee and coffee substitutes, tea, herbal infusions, and other cereal beverages excluding cocoa.
- Wines, Alcoholic Beverage
- Fruits and Vegetables products except those where Monosodium Glutamate is permitted under these Regulations.

- Baking Powder, Arrowroot
- Plantation Sugar, Jaggery and Bura,
- Ice-Candies, Ice cream and Frozen desserts.
- Cocoa Butter
- Malted Milk Food and Milk based foods
- Bread
- Vinegar
- Sugar Confectionery, Toffee, Lozenges, Chocolate
- Pan Masala

Reference:
https://foodsafetyhelpline.com/what-are-flavouring-agents-what-do-fssai-regulations-say/

Food Additives: Emulsifiers

Oil and water don't mix — until an emulsifying agent is added.

Emulsifiers made from plant, animal and synthetic sources commonly are added to processed foods such as mayonnaise, ice cream and baked goods to create a smooth texture, prevent separation and extend shelf life. However, in this era of "clean labels," consumers question the necessity of additives in food.

Definition

A food emulsifier, also called an emulgent, is a surface-active agent that acts as a border between two immiscible liquids such as oil and water, allowing them to be blended into stable emulsions. Emulsifiers also reduce stickiness, control crystallization and prevent separation.

Functions, Names and Labeling

Emulsifiers create two types of emulsions: either droplets of oil dispersed in water or droplets of water dispersed in oil. Within the emulsion, there is a continuous and dispersed phase. In an oil-in-water emulsion, the continuous phase is the water and the dispersed phase is the oil; conversely, in a water-in-oil emulsion, the oil is the continuous phase.

Emulsions also can be made by applying mechanical force from a blender or homogenizer, which breaks down the dispersed phase into tiny droplets that become suspended in the continuous phase.

Low-fat spreads, ice cream, margarine, salad dressings and many other creamy sauces are kept in stable emulsions with the addition of emulsifiers. These additives also are widely used in other foods such as peanut butter and chocolate.

“Emulsifiers enhance the structure of baked goods by increasing whip-ability of batters, conditioning of dough and helping foods like pasta be more resistant to overcooking,” says food scientist Kantha Shelke, PhD, CFS.

Commonly used emulsifiers in modern food production include mustard, soy and egg lecithin, mono- and diglycerides, polysorbates, carrageenan, guar gum and canola oil.

Lecithin in egg yolks is one of the most powerful and oldest forms of an animal-derived emulsifier used to stabilize oil in water emulsions, for example, in mayonnaise and hollandaise sauce.

Emulsifiers are required by law to be included on a food’s ingredient list.

Oversight

Safety of emulsifiers is carefully regulated and tested by the U.S. Food and Drug Administration. Emulsifiers can be found on the Generally Recognized As Safe, or GRAS, list and are allowed in specific types of food and beverages at precise levels.

However, “FDA processes do not take into consideration individual diets of people who rely heavily on packaged foods,” Shelke says.

Although GRAS substances technically must meet the same safety standards as approved food additives, the GRAS process has evolved into a voluntary notification program and many GRAS additives have not been tested.

Congress defines safe as “reasonable certainty that no harm will result from use” of an additive. Additives are never given permanent approval. The FDA continually reviews the safety of approved additives, based on the best scientific knowledge, to determine if approvals should be modified or withdrawn.

Earlier in 2017, the FDA reviewed and confirmed the safety of carrageenan, an emulsifier whose safety has been questioned.

Safety

Most concerns about food additives target synthetic ingredients that are added to foods. Published peer-reviewed intervention studies involving emulsifiers are limited to animals. A 2015 mouse study published in Nature found that two common synthetic emulsifiers, carboxymethylcellulose (CMC) or polysorbate 80 (P80), triggered weight gain and low-grade symptoms of inflammation and metabolic syndrome after 12 weeks.

"We suspect some emulsifiers act like detergents, upsetting the friendly bacteria in the microbiota, which triggers low-grade inflammation and causes excess eating," says co-author Andrew Gewirtz, PhD.

A follow-up study by Gewirtz, a professor of biomedical sciences at Georgia State University, and his colleagues, published in Cancer Research, suggested the changes in gut bacteria from emulsifiers could trigger bowel cancer. A small clinical trial currently is underway to evaluate the role of CMC in humans.

In response to questions about the safety of some emulsifiers, a team of FDA scientists conducted a review of seven emulsifiers commonly used in food, including CMC and P80, to determine whether these ingredients pose any risk to human health. Their findings, published in 2017, confirmed that emulsifiers remained safe at the estimated exposure levels.

Final Thoughts

Food additives, including emulsifiers, play an important role in our food supply. Consumers who are concerned about these ingredients are encouraged to read labels and consume more minimally processed foods.

Kathleen Zelman

Kathleen Zelman, MPH, RDN, is the nutrition director of WebMD.

Reference:
https://foodandnutrition.org/november-december-2017/food-additives-emulsifiers/

Stabiliser (food)

From Wikipedia, the free encyclopedia

Pectin is used as a stabiliser in foods such as yogurt

A **stabiliser** is an additive to food which helps to preserve its structure. Typical uses include preventing oil, water emulsions from separating in products such as salad dressing; preventing ice crystals from forming in frozen food such as ice cream; and preventing fruit from settling in products such as jam, yoghurt and jellies. The following hydrocolloids are the most common ones used as stabilisers:[1]

- alginate
- agar
- carrageen
- cellulose and cellulose derivatives
- gelatin
- guar gum

- gum Arabic
- locust bean gum
- pectin
- starch
- xanthan gum

Consumers demand a certain quality and eating experience from processed foods. This is achieved, in part, by the addition of stabilizers, thickeners and gelling agents, which give foods consistent texture, taste and mouth feel.

Definition

Extracted primarily from natural substances, stabilizers, thickeners and gelling agents are approved direct additives incorporated into foods to provide structure, viscosity, stability and other qualities, such as maintaining existing color.

Functions, Names and Labeling

Thickeners, stabilizers and gelling agents are classified separately but overlap in functionality. When dissolved or added to foods, they create stiffness, stabilize emulsions or form gels.

Thickeners range from flavorless powders to gums and are chosen for their ability to work in a variety of chemical and physical conditions. Variables affecting choice of thickener include pH, frozen state, clarity and taste. Starches, pectin and gums are the most common commercial thickeners used in soups, sauces and puddings.

Stabilizers are substances that increase stability and thickness by helping foods remain in an emulsion and retain physical characteristics. Ingredients that normally do not mix, such as oil and water, need stabilizers. Many low-fat foods are dependent on stabilizers. Lecithin, agar-agar, carrageenan and pectin are common in ice cream, margarine, dairy products, salad dressings and mayonnaise.

Gelling agents also function as stabilizers and thickeners to provide thickening without stiffness through the formation of gel in jellies, jams, desserts, yogurts and candies. Gums, starches, pectin, agar-agar and gelatin are common gelling agents.

Home cooking achieves the same structural changes with the addition of familiar starches, grains, egg yolks, yogurt, gelatin, mustard and vegetable purees.

Thickening agents also are used in treating medical conditions, such as dysphagia, to make swallowing easier and reduce the risk of aspiration.

To help consumers understand the function of the food additive, a classification is indicated on food labels — for example, "pectin (gelling agent)." Most direct additives are identified on the ingredient label of foods.

Thickeners, stabilizers and gelling agents are largely polysaccharides or derived from protein sources, for example:

Polysaccharides

- Starches: arrowroot, cornstarch, potato starch, sago, tapioca
- Vegetable gums: guar gum (extracted from guar bean), xanthan gum (from microbial fermentation used in gluten-free baked goods), locust bean gum (from carob tree)
- Pectin (from apples or citrus fruit)

Protein

- Collagen, egg whites, gelatin (from animal collagen), whey

Others

- Sugars: agar (from algae), carrageenan (from seaweeds and used to prevent separation in dairy products and ice cream)
- Sodium pyrophosphate (used in common foods such as canned fish and instant pudding)
- Lecithin (found in egg yolk, legumes and corn)
- Mono- and diglycerides (stabilizers naturally present in many seed oils)

Sources are primarily natural (arrowroot, gelatin, starches) but also can be synthetic (carboxymethyl cellulose, methyl cellulose).

Oversight

Thickeners, stabilizers and gelling agents must be authorized by the Food and Drug Administration before use. Standards for food additives are clearly defined with strict criteria, and there must be a

documented need for their use before approval is granted. Maximum usage levels vary depending on the additive and the food in which it is used.

For example, stabilizers in frozen dairy desserts, fruit and water ices and in confections and frostings cannot exceed 0.5 percent by weight of the final product. Emulsifier, flavoring adjuvant, stabilizer or thickener in baked goods have the same 0.5 percent by weight limit.

Safety

Today, food additives are scrutinized, regulated and monitored more closely than at any other time. All new food additives undergo a rigorous testing and safety assessment to minimize potential adverse effects to human health. However, side effects and nutrient-drug interactions may result from large doses. For example, consuming more than 15 grams of xanthan gum may cause nausea, flatulence and bloating. Food-drug interactions are possible with certain medications; carrageenan may cause adverse side effects in people taking anticoagulants and antihypertensive drugs, and pectin may interact with antibiotics and cholesterol-lowering drugs.

Final Thought

Registered dietitian nutritionists can help consumers feel more comfortable about food additives by communicating their similar role in home kitchens.

“While the name of the ingredient may be unfamiliar, the mode of action in the food matrix is similar,” says Heather Dover, RDN, research assistant at the Center for Research on Ingredient Safety at Michigan State University.

“Most concerns about food additives are related to synthetic ingredients added to foods, yet 99 percent of these additives are derived from natural sources and meet all of the FDA standards for safety, intended use and populations,” says Roger Clemens, DrPH, and former president of the Institute of Food Technologists. “For over 100 years of usage, these direct additives have posed no adverse effect for any population.”

Reference:
https://en.wikipedia.org/wiki/Stabiliser_(food)
https://foodandnutrition.org/may-june-2017/stabilizers-thickeners-gelling-agents/

Sweetener (food)

Sweetener, any of various natural and artificial substances that provide a sweet taste in food and beverages. In addition to their sweetening power, they may be used for such processes as food preservation, fermentation (in brewing and wine making), baking (where they contribute to texture, tenderization, and leavening), and food browning and caramelization. Natural sweeteners may be both nutritive and flavorable and thus popular both as food and flavouring. However, because common sugar and other nutritive sweeteners such as honey and corn syrup are associated with health problems (such as obesity and tooth decay) or are even a threat to life (for diabetics), there have been efforts since the 19th century to produce nonnutritive sweeteners that are not subject to metabolism and contain little or no caloric value. Nonnutritive sweeteners, which may be either artificial (synthetic) or derived from plants, include such compounds as saccharin, aspartame, cyclamates, and thaumatin.

Sugar is a generic term for a category of carbohydrate compounds known as sucrose, or saccharose ($C_{12}H_{22}O_{11}$). A group of related compounds are corn sugar (called glucose, or dextrose), fruit sugar (fructose, or levulose), milk sugar (lactose), and malt sugar (maltose). Sucrose is a disaccharide; that is, it is made up of two simple sugars, or monosaccharides—glucose and fructose. It is one of the sweetest of sugars. If sucrose is taken as a standard of 1, the sweetness of glucose is 0.5–0.6, that of lactose is 0.27, and that of maltose is 0.6; fructose, found in fruits and honey, is the sweetest, being 1.1 to 2.0 times as sweet as sucrose.

Sucrose is commercially derived chiefly from sugarcane and sugar beets but also comes from such sources as maple trees, sugar palms (especially date palms), and sorghum. Sucrose is found in all plants: an apple is about 4 percent sucrose, 6 percent fructose, and 1 percent glucose (by weight); a grape is about 2 percent sucrose, 8 percent fructose, 7 percent glucose, and 2 percent maltose (by weight). Honey is composed principally of fructose and glucose, the composition depending on the original nectar collected by the honeybee and on the amount of processing and storage time.

In the development of low-calorie sweeteners, the problems are several and are not limited to sweetness. Some sweeteners lose their sweetness at high temperatures (making them often unsuitable in cooking) or lose the sweetness over time (giving them a short shelf life). Some nonnutritive sweeteners have an undesirable aftertaste. Sugar furthermore has functional properties not found wholly in any other sweeteners. Sugar adds bulk and texture to baked goods; it helps in forming the structure of the baked food, provides moistness, tenderness, and antitoughening characteristics, and

contributes to leavening. In addition it has a preservative effect (as in jellies and preserves) and helps generally to prevent spoilage. It serves as food for fermenting organisms that are important in making such things as alcoholic beverages, breads, and pickles. In soft drinks, in addition to providing sweetness, sugar provides “mouthfeel” and body and helps to stabilize the carbon dioxide. Sugar, in sum, has many functional properties in food, and no other sweetener has so far been developed to duplicate all or even many of them.

The artificial sweetener saccharin (ortho-sulfobenzoic acid imide) was discovered in 1879 by two German researchers, I. Remsen and C. Fahlberg, and has about 300 to 500 times the sweetening power of cane sugar. It is manufactured on a large scale in several countries in the form of saccharin, sodium saccharin, and calcium saccharin. Although its safety was the subject of controversy during the 1970s and ’80s, it is still widely used.

Cyclamates, a group of synthetic sweeteners derived from cyclohexylamine or cyclamic acid, were discovered in 1937 and are about 30 times as sweet as sucrose. Although used in several countries, cyclamates were banned in some countries (notably the United States, in 1969) after being suspected of carcinogenicity.

Aspartame, or aspartylphenylalanine (marketed as NutraSweet, Equal, Egal, or Canderal), was discovered in 1965. It has some caloric value (though negligible) and is about 150–200 times as sweet as sucrose. Its safety remains controversial, but it is now the most popular sweetening ingredient in diet soft drinks. It tends to lose its sweetness over long periods, but manufacturers have taken measures to enhance the stability through additives.

Thaumatin, a protein extracted and purified from *Thaumatococcus danielli*, a plant found in western Africa, has found increasing use in Japan since its approval there in 1979. It combines well with monosodium glutamate and is used in typical Japanese seasonings as well as in chewing gum.

Acesulfame potassium (marketed as Sunette) was approved in the United States in 1988. It is about 130–200 times as sweet as sucrose, has good shelf life and high stability, and was initially used in dry food mixes.

Stevioside, derived from the plant *Stevia rebaudiana*, has been used in Japan, Paraguay, and a few other countries as a low-calorie sweetener. It is about 300 times as sweet as sucrose.

A potential noncaloric sweetener, patented in the United States in 1981, is “left-handed” sugar, or L-sugar. It is chemically identical to sucrose except that its molecular structure is an opposite mirror image of standard “right-handed” sucrose; it is said to look, act, and taste like sucrose, but the human body seems not to recognize it and metabolize it, so that it passes from the body basically unchanged. The manufacture of L-sugar, however, has proved prohibitively difficult and expensive.

Research—mainly in Europe, North America, and Japan—continues on hundreds of potential sweeteners.

Reference:
https://www.britannica.com/topic/sweetener

Non-nutritive Sweeteners

These sweeteners are not metabolized by the body. Because they do not contribute calories or energy to the diet, they are appropriate in the diets of those on calorie restricting diets, such as persons with some types of diabetes and those who are overweight. Non-nutritive sweeteners also work well in preventing dental caries.

Here are the non-nutritive sweeteners approved by the Food and Drug Administration (FDA) for use in food and/or drinks:

- Aspartame
- Acesulfame-K
- Neotame
- Saccharin
- Sucralose

Reference:
https://www.ksre.k-state.edu/kvafl/ingredients/sweeteners.html

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DEPARTMENT OF BIOTECHNOLOGY

UNIT – III – Food Biotechnology – SBB2203

1. Food borne diseases (Food Poisoning)

Human illnesses caused by foodborne microorganisms are popularly referred to as food poisoning. The common use of a single classification is due primarily to similarities of symptoms of various food-related diseases (see Table 5). Apart from illness due to food allergy or food sensitivity, foodborne illness may be divided into two major classes, food infection and food intoxication. Food infection results when foods contaminated with pathogenic, invasive, food poisoning bacteria are eaten. These bacteria then proliferate in the human body and eventually cause illness. Food intoxication follows the ingestion of preformed toxic substances which accumulate during the growth of certain bacterial types in foods.

The period of time between the consumption of contaminated foods and the appearance of illness is called the incubation period. The incubation period can range anywhere from less than one hour to more than three days, depending on the causative organisms or the toxic product.

Table. Characteristics of the important bacterial food intoxications and foodborne infections. (NAS-NRC, 1975)*

Disease	**Etiologic Agent**	**Incubation Period**	**Symptons**
Botulism	*Clostridium botulinum* A.B.E.F toxin	Usually 1 to 2 days; range 12 hours to more than 1 week	Difficulty in swalling, double vision, difficulty in speech. Occasionally nausea, vomiting, and diarrhea in early stages. Constipation and subnormal temperature. Respiration becomes difficult, often followed by death from paralysis of muscles of respiration.
Staphylococcal food poisoning	Staphyloccal enterotoxin	1 to 6 hours; average 3 hours	Nausea, vomiting, abdominal cramps, diarrhea, and acute prostration. Temperature subnormal

			during acute attack, may be elevated later. Rapid recovery-usually within 1 day.
Salmonellosis	Specific infection by *Salmonella* spp.	Average about 18 hours; range 7 to 72 hours	Abdominal pains, diarrhea, chills, fever, frequent vomiting, prostration. Duration of illness: 1 day to 1 week.
Shigellosis (bacillary dysentery)	Shigella sonnei, s. flexneri, s. dysenteriae, s. boydii	Usually 24 to 48 hours; range 7 to 48 hours	Abdominal cramps, fever, chills, diarrhea, watery stool (frequently containing blood, mucus, or pus), spasm, headache, nausea, dehydration, prostration. Duration: a few days.
Enteropathogenic *Escherichia coli* infection	*Escherichia coli* serotypes associated with infant and adult infections	Usually 10 to 12 hours; range 5 to 48 hours	Headache, malaise, fever, chills, diarrhea, vomiting, abdominal pain. Duration: a few days.
Clostridium perfringens food poisoning	*Clostridium perfringens*	Usually 10 to 12 hours; range 8 to 22 hours	Abdominal cramps and diarrhea, nausea, and malaise, vomiting very rare. Meat and poultry products usually involved. Rapid Recovery.
Bacillus cereus food poisoning	*Bacillus cereus*	Usually about 12 hours;	Similar to *Clostridium perfringens* poisoning

		range about 8 to 16 hours	
Vibrio Parahaemolyticus food poisoning	*Vibrio Parahaemolyticus*	Usually 12 to 14 hours; range 2 to 48 hours	Abdominal pain, server watery diarrhea, usually nausea and vomiting, mild fever, chills and headache. Duration: 2 to 5 days.

*Repeated from Prevention of Microbial and Parasitic Hazards Associated with Processed Foods, pages 6-7, with the permission of the National Academy of Sciences, Washington, DC.

Foodborne Disease Organisms

Escherichia coli

A few of the E. Coli strains found in human feces are in themselves pathogenic, causing infection and disease. These are called Enteropathogenic E. Coli or EEC. In one extensive study of the feces of food handlers (Hal and Hause, 1966), 6.4% of the workers harbored the EEC organisms as carriers.

Staphylococcus aureus

S. aureus, commonly referred to as "staph," is normally present on the skin, the mucous membranes, and in pimples and boils of human beings and other animals. It is nearly always present in small numbers in raw meats and in foods handled extensively by human hands. The food poisoning strains generally come from human sources. Pasteurizing or cooking destroys the organism, but not its toxin. Foods contaminated by staph organisms can cause food poisoning after the organisms have been destroyed by heat.

The presence of staph in a cooked food has two levels of significance.

1. Low numbers (not over a few hundred per gram) indicate the degree of contact with human skin or nasal mucous, cross-contamination from raw meat, or survivors of a larger population.

2. High numbers (100,000 or more per gram) indicate that the bacteria were allowed to grow in the food, thereby creating the potential serious hazard of the presence of toxin.

   Keeping foods completely free from staph contamination is often difficult or impossible. Therefore, the processor should store the food at temperatures that preclude the growth of staph (see Table 1). It is only during growth that staph forms the toxin. An epidemiological investigation to determine the source of the organism is tedious, but visual inspection of workers' hands can be useful. The well-informed sanitarian will also seek time-temperature abuses of foods contaminated with staph.

   The National Academy of Science's National Research Council has listed the following steps to limit the incidence and level of staph in foods (NAS-NRC, 1975):

1. Reduce direct and indirect exposure of foods, particularly cooked foods, to human contact as much as possible. If handling is necessary, use sanitary rubber or plastic gloves, or sanitize hands. Persons with infected cuts, abrasions, boils, or pimples should never handle cooked foods.
2. Test raw materials and eliminate production lots that contain high levels of S. aureus.
3. Process to destroy the microorganisms.
4. Eliminate cross-contamination from raw to cooked food.
5. Keep cooked foods no longer than 2 to 3 hours between 40°F and 140°F.

   Control of staph growth in fermented foods, such as cheese or sausages, requires controlling a number of processing factors (see NAS-NRC, 1975). Low pH, relatively high levels of lactic bacteria, salt, and nitrite help to inhibit toxin formation.

Salmonella

Salmonella infection, or salmonellosis, is almost always caused by eating contaminated food or drink. Contamination originates from the intestinal tract of human beings or animals who harbor Salmonella organisms. Most adults can resist infection from a few cells, but become ill when ingesting millions. Infants, the aged, and the infirm are much more sensitive and can be affected by a few Salmonella cells. After recovery, the victim may remain a carrier for a period varying from a week to permanency.

Domestic animals, such as dogs, poultry, swine, horses, sheep, and cattle are carriers of these pathogens. Carriers show no outward symptoms of the disease at the time of slaughter. As long as abattoirs continue to receive Salmonella carriers for slaughter, Salmonella contamination of the finished raw meat is inevitable. Even with apparently satisfactory sanitation, slaughtering and dressing procedures may spread traces of feces from a carrier animal to subsequently slaughtered animals by way of equipment, water, and hand contact (NAS-NRC, 1969).

Salmonella is often discussed as if it were a single organism. There are actually more than 1,300 serotypes identified within the genus Salmonella. All are quite sensitive to heat, so freshly pasteurized or cooked foods are free of the organism (USDA, 1966). The principal routes of its entry into cooked foods are cross-contamination from raw foods or animals (via hands, equipment, air, water), recontamination from human carriers, or gross undercooking. Regulatory agencies are quick to institute seizures, recalls, and other legal action against products and firms shipping Salmonella-contaminated processed foods.

Dry and semi-dry fermented sausages rarely cause food borne diseases. However, recent investigations by USDA have shown that Salmonella can survive the fermentation and drying process (Smith et. al., 1975). Salmonella in natural animal casings likewise survives short periods of salting, but dies more rapidly in acidified or alkalized casings (Gabis and Silliker, 1974).

Salmonella can also grow outside the animal body when conditions are favorable. For this reason, it has appeared in a wide variety of foods and feeds, in addition to meat and poultry products. Some of these are brewers yeast, coconut meat, cochineal dye, dried or frozen eggs, noodles, custards, dried animal feeds, cottonseed flour, candy, chocolate, dried milk, fish and shellfish, cream-filled pastries, sausage casings, and watermelon. The NAS-NRC (1969-1975) has made extensive recommendations for evaluation, control, and eradication of the Salmonella problem.

Costridium botulinum

C. botulinum produces a rare but often fatal disease called botulism. It is caused by a neurotoxin produced during growth in the absence of air. Except in the case of infantile botulism the intact spores are harmless. Infants ingesting spores, usually from honey, have developed symptoms of botulism. Botulism usually occurs after a food containing the preformed toxin has been eaten, but sometimes the organism infects wounds, forming the toxin in the muscle of the victim. There are seven types of

C. botulinum (A to G), of which four (A, and B associated with meats and vegetables, E, marine environment and F) cause human disease. Only once has type C been reported to cause human illness. Type G is a new incompletely studied discovery (Schmidt, 1964, USPHS, 1974).

Fortunately, the toxins, regardless of type, have very little resistance to heat and are inactivated by boiling for 10 minutes. Thus, all freshly, but adequately, cooked foods are safe (Riemann, 1973). All C. botulinum strains can form spores which exhibit varying resistance to heat. The spores of types A and B are highly resistant. Spores of type E die in a fraction of a minute at 212°F (Perkins, 1964). The canning industry, under the technical leadership of the National Food Processors Association (formerly the National Canners Association), has established times and temperatures of retorting necessary to insure the commercial sterility of low-acid canned foods (NCA, 1968, 1971b, 1976b). The NFPA also submitted to the FDA the initial petition which eventually developed in the GMP regulations for low-acid canned foods.

Botulinum spores are widely distributed in soils. Type A predominates in the western states and in New England; type B, in the eastern and southern states. Type E is usually associated with marine or fresh water environments throughout the world and is psychrotropic (Riemann, 1973). Type F has been isolated too rarely to establish its distribution pattern (Eklund et.al., 1967).

C. botulinum will not grow below pH 4.8. Therefore, botulism is a concern only in low acid foods, which are defined as foods with a finished equilibrium pH greater than 4.6. The majority of outbreaks occur from home canned vegetables, meats, fish, and over-ripe fruits (USPHS, 1974).

Canned cured meats contain salt and nitrite. The preservatives protect against the outgrowth of botulinum spores that may have survived the minimal processing, which is frequently at or below boiling (Halvorson, 1955; Ingram and Hobbs, 1954; Pivnick et. al, 1969).

There have been 34 outbreaks of type E botulism among fish products prepared in the U.S. and Canada (Lechowich, 1972). Most have been smoked or lightly salted products. The FDA isolated botulinum types B, E, and F from pasteurized meat of the blue crab (Kautter et. al., 1974). The NAS-NRC (1975) has reviewed steps to minimize the possibility of out-breaks from smoked fish and FDA has published regulations designed to control the problem (FDA, 1970).

Clostridium perfringens

C. perfringens is a spore-forming organism which, like botulinum, grows only in the absence of air. It grows best in meat or poultry dishes, stews, or gravies kept warm. Such foods meet its exacting nutritional requirements and the warm holding temperature, up to 122°F, encourages its growth. The spores themselves are harmless, but the vegetative cells, which can grow to enormous numbers in these foods, form spores in the intestinal tract of the victim. During the sporulation process, the remainder of the vegetative cell dissolves, releasing the poison that causes illness.

The vegetative cells which cause the disease are very delicate. They can be destroyed or reduced to low, safe levels by cooking or freezing. The spores are widely distributed in nature and are present in small numbers in various foods (Hall and Angelotti, 1965; Strong et. al., 1963). They occur in feces, soils, dust, water, marine sediments, raw foods, and even cooked foods.

C. perfringens poisoning is a problem specific to the food service industry. Only proper temperature control prevents the problem. A good rule of thumb is to keep ready-to-eat moist foods below 40°F or above 140°F. Time-temperature abuse is a severe health hazard. Since the spores are everywhere, epidemiologic investigation of strains to determine the source of spores is a relatively futile exercise. However, if serological tests show that the same types are present in the victim's food and feces, a particular dish can be incriminated. Unfortunately, the biological materials (antisera) for this purpose are not yet commercially available. Therefore, the determination that large numbers of C. perfringens cells are present remains the most suitable investigative test.

Bacillus cereus

B. cereus is a spore-forming organism that grows in the presence of oxygen and is widely distributed in most raw foods. Since the spores survive boiling for several minutes, they remain viable in cooked foods in small numbers. The organism does not compete well with other bacteria in raw foods, but in moist, cooked dishes held warm (up to 122°F), it grows to millions per gram in a few hours. Under these conditions the food becomes poisonous. B. cereus grows well in a wide variety of cooked foods, such as meats, poultry, sauces, puddings, soups, rice, potatoes, and vegetables. The disease is similar to that of perfringens (see Table 5), although the mechanism of the disease is unknown. Adults have rather mild symptoms, but small children may become seriously ill. In most instances, the victims recover quickly and do not seek medical attention. Therefore, only large outbreaks are reported and become part of the statistical record.

Similar to C. perfringens, B. cereus is primarily a concern of the food service industries. The appropriate control is to keep hot foods hot (over 140°F) and cold foods cold (under 40°F). Epidemiologic investigation of strains to determine the source of the spores proves equally futile.

Vibrio parahaemolyticus

V. parahaemolyticus is a non-spore forming, slightly curved rod, closely related to the organism that causes cholera. It is widely distributed and grows in brackfish waters, estuarine sediments, raw fish, and shellfish throughout the world. It competes well with spoilage organisms at temperatures of 41°F or above. It occurs in greatest numbers in the summer when higher temperatures engender rapid growth.

V. parahaemolyticus is the principal cause of food poisoning in Japan where raw fish is regularly consumed. Elsewhere, the disease occurs less frequently because the organism dies readily during pasteurization or cooking. Nevertheless, cooked seafoods can be recontaminated from water or raw seafood. The first confirmed outbreaks in the United States occurred in 1971 and 1972 from crabmeat, shrimp, and lobster. In one Japanese outbreak, 22 people died and 250 others became ill.

The human pathogenicity of the organism is determined by culturing it on a special medium, a salt agar containing human blood. If the organism can grow and destroy blood cells on this medium, the so-called Kanagawa test, it is labeled “Kanagawa positive” and assumed capable of causing human disease. The Japanese have found that about 1% of the strains of V. parahaemolyticus from waters near their shores are Kanagawa positive (Sakazaki et. al., 1968). On the other hand, Twedt et. al. (1970) reported that up to 90% of the strains from U.S. estuarial waters are Kanagawa positive. However, the significance of the Kanagawa test is not fully understood.

To reduce the incidence of these outbreaks, the seafood industry should:

- Hold raw seafoods at or below 40°F;
- Keep cooked seafoods carefully apart from raw seafood, sea water, insanitary equipment, and unclean containers; and
- Hold cooked seafood below 40°F or above 140°F

Listeria

Before the 1980's most problems associated with diseases caused by Listeria were related to cattle or sheep. This changed with food related outbreaks in Nova Scotia, Massachusetts, California and Texas. As a result of its widespread distribution in the environment, its ability to survive long periods of time under adverse conditions, and its ability to grow at refrigeration temperatures, Listeria is now recognized as an important food borne pathogen.

Immunocompromised humans such as pregnant women or the elderly are highly susceptible to virulent Listeria. Listeria monocytogenes is the most consistently pathogenic species causing listeriosis. In humans, ingestion of the bacteria may be marked by a flu-like illness or symptoms may be so mild that they go unnoticed. A carrier state can develop.

Following invasion of macrophages virulent strains of Listeria may then multiply, resulting in disruption of these cells and septicimia. At this time the organism has access to all parts of the body. Death is rare in healthy adults; however, the mortality rate may approximate 30% in the immunocompromised, newborn or very young.

As mentioned earlier Listeria monocytogenes is a special problem since it can survive adverse conditions. It can grow in a pH range of 5.0-9.5, in good growth medium. The organism has survived the pH 5 environment of cottage cheese and ripening Cheddar. It is salt tolerant surviving concentrations as high as 30.5% for 100 days at 39.2°F. But only 5 days if held at 98.6°F.

The key point is that refrigeration temperatures do not stop growth of Listeria. It is capable of doubling in numbers every 1.5 days at 39.2°F. Since high heat, greater than 175°F, will inactivate the Listeria organisms, post-process contamination from environmental sources then becomes a critical control point for many foods.

Yersinia enterocolitica

Even though Yersinia enterocolitica is not a frequent cause of human infection in the U.S., it is often involved in illness with very severe symptoms. Yersiniosis, infection caused by this microorganism, occurs most commonly in the form of gastroenteritis. Children are most severely affected. Symptoms of pseudo-appendicitis have resulted, in many unnecessary appendectomies. Death is rare and recovery is generally complete in 1 – 2 days. Arthritis has been identified as an infrequent but significant sequela of this infection.

Y. enterocolitica is commonly present in foods but with the exception of pork, most isolates do not cause disease. Like Listeria this organism is also one that can grow at refrigeration temperatures. It is sensitive to heat (122 F., sodium chloride (5%) and acidity (pH 4.6), and will normally be inactivated by environmental conditions that will kill salmonellae.

Campylobacter jejuni

C. jejuni was first isolated from human diarrheal stools in 1971. Since, then it has continually gained recognition as a disease causing organism in humans.

C. jejuni enteritis is primarily transferred from animal origin foods to humans in developed countries. However, fecal contamination of food and water and contact with sick people or animals predominates in developing countries.

Although milk has been most frequently identified throughout the world to be a vehicle for Campylobacter, one anticipates that future investigations will identify poultry and its products and meats (beef, pork and lamb) as major reservoirs and vehicles.

C. jejuni dies off rapidly at ambient temperature and atmosphere, and grows poorly in food.

The principles of animal science will play a significant role in the control of this ubiquitous organism. Hygienic slaughter and processing procedures will preclude cross-contamination while adequate cooling and aeration will cause a decrease in the microbial load. In addition, thorough cooking of meat and poultry products followed by proper storage should assist in maintaining food integrity and less contamination.

Mycotoxins

Mycotoxins are harmful byproducts from molds that grow on foods and feeds. They have caused severe illness and death in animals for centuries. They first came to the attention of modern scientists in 1960 when 100,000 turkey poults died in England after eating moldy peanut meal from Africa and South America. The mycotoxins involved were later shown to be aflatoxins, a group of closely related organic compounds that can cause acute disease and death. Stimulated by these first discoveries and by research in antibiotics, investigators have discovered dozens of mold strains which produce a wide variety of mycotoxins that affect animals. There are now about 60 identified toxins. Of these, only a

few have been designated human food contaminants. These numbers will likely increase as mycotoxin investigations continue and identification methods are improved.

Historically, mycotoxins have been associated with human poisoning and even death. Ergot is among the first mycotoxins recognized as affecting human beings. It is produced by a mold growing on cereal grains. Ergot poisoning occurred in the Rhine Valley in the year 857 and has been reported several times since. The most recent outbreak was in 1951 in southern France. Many Russians died during World War II from eating moldy grains. The Japanese have reported human toxicity from eating moldy rice; the disease caused severe liver damage, hemorrhaging, and some fatalities (Mirocha, 1969).

Although such incidents are rare occurrences, there is evidence that low dietary levels of aflatoxins contribute to cancer of the liver in human beings. Extensive laboratory studies have also shown that even at very low dietary levels, aflatoxin can produce liver cancer in rats, mice, monkey, ducks, ferrets, and rainbow trout. Epidemiological studies in Southeast Asia and Africa have related a high incidence of human liver cancer to aflatoxin levels up to 300 parts per billion (ppb) in 20% of the food staples, and 3 to 4 ppb in 7% of the foods as eaten. In one geographical area, 95% of the corn and 80% of the peanuts contained aflatoxin at an average level of 100 ppb.

Although there is no direct evidence that aflatoxins cause human liver cancer in the United States, FDA is concerned about the effect of long-term, low-level consumption of a known, highly carcinogenic substance in our food supply. FDA established an informal defect action level tolerance of 30 ppb on peanuts and peanut products in 1965. With improved harvesting, storage, and sorting practices developed by USDA and industry, the level of aflatoxins contamination gradually declined and FDA lowered the informal action level to 20 ppb in 1969. FDA proposed in the Federal Register of December 6, 1974, a regulation establishing a tolerance of 15 ppb for total aflatoxins in shelled peanuts and peanut products used as human food. Today the limits are 0.5 ppb for milk, 20 ppb for food, and 100 ppb for feed.

Molds which form mycotoxins can be present on any food not heated in a closed container. One must assume, therefore, that they are present and capable of producing, toxin if conditions permit. But finding a toxigenic mold in a food does not imply that the food contains a mycotoxin. Conversely, the absence of visible growth of an aflatoxin producing mold does not mean toxin is absent since aflatoxins may be produced when there is little visible mold growth.

There are several ways to determine whether molds growing in an abused food will produce mycotoxins. The food can be held with its naturally contaminating molds, or inoculated with a toxigenic strain, and kept until the molds develop. The food can then be tested for the presence or absence of toxin. Such experiments have demonstrated that molds produce mycotoxins on a large variety of cereal grains and seeds, dry beans and fruits, spices, nuts, and cured meats. As do bacteria, molds have moisture, temperature, and nutritional requirements for optimal growth and toxin production. In most cases the initial mold invasion occurs in the fields before or during harvest. Mold growth continues during storage if the moisture content and storage temperatures remain high.

Aflatoxin has been found throughout the world on corn, barley, copra, cassava, spices, dry milk, tree nuts, cottonseed, peanuts, rice, wheat, and grain sorghum. In the U.S. it has been found in corn, figs, grain sorghum, cottonseed, peanuts, and certain tree nuts.

The industry has relied on electronic and visual sorting methods, as well as blowing and vacuuming, to control aflatoxin levels in walnuts arid pecans. Corn mill operators use a high intensity ultraviolet ("black") light to detect possible aflatoxin contamination. Roasting reduces the level of aflatoxin up to 50% in some cases (Escher et. al., 1973).

The universal solution to the problem is eliminating conditions that permit mold growth, whenever it is feasible to do so, and thereby preventing the formation of mycotoxins. In some cases (corn, peanuts) mold growth and toxin production occur before harvest. Insect and bird damaged corn kernels are very susceptible; therefore, controlling these pests will help alleviate mold problems. For most susceptible foods, the critical period is immediately following harvest, during storage and initial drying when the moisture content is high enough to allow mold growth.

Spoilage

The most prevalent microbiological problem facing the food industry is simple spoilage by bacteria, yeasts, or molds that are not hazardous to health. Chilling slows spoilage; proper freezing, drying, canning, and pickling arrest it completely. Chilled foods must be transported to the consumer before spoilage microorganisms make them unfit for consumption. The problems of spoilage in the other processes arise only upon departure from established techniques. The incidence of product spoilage can be greatly reduced and shelf-life extended by taking appropriate precautions.

Refrigerated Foods

The popularity of refrigerated/chilled foods is increasing at a surprising rate. Most of these products are convenient to use and have a "close to fresh" image. Some of these products are partially cooked or processed prior to chilling. This heat reduces the microbial population but does not render it "commercially sterile." Because of this, refrigerated foods have a limited shelf-life. That is affected by temperature and customer abuse.

Refrigerated foods have been in our stores for many years. Products such as milk, cheese, yogurt and other dairy products, cookie and biscuit doughs, eggs, salads and processed meats are commonly found in the refrigerated section or deli. The optimum storage temperature is 33°F. or as close to freezing as possible. However, most refrigerated cases are holding near 45 or even 45°F. This temperature fluctuation reduces shelf-life of the products, and can lead to a problem of public health significance.

The Refrigerated Foods and Microbiological Criteria Committee of the National Food Processors Association has published a paper on "Safety Considerations for New Generation Refrigerated Foods" in the January, 1988 issue of Dairy and Food Sanitation. Many of the points considered in this section were derived from that paper.

Several important points on preparation, handling and distribution need to be considered. First of all, always assume pathogenic organisms are present in a food product. Secondly, refrigeration temperatures may slow or prevent replication of most pathogenic microorganisms, but some will continue to multiply (psychrotrophs). Psychrotropic pathogens include Yersinia enterocolitica, Listeria monocytogenes, non-proteolytic strains of C. botulinum some strains of enterotoxigenic E. coli and Aeromonas hydrophilia. Several other food borne disease organisms capable of growth at slightly above 41°F include: Vibrio parahemolyticus; Bacillus cereus; Staphylococcus aureus and certain strains of Salmonella. Thirdly, manufacturers should expect some temperature abuse of the foods during storage and distribution; this includes handling at the consumer level.

The last two points for consideration deals with labeling. A "Keep Under Refrigeration" statement must be prominent on the product label and outside carton. In addition, a "Sell By" or "Use By" date needs to be used on these products. This will help processors control their product, but it is not a

guarantee against problems. If the stock is not rotated properly, the out of date product will still get out.

A processor of refrigerated foods needs to incorporate as many treatments as possible that will help reduce the microbial population and minimize reproduction. Some of these treatments include: heat, acidification, preservatives, reduced water activity, and modified atmosphere packaging. Even though modified atmosphere is included as a potential barrier, it must be noted that reduced oxygen atmospheres may actually favor anaerobic pathogens. For many products modified atmosphere is really an aid to enhance product quality rather than safety.

One example of a product which successfully employs the multiple barrier principle is pasteurized cheese spread. The product uses a combination of reduced water activity (added salt and phosphates) and mild heat treatment to eliminate non-spore forming pathogens and inhibit growth of spore forming pathogenic microorganisms.

Any manufacturer who considers marketing a refrigerated food should have extensive shelf-life studies done by persons knowledgeable in the area of food microbiology.

Canned Foods

The shelf-life of canned foods results from the destruction of microorganisms capable of growth within the container during normal handling and storage. To attain this optimum situation, canners should:

- Follow the GMP regulations for low-acid foods.
- Reduce the spore level in the food by maintaining a sanitation program, particularly for blanchers and elsewhere where thermophilic spore formers thrive, and by monitoring ingredients for spore forming bacteria. As a general rule, food with a high spore level requires more retort time and/or temperature in the same or similar operations (Figure 6 and Table 4). A process approved by a processing authority must be filed with FDA on each low-acid and acidified food sold in the U.S. Assuming the same retort time and/or temperature, the incidence of spoilage will be higher in the canned food with a high initial spore level when all other factors are the same (Table 6).
- Follow good sanitation and good container handling techniques during the container cooling and post-cooling period. It is also important to cool heat processed containers quickly to about l00°F

(38C) since thermophilic outgrowth may occur with low spore numbers if containers are stacked or cased while hot.

- Maintain good seams on cans and tight lids on glass containers by regular control and testing.

Table 6. Effect of level of flat-sour spores on incidence of spoilage of canned vegetables. (Reed and Bohrer, 1961).

Product	Spores per can before processing (number)	Incidence of spoilage (percent)*
Canned peas	2,160	0
	13,000	66
Canned corn	900	16.7
	38,000	100

*After incubation of processed cans at 130°F (54.4°C)

Dry Foods

Dry foods do not spoil from microbial activity once they are adequately dry. Most foods require natural or artificial drying before they become stable. Adding sugar or salt, as in candied fruits or salted fish, accomplishes the same purpose since moisture becomes unavailable for use by microorganisms. The appropriate term to express the availability of water to microorganisms is water activity (a_w).

Although microorganisms cannot grow on dry foods, those that survive the drying process remain alive for prolonged periods. They quickly resume their activity upon rehydration. Under adverse conditions of storage that permit water to enter the product, molds are usually the first to grow because of their wider range of tolerance to low a_w (Watson and McFarlane, 1948) and they also have less competition from other organisms.

Fermented and Pickled Foods

Fermented and pickled foods owe their stability to the microbial development of organic acids by lactic bacteria or the addition of such acids to the foods, especially in the presence of a relatively high level of salt. Spoilage can occur either during the fermentation period or upon storage of the final product. The fermentation can fail if bacteriophage attacks the starter culture, if the temperature is unsuitable, or if the amount of fermentable carbohydrate is inadequate.

To prevent spoilage during the fermentation period:

1. Add lactic bacteria as a starter. Keep the starter in pure culture to help eliminate bacteriophage.
2. Add fermentable carbohydrate or organic acid.
3. Maintain the salt level high enough to inhibit spoilage bacteria and to permit the more salt-tolerant lactics to grow.
4. Control the temperature to favor lactics.

To reduce or eliminate spoilage during storage of the pickled or fermented food:

1. Add chemical preservatives, such as benzoates, sorbates, or propionates suitable to the product and acceptable to regulatory authorities.
2. Pasteurize the product, if practicable, to destroy or inhibit spoilage organisms.
3. Store pickles fully covered with brine to inhibit molds and impede yeast development.

Reference:

https://aggie-horticulture.tamu.edu/food-technology/food-processing-entrepreneurs/microbiology-of-food/

Food Allergens (Food allergies)

Food allergies and other types of food hypersensitivities affect millions of Americans and their families. Food allergies occur when the body's immune system reacts to certain proteins in food. Food allergic reactions vary in severity from mild symptoms involving hives and lip swelling to severe, life-threatening symptoms, often called anaphylaxis, that may involve fatal respiratory problems and shock. While promising prevention and therapeutic strategies are being developed, food allergies

currently cannot be cured. Early recognition and learning how to manage food allergies, including which foods to avoid, are important measures to prevent serious health consequences.

To protect those with food allergies and other food hypersensitivities, the FDA enforces regulations requiring companies to list ingredients on packaged foods and beverages. For certain foods or substances that cause allergies or other hypersensitivity reactions, there are more specific labeling requirements.

The FDA provides guidance to the food industry, consumers, and other stakeholders on best ways to assess and manage allergen hazards in food. The FDA also conducts inspections and sampling to check that major food allergens are properly labeled on products and to determine whether food facilities implement controls to prevent allergen cross-contact (the inadvertent introduction of a major food allergen into a product) and labeling controls to prevent undeclared allergens during manufacturing and packaging. When problems are found, the FDA works with firms to recall products and provide public notification to immediately alert consumers. In addition, the FDA has the authority to seize and remove violative products from the marketplace or refuse entry of imported products.

Major Food Allergens

Congress passed the Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA). This law identified the following eight foods as major food allergens:

- Milk
- Eggs
- Fish
- Shellfish
- Tree nuts
- Peanuts
- Wheat
- Soybeans

At the time of the law's passage, the eight major allergens accounted for 90 percent of food allergies and serious allergic reactions in the U.S. FALCPA requires that foods or ingredients that contain a "major food allergen" be specifically labeled with the name of the allergen source. Congress passed this law to make it easier for consumers who are allergic to foods and their caregivers to identify and avoid foods that contain major food allergens. The FDA enforces the provisions of this law in most packaged food products. This includes dietary supplements but does not include meat, poultry, and egg products (which are regulated by the U.S. Department of Agriculture); alcoholic beverages subject to Alcohol and Tobacco Tax and Trade Bureau labeling regulations; raw agricultural commodities; drugs; cosmetics; and most foods sold at retail or food service establishments that are not pre-packaged with a label.

Food Labels and Allergens

People with food allergies should read labels and avoid the foods they are allergic to. The law requires that food labels identify the food source of all major food allergens used to make the food. This requirement is met if the common or usual name of an ingredient already identifies that allergen's

food source name (for example, buttermilk). Otherwise, the allergen's food source must be declared at least once on the food label in one of two ways.

The name of the food source of a major food allergen must appear:

In parentheses following the name of the ingredient.
Examples: “lecithin (soy),” “flour (wheat),” and “whey (milk)”

— OR —

Immediately after or next to the list of ingredients in a “contains” statement.
Example: “Contains wheat, milk, and soy.”

FALCPA's labeling requirements extend to retail and food-service establishments that package, label, and offer products for human consumption. However, FALCPA's labeling requirements do not apply to foods that are placed in a wrapper or container (such as paper or a box for a sandwich) following a customer’s order at the point of purchase.

Consumers may also see advisory statements such as “may contain [allergen] or “produced in a facility that also uses [allergen].” These are used to address “cross-contact,” which can occur when multiple foods with different allergen profiles are produced in the same facility using shared equipment or on the same production line, as the result of ineffective cleaning, or from the generation of dust or aerosols containing an allergen.

FDA guidance for the food industry states that advisory statements should not be used as a substitute for adhering to current good manufacturing practices and must be truthful and not misleading.

Other Allergens or Allergenic Substances

More than 160 foods have been identified to cause food allergies in sensitive individuals. There are also several food ingredients that cause nonallergic hypersensitivity reactions in sensitive individuals that require specific labeling. For example, in addition to the eight major food allergens identified by law, the FDA monitors the food supply to determine if other allergens, food ingredients, or food additives pose a significant health risk and acts accordingly. Gluten, certain additives (e.g., yellow 5, carmine, sulfites), and emerging food allergens, such as sesame, are examples of other substances the FDA monitors and, in some cases, requires specific labeling for.

Gluten

Gluten describes a group of proteins found in certain grains (e.g., wheat, barley, and rye). In people with celiac disease, foods that contain gluten trigger an immune response that attacks and damages the lining of the small intestine. Such damage may not only limit the ability of celiac disease patients to absorb nutrients, leading to problems such as iron deficiency anemia, osteoporosis, and malnutrition, but it puts them at increased risk for potentially serious health problems, including intestinal cancers and autoimmune diseases such as diabetes. On August 2, 2013, the FDA issued a final rule defining "gluten-free" for food labeling, which helps consumers, especially those living with celiac disease, be confident that items labeled "gluten-free" meet a defined standard for gluten content. On August 12, 2020, the FDA issued a final rule to establish compliance requirements for fermented and hydrolyzed foods, or foods that contain fermented or hydrolyzed ingredients, bearing the "gluten-free" claim.

Color and Food Additives

Some individuals may have hypersensitivity reactions to a color additive. For example, FD&C Yellow No. 5, widely found in beverages, desserts, processed vegetables, drugs, makeup, and other products, may cause symptoms such as itching and hives in some people. The FDA requires all products containing FD&C Yellow No. 5 to identify it on their labels so consumers who are sensitive to the dye can avoid it. Color additives made from cochineal extract and carmine, which are derived from insects, have been identified as allergenic substances that must be declared on the label of all food and cosmetic products. Various sulfiting agents, including sodium bisulfite, are allowed as food ingredients. But due in part to adverse reactions to them, such as asthma in sensitive individuals, they

must be declared on food labels when present in food and the concentration in the food is ≥10 parts per million total sulfur dioxide.

Sesame

Because sesame is not one of the eight major food allergens, manufacturers do not have to list it as an allergen based on the requirements of FALCPA, although in most cases it must appear in the ingredient statement. An exception is when sesame is part of a natural flavoring or spice. Another exception is when sesame is not in the common or usual name of a food (e.g., tahini, which is made from sesame seeds). Because sesame allergies in the U.S. population appear to have increased and severe reactions have been reported, the FDA issued a Request for Information to gather additional information on the prevalence and severity of sesame allergies in the United States to determine if additional steps are needed. In November 2020, to help consumers who are allergic or sensitive to sesame to avoid these products, the FDA issued a draft guidance to encourage manufacturers to voluntarily declare sesame in the ingredient list when it is used as a "flavoring" or "spice" or when the common or usual name (such as tahini) does not specify sesame.

FDA Activities

The FDA takes several measures to make sure that consumers are protected from ingredients and foods they may be allergic to. These include establishing regulatory requirements, providing industry guidance, conducting surveillance, and taking regulatory actions when appropriate.

Guidance Documents and FDA Regulations

The FDA issues guidance documents to provide industry with its current thinking about various issues. Many FDA guidance documents contain information about allergens. Certain food safety regulations also contain provisions related to allergens and other ingredients that may cause sensitivities.

Inspections

The FDA's "Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food" rule (CGMP & PC rule, 21 CFR part 117) establishes requirements applicable to establishments that manufacture, process, pack, or hold human food. The CGMP & PC rule includes requirements for allergen preventive controls to prevent allergen cross-contact in

manufacturing and packaging and to prevent undeclared allergens. For example, the FDA requires facilities to put written procedures in place to control allergen cross-contact between products that contain allergens and those that are not supposed to contain them and to ensure that the products are accurately labeled with respect to allergens. The FDA inspects food manufacturers according to the applicable requirements of 21 CFR part 117 to determine whether allergen cross-contact has been minimized or prevented and whether a food facility has appropriate controls for allergen labeling.

Monitoring

The FDA monitors reports of food allergic reactions and reports related to ingredients and food hypersensitivities (including gluten) that come into the FDA Consumer Complaint System. The FDA looks at every complaint to determine the appropriate course of action. Based on an evaluation of the potential safety concern, the FDA may take regulatory action(s) to improve product safety and protect the public health, communicate new safety information to the public, or, in certain cases, remove a product from the market.

The FDA also receives reports from industry regarding undeclared allergens through the Reportable Food Registry (RFR). For example, from September 2009 to September 2014, about one-third of foods reported to the FDA through the RFR as serious health risks involved undeclared allergens. Of the major food allergens, milk represents the most common cause of recalls due to undeclared allergens. The five food types most often involved in food allergen recalls were bakery products, snack foods, candy, dairy products, and dressings (such as salad dressings, sauces, and gravies). Within the candy category, the FDA has received many reports of undeclared milk in dark chocolate products, highlighting this food type as a higher risk product for consumers allergic to milk.

Testing

The FDA conducts periodic surveys and sampling assignments to gather information about specific foods. For example, in 2013 and 2014, the FDA conducted a survey to estimate the prevalence of undeclared milk allergen in dark chocolate products. A second survey of samples collected in 2018 and 2019 was conducted to understand the extent to which dark chocolate bars and dark chocolate chips labeled as “dairy free” contained levels of milk that would be potentially hazardous to consumers with milk allergies. In 2015 and 2016, the FDA conducted sampling of a variety of foods to determine compliance with “gluten-free” labeling requirements.

To test for allergens in foods, the FDA uses enzyme-linked immunosorbent assay (ELISA) testing, through which antibodies attach to various allergens. The FDA tests food samples using two different types of ELISA kits before confirming the results. Other allergen testing methodologies include the DNA-based polymerase chain reaction and mass spectrometry. The FDA has developed the xMAP food allergen detection assay that can rapidly detect up to 14 allergens, including all major allergens except fish, with one sample. The FDA is currently evaluating the use of this new assay for future sampling assignments.

Regulatory Action

The FDA can carry out a number of regulatory actions if a food label lacks required allergen information for a food ingredient, if a food product is found to inadvertently contain a food allergen due to cross-contact, or if a food product does not qualify to be labeled as "gluten-free." The FDA considers such products misbranded or adulterated, depending on the circumstances, and subject to enforcement actions such as recalls, import refusal, and seizure. The agency may also issue warning letters to facilities making such foods, or may place foods imported from other countries on import alert for these violations. When there is a problem that justifies a recall, firms generally recall such food products from the marketplace voluntarily. Consumers can learn what products have been recalled recently on the FDA's website, or by signing upExternal Link Disclaimer to receive Recalls, Market Withdrawals and Safety Alerts emails.

What to Do If Symptoms of an Allergic Reaction Occur

Symptoms of food allergies typically appear from within a few minutes to a few hours after a person has eaten the food to which he or she is allergic. A severe, life-threatening allergic reaction is called anaphylaxis.

Symptoms of allergic reactions can include:

- Hives
- Flushed skin or rash
- Tingling or itchy sensation in the mouth
- Face, tongue, or lip swelling
- Vomiting and/or diarrhea
- Abdominal cramps

- Coughing or wheezing
- Dizziness and/or lightheadedness
- Swelling of the throat and vocal cords
- Difficulty breathing
- Loss of consciousness

People with a known food allergy who begin experiencing any of these symptoms should stop eating the food immediately, evaluate the need to use emergency medication (such as epinephrine) and seek medical attention. Some of these symptoms are not always due to a food allergen. So, it is important to seek proper care and diagnosis from a healthcare provider to determine if the symptoms or reaction experienced was due to a food allergen.

Reporting Adverse Reactions and Labeling Concerns

If you think that you or a family member has had an allergic reaction or injury that might be associated with a problem of having eaten a particular food product, discuss this with your healthcare provider. If a product has unclear labeling or you believe contains an allergen that isn't labeled, the FDA would like to know. Keep any food packages because they may contain important information. You may want to contact the manufacturer about the problem. Also, report the problem to the FDA in either of these ways:

Consumers and manufacturers can submit reports detailing product reactions or labeling concerns to an FDA Consumer Complaint Coordinator for the state where the food was purchased. You can also call FDA at 1-888-SAFEFOOD.

Consumers can submit a report using FDA's MedWatch Online reporting form for consumers.

Reports submitted to the FDA should include as much information as possible:

- Who is reporting the incident and who was affected? Please provide names, addresses, and phone numbers.
- The name and address of the place where the product was purchased
- A clear description of the reaction, including:
    - Date the reaction occurred.

- All symptoms experienced.
- How long after you ate or drank the product that the reaction occurred.
- Medications used to treat symptoms.
- Whether the reaction required further medical care, and if so, what kind. Please provide contact information for the doctor or hospital.

- A complete description of the product, including:
  - Date of purchase.
  - Any codes or identifying marks on the label or container, such as lot number, expiration date, and UPC code.
  - Photos of the product, label, ingredient statement, and lot code.

Reference:

https://www.fda.gov/food/food-labeling-nutrition/food-allergies

Microbiological Examination of Foods:

The following points highlight top seven methods for the microbiological examination of foods. The methods are:- 1. Indicator Organisms 2. Direct Examination 3. Cultural Techniques 4. Enumeration Methods 5. Alternative Methods 6. Rapid Methods for the Detection of Specific Organisms and Toxins 7. Laboratory Accreditation.

Method # 1. Indicator Organisms:

It may be necessary to carry out a microbiological examination of a food for one or more of a number of reasons. The determination of the microbiological quality of a food or food constituent may be required in order to estimate its shelf-life or its suitability for human consumption.

Although an estimate of the total viable count may be desirable, it is often more useful to obtain an estimate of the numbers of a particular component of the total flora such as moulds in a cereal, psychrotrophic bacteria in a product to be stored at low temperature, anaerobes in a vacuum- packed food, or yeasts in a fruit beverage. Or it may be required to determine that a food meets established microbiological criteria.

The total mesophilic plate count is widely used as an indication of the microbiological quality of foods unless they are known to contain large numbers of bacteria as a natural consequence of their preparation such as fermented milk and meat products.

A quite different reason for a microbiological examination of a food may be to determine the cause of spoilage or the presence of a pathogen where a food has been implicated in foodborne illness. The methods for determining an estimate of the total mesophilic count are very different from those required for demonstrating the presence of a pathogen, or its isolation for further study.

The isolation of specific pathogens, which may be present in very low but significant numbers in the presence of larger numbers of other organisms, often requires quite elaborate procedures.

It may involve enrichment in media which encourage growth of the pathogen while repressing the growth of the accompanying flora, followed by isolation on selective diagnostic media, and finally the application of confirmatory tests.

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Though microbiological criteria or the investigation of an outbreak of foodborne illness may often require the monitoring of certain products for specific pathogens, the difficulties associated with detecting low numbers of pathogens make it impracticable as a routine procedure to be applied without good cause.

An alternative to monitoring for specific pathogens is to look for an associated organism present in much larger numbers – an indicator organism. This is a concept developed originally for pathogens spread by the faecal-oral route in water and which has since been applied to foods, often rather uncritically.

A good indicator organism should always be present when the pathogen may be present, it should be present in relatively large numbers to facilitate its detection, it should not proliferate in the environment being monitored and its survival should be similar to that of the pathogen for which it is to be used as an indicator.

Escherichia coli is a natural component of the human gut flora and its presence in the environment, or in foods, generally implies some history of contamination of faecal origin. In water microbiology

in temperate climates E. coli meets these criteria very well and has proved a useful indicator organism of faecal pollution of water which may be used for drinking or in the preparation of foods.

There are, however, limitations to its use in foods where there appears to be little or no correlation between the presence of E. coli and pathogens such as Salmonella in meat, for example. Although E. coli cannot usually grow in water in temperate countries, it can grow in the richer environment provided by many foods.

Testing for E. coli can itself be relatively involved and a number of simpler alternatives are often used. These are less specific and therefore the relationship between indicator presence and faecal contamination becomes even more tenuous. Traditionally the group chosen has been designated the coliforms – those organisms capable of fermenting lactose in the presence of bile at 37 °C.

This will include most strains of E. coli but also includes organisms such as Citrobacter and Enterobacter which are not predominantly of faecal origin (Table 10.1).

The faecal coliforms, a more restricted group of organisms, are those coliforms which can grow at higher temperatures than normal, i.e. 44-44.5 °C and the methods developed for their detection were intended to provide rapid, reproducible methods for demonstrating the presence of E. coli without having to use time-consuming confirmatory tests for this species.

Faecal coliforms contain a higher proportion of E. coli strains and the test can be made even more specific for E. coli type 1 by including a test for indole production from tryptophan to exclude other thermo-tolerant coliforms. Further specificity can be introduced by using a medium diagnostic for β-glucuronidase activity; an enzyme possessed by most, but not all, strains of E. coli and relatively uncommon in other bacteria.

One criticism of using coliforms and faecal coliforms is that their absence could give a false reassurance of safety when lactose-negative organisms predominate. The lactose-negative organisms include not only Salmonella and Shigella, but also enteropathogenic strains of E. coli itself such as O124.

For this reason tests for the whole of the Enterobacteriaceae are increasingly being used. The Enterobacteriaceae includes even more genera of non-faecal origin than the coliforms, such as species of Erwinia and Serratia which are predominantly plant associated.

For this reason Enterobacteriaceae counts are used more generally as an indicator of hygienic quality rather than of faecal contamination and therefore say more about general microbiological quality than possible health risks posed by the product.

For instance, the presence of high numbers of Enterobacteriaceae in a pasteurized food would be cause for concern although it would not necessarily imply faecal contamination, and one would expect to find Enterobacteriaceae on fresh vegetables without the product necessarily being hazardous. The potential significance of genera of the Enterobacteriaceae is summarized in Table 10.1.

Table 10.1 *Significance of genera of the Enterobacteriaceae in the monitoring of foods*

Genus	*Predominantly faecal origin*	*Usually detected in 'coliform tests'*	*Typically enteropathogenic in humans*
Citrobacter	No	Yes	No
Edwardsiella	Yes	No	No
Enterobacter	No	Yes	No
Erwinia	No	No	No
Escherichia	Yes	Yes	No
Hafnia	No	No	No
Klebsiella	No	Yes	No
Proteus	No	No	No
Salmonella	Yes	No	Yes
Serratia	No	No	No
Shigella	Yes	No	Yes
Yersinia	Yes	No	No

These comments are generalizations and there are exceptions to most of them. Adapted from 'Micro-organisms in Foods 1: Their Significance and Methods of Enumeration'. 2nd Edition ICMSF, University of Toronto Press, 1978.

Some food microbiologists have tried to distinguish between 'indicator' organisms, which relate to general microbiological quality, and so-called 'index' organisms, which suggest that pathogens may be present. As will be apparent from the discussion above, this is not a simple distinction to make and the terminology has not been widely adopted.

Method # 2. Direct Examination:

When examining foods, the possibility of detecting the presence of micro-organisms by looking at a sample directly under the microscope should not be missed.

A small amount of material can be mounted and teased out in a drop of water on a slide, covered with a cover slip, and examined, first with a low magnification, and then with a x 45 objective. The condenser should be set to optimize contrast even though that may result in some loss of resolution.

Alternatively dark-field illumination or phase-contrast microscopy may be used. It is usually relatively easy to see yeasts and moulds and with care and patience it is possible to see bacteria in such a preparation.

The high refractive index of bacterial endospores makes them particularly easy to see with phase-contrast optics and, if the preparation is made as a hanging drop on the cover glass mounted over a cavity slide, it should also be possible to determine whether the bacteria are motile.

Since only a small sample of product is examined in this way, micro-organisms will not be seen unless present in quite large numbers, usually at least 10^6 ml^{-1}. In the case of some liquid commodities, such as milk, yoghurt, soups and fruit juices, it may be possible to prepare and stain a heat-fixed smear.
But the food constituents often interfere with the heat fixing and care is needed to prevent the smear being washed away during staining.

It may be necessary to dilute the sample with a little water, although that will reduce the concentration of micro-organisms further. The great advantage of such techniques is their rapidity, although in their simplest forms they do not distinguish between live and dead cells.

The direct epifluorescent filter technique or DEFT is a microscopy technique which has been applied to the enumeration of micro-organisms in a range of foods, although it was originally developed for estimating bacterial counts in raw milk. The technique was developed in response to the need for a rapid method for judging the hygienic quality of farm milks.

It achieves a considerably increased sensitivity over conventional microscopy techniques by concentrating bacteria from a significantly larger volume of sample by filtering it through a polycarbonate membrane filter.

The retained bacteria are then stained on the membrane with acridine orange and counted directly under the epifluorescence microscope. It may be necessary to pretreat the sample to allow filtration thus, for example, milk can be pretreated with detergent and a protease enzyme.

It is also essential to ensure that the bacteria are trapped in a single focal plane because of the limited depth of focus of the microscope at the magnifications required.

This is achieved by using a polycarbonate membrane where relatively uniform pores are produced following neutron bombardment of a plastic film, rather than cellulose acetate filters which have tortuous pores where bacteria will be held at different levels.

Acridine orange is a metachromatic fluorochrome, fluorescing either green or orange depending on the nature of the molecules within the cell to which it is bound. When bound to double-stranded DNA it fluoresces green but when bound to single-stranded RNA it fluoresces orange, as long as there is an adequate concentration of dye to saturate all the binding sites.

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Generally it is assumed that those cells which fluoresce yellow are viable while those that fluoresce green are non-viable. This is certainly not always true. The actual colour of an individual cell depends on many factors but, probably the most important is the concentration of acridine orange within the cell.

In many micro-organisms the integrity of the cell membrane restricts the passage of the dye into the cell and it is often the case that viable micro-organisms will fluoresce green and dead micro-organisms, in which the membrane is more leaky, will fluoresce orange.

Thus, although there are limitations to the use of acridine orange as a vital stain, the method has been adapted for the enumeration of micro-organisms in a range of food commodities including fresh meat and fish, meat and fish products, beverages and water samples.

In a modification of the technique, specific groups of micro-organisms can be enumerated. The membrane filter is incubated on an appropriate medium containing optical brighteners and the micro-colonies that develop on the membrane enumerated using the fluorescence microscope.

Method # 3. Cultural Techniques:

Although there is clearly a place for the direct examination of a food for microorganisms, the full microbiological examination usually requires that individual viable propagules are encouraged to multiply in liquid media or on the surface, or within the matrix, of a medium solidified with agar.

Agar is a polysaccharide with several remarkable properties which is produced by species of red algae. Although it is a complex and variable material, a major component of agar is agarose which is made of alternating units of 1,4-linked 3,6-anhydro-L-galactose (or L-galactose) and 1,3-linked D-galactose (or 6-0- methyl-D-galactose).

The properties of agar which make it so useful to microbiologists include the ability to form a gel at low concentrations (1.5-2%) which does not significantly influence the water potential of the medium. Such a gel is stable to quite high temperatures and requires a boiling water bath, or autoclave temperatures, to 'melt' it.

Once molten however, agar solutions remain liquid when cooled to relatively low temperatures (ca. 40 °C) making it possible to mix it with samples containing viable organisms before, or during, dispensing.

A further convenient property of agar is its stability to microbial hydrolysis, despite being a polysaccharide. Only a relatively small group of micro-organisms are able to degrade agar, presumably due to the presence of the unusual L-form of galactose in the polymer.

A very wide range of media are available to the microbiologist and details of their formulation, and how they are used, may be found in a number of readily available books and manuals. A selection of some commonly used media is listed in Table 10.2.

Table 10.2 *A selection of media commonly used in food microbiology*

Medium	*Use*
Plate Count Agar	Aerobic mesophilic count
MacConkey Broth	MPN of coliforms in water
Brilliant Green/Lactose/Bile Broth	MPN of coliforms in food
Violet Red/Bile/Glucose Agar	Enumeration of Enterobacteriaceae
Crystal Violet/Azide/Blood Agar	Enumeration of faecal streptococci
Baird–Parker Agar	Enumeration of *Staphylococcus aureus*
Rappaport–Vassiliadis Broth	Selective enrichment of *Salmonella*
Thiosulfate/Bile/Citrate/Sucrose Agar	Isolation of vibrios
Dichloran/18% Glycerol Agar	Enumeration of moulds
Rose Bengal/Chloramphenicol Agar	Enumeration of moulds and yeasts

The formulation of a medium will depend, not only on what group of organisms is being studied, but also on the overall purpose of the study; whether it be to encourage good growth of the widest possible range of organisms, to be selective or elective for a single species or limited group, to resuscitate damaged but viable propagules, or to provide diagnostic information.

General purpose media such as nutrient agar and plate count agar for bacteria, or malt extract agar and potato/dextrose agar for fungi, have evolved to provide adequate nutrition for the growth of non-fastidious, heterotrophic micro-organisms. They do not deliberately contain any inhibitory agents but they may nevertheless be selective because of the absence of specific nutrients required by more fastidious organisms.

Selective media contain one or more compounds which are inhibitory to the majority of organisms but significantly less so to the species, or group of species, which it is required to isolate.

It must be noted that all selective media, because they are based on the presence of inhibitory reagents, will generally be inhibitory to some extent to the organisms to be selected. If cells of the target organism have been subject to sub-lethal injury, then they may not be able to grow on the medium without a resuscitation step to allow them to repair.

Elective media on the other hand, are designed to encourage the more rapid growth of one species or group of micro-organisms so that they out-compete others even in the absence of inhibitory agents. Thus cooked-meat broth incubated at 43 to 45 °C allows rapid growth of Clostridium perfringens so that it may become the dominant organism after only 6-8 hours incubation.

The difference between selective and elective media must be seen from the viewpoint of the organism which it is desired to recover. By ensuring optimal growth in the elective medium for one organism, it is desirable that conditions are sub-optimal, or even inhibitory, to others.

A problem in the use of elective media is that growth of the desirable species may change the medium in a manner which now encourages the growth of other species. On the other hand a selective medium, if well designed, should remain inhibitory to unwanted organisms even when the organisms required are growing.

Resuscitation media are designed to allow the recovery of propagules which are sub-lethally damaged by some previous condition such as heat treatment, refrigeration, drying or exposure to irradiation. Such damaged micro-organisms may not only be more sensitive to inhibitory agents present in selective media, but may be killed if exposed to conditions encouraging rapid growth of healthy cells.

Typically resuscitation media are nutritionally weak and may contain compounds which will scavenge free radicals such as those which may be generated by the metabolism of oxygen.

Diagnostic media contain a reagent or reagents which provide a visual response to a particular reaction making it possible to recognize individual species or groups because of the presence of a specific metabolic pathway or even a single enzyme.

Many media used in practice combine selective reagents, elective components and diagnostic features. An interesting example is the Baird-Parker agar used for the presumptive isolation of Staphylococcus aureus. The selective agents are sodium tellurite and lithium chloride, the elective agents are sodium pyruvate and glycine and the diagnostic features are provided by the addition of egg yolk.

The production of black colonies due to the reduction of tellurite is characteristic of S. aureus as well as several other organisms able to grow on this medium such as other species of Staphylococcus, Micrococcus and some species of Bacillus.

The additional diagnostic feature shown by most strains of S. aureus is the presence of an opaque zone due to lecithinase activity surrounded by a halo of clearing due to proteolytic activity.

Method # 4. Enumeration Methods:

Plate Counts:

It has already been suggested that to count micro-organisms in a food sample by direct microscopy has a limited sensitivity because of the very small sample size in the field of view at the magnification needed to see micro-organisms, especially bacteria. In a normal routine laboratory the most sensitive

method of detecting the presence of a viable bacterium is to allow it to amplify itself to form a visible colony.

This forms the basis of the traditional pour plate, spread plate or Miles and Misra drop plate still widely used in microbiology laboratories. Table 10.3 compares the sample size examined and potential sensitivity of all these methods. In the pour plate method a sample (usually 1 ml) is pipetted directly into a sterile petri dish and mixed with an appropriate volume of molten agar.

Even if the molten agar is carefully, tempered at 40-45 °C, the thermal shock to psychrotrophs may result in them not producing a visible colony. The spread-plate count avoids this problem and also ensures an aerobic environment but the sample size is usually limited to 0.1 ml.

Table 10.3 *A comparison of the sensitivity of methods of enumeration*

Method	*Volume of sample (ml)*	*Count (cfu g^{-1}) corresponding to a single organism or colony seen**
Direct microscopy	5×10^{-6}	2×10^{6}
Miles and Misra	0.02	5×10^{2}
Spread plate	0.1	10^{2}
Pour plate	1.0	10
MPN	3×10.0 $+ 3 \times 1$ $+ 3 \times 0.1$	0.36

* Based on a 10^{-1} dilution of a sample obtained by, for example, stomaching 1g (or ml) of food with 9 ml of diluent.

In a thoroughly mixed suspension of particles such as micro-organisms, the numbers of propagules forming colonies on replicate plates is expected to have a Poisson distribution, a property of which is that the variance is equal to the mean i.e.

$\bar{X} = \text{var} = s^2$ ……..(10.1)

A consequence of this is that the limiting precision of a colony count is dependent on the number of colonies counted. The 95% confidence limits (CL) can be estimated as approximately

$2_s = 2\sqrt{x}$ ……..(10.2).

(if x is the count on a single plate and has to be our estimate of the mean). Thus for a plate with only 16 colonies, the 95% CL would be approximately $\pm$50% (i.e. we would have 95% confidence that the count lies between 8 and 24). For a count of 30, it would be $\pm$37% and for a count of 500 only $\pm$9%.

However if the number of colonies on a plate was as high as 500, it would not only be difficult to count them accurately, but such a crowded plate is likely to result in many colony-forming units never forming a visible colony leading to an underestimate. Thus it is widely accepted that reasonably accurate results are obtained when plates contain between 30 and 300 colonies.

To obtain plates with this number of colonies it is often necessary to dilute a sample before enumeration. The most widely used dilution technique is the ten-fold dilution series.

With a completely unfamiliar sample it is necessary to plate-out a number of dilutions to ensure that some plates are obtained with counts in the desired range, but with experience of a particular material plating only one dilution may be sufficient.

The diluent used must not cause any damage, such as osmotic shock, to the micro-organisms. Sterile distilled water is therefore unsuitable. A commonly used diluent, known as maximum recovery diluent, contains 0.1% peptone and 0.85% sodium chloride.

Traditional plate counts are expensive in Petri dishes and agar media, especially if adequate replication is carried out, and the Miles and Misra drop count and spiral plater have been developed to reduce this. In the Miles-Misra technique materials are conserved by culturing a smaller volume of each dilution, usually 20 µl.

This way a number of dilutions can be grown on a single plate by dividing it into sectors each representing a different dilution. The spiral plater employs a mechanical system which dispenses 50 µl of a liquid sample as a spiral track on the surface of an agar plate. The system is engineered so that most of the sample is deposited near the centre of the plate with a decreasing volume applied towards the edge.

This produces an effect equivalent to a dilution of the sample by a factor of 10^3 on a single plate, thus producing a two-thirds' saving on materials as well as saving the time required in preparing and plating extra dilutions.

After incubation, colonies are counted using a specially designed grid which relates plate area to the volume applied, thus enabling the count to be determined. The system is not suitable for all food samples though, as particulate material can block the hollow stylus through which the sample is applied.

The limit in sensitivity of the traditional plate count arises from the small volumes used and clearly, the sensitivity can be increased by increasing the volume size and the number of replicate counts or plates. It may be possible to filter a larger volume through a membrane, which retains the viable organisms, and then lay the membrane onto an appropriate medium.

In all of these methods of enumeration it is essential to appreciate the statistical background to sampling and to recognize that extrapolations from colony counts depend on several assumptions that may not be justified.

Thus a colony may not be derived from a single micro-organism, but from a clump of micro-organisms, and the material being examined may not be homogeneous so that the subsample actually studied is not representative of the whole.

Most Probable Number Counts:

An alternative method of enumerating low numbers of viable micro-organisms is that referred to as the Most Probable Number (MPN) method. The method is usually based on inoculating replicate tubes of an appropriate liquid medium (usually 3, 4 or 5) with three different sample sizes or dilutions of the material to be studied (e.g. 10 g, 1.0 g and 0.1 g).

The medium used has to be designed to make it possible to decide whether growth or no growth has occurred and the number of positives at each sample size or dilution is determined after incubating the tubes. The MPN is obtained by referring to a table such as that shown in Table 10.4.

There are computer programmes for generating MPN values from different designs of the experiment and these programmes can also provide confidence limits for the MPN and suggest what the likelihood of particular combinations of positive results should be.

Table 10.4 *A selection of MPN values**

Number of positive tubes	*MPN*	*95% Confidence Limits*
0 0 0	<0.30	
1 0 0	0.36	0.02 to 1.7
2 0 0	0.92	0.15 to 3.5
2 1 0	1.5	0.4 to 3.8
3 0 0	2.3	0.5 to 9.4
3 1 0	4.3	0.9 to 18.1
3 1 1	7.5	1.7 to 19.9
3 2 0	9.3	1.8 to 36
3 2 1	15	3.0 to 38
3 3 0	24	4.0 to 99
3 3 1	46	9.0 to 198
3 3 2	110	20.0 to 400
3 3 3	>110	

* Based on 3 × 1 g(ml) + 3 × 0.1 g(ml) + 3 × 0.01 g(ml) samples (expressed as organisms per 1 g)

A modern variation on the MPN theme is the use of the hydrophobic grid membrane filter (HGMF). A sample is filtered through the HGMF which is divided by a hydrophobic grid into a number (normally 1600) of small cells or growth compartments.

After incubation of the filter on an appropriate medium, each of these cells is scored for growth or no-growth. This can be done either manually or automatically and the count in the original sample determined as equivalent to a single dilution MPN using 1600 tubes.

The enumeration of micro-organisms assumes that there are distinctive propagules to be counted. This is acceptable for single-celled organisms such as the majority of bacteria or yeasts but in the case of filamentous fungi there may be a problem in interpreting the significance of numbers of colony- forming units.

To assess the quantity of fungal biomass in a food commodity may require quite different techniques.

One possibility is to make a chemical analysis for a constituent which is specifically associated with fungi, such as chitin, which is a constituent of the cell walls of zygomycetes, ascomycetes, basidiomycetes and deuteromycetes (also present in insect exoskeleton), or ergosterol which is a major constituent of the membranes of these groups of fungi.

Some moulds, such as species of Aspergillus and Penicillium associated with the spoilage of cereals, produce volatile metabolites such as methyl-furan, 2- methyl-propanol, 3-methyl-butanol and oct-1-en-3-ol (this last compound having a strong mushroomy smell). It would be possible to detect and analyse these compounds in the head-space gases of storage facilities using gas-liquid chromatography.

Method # 5. Alternative Methods:

Cultural methods are relatively labour intensive and require time for adequate growth to occur. Many food microbiologists also consider that the traditional enumeration methods are not only too slow but lead to an overdependence on the significance of numbers of colony-forming units.

Food manufacturers require information about the microbiological quality of commodities and raw materials rapidly and it could be argued that an assessment of microbial activity is as important as a knowledge of numbers. A number of methods have been developed which aim to give answers more quickly and hence are often referred to as ‘Rapid Methods’.

Dye-Reduction Tests:

A group of tests which have been used for some time in the dairy industry depend on the response of a number of redox dyes to the presence of metabolically active micro-organisms. They are relatively simple and rapid to carry out at low cost. The redox dyes are able to take up electrons from an active biological system and this results in a change of colour.

Usually the oxidized form is coloured and the reduced form colourless but the triphenyltetrazolium salts are an important exception. Figure 10.1 shows the structures of the oxidized and reduced forms of the three most widely used redox dyes, methylene blue, resazurin and triphenyltetrazolium chloride.

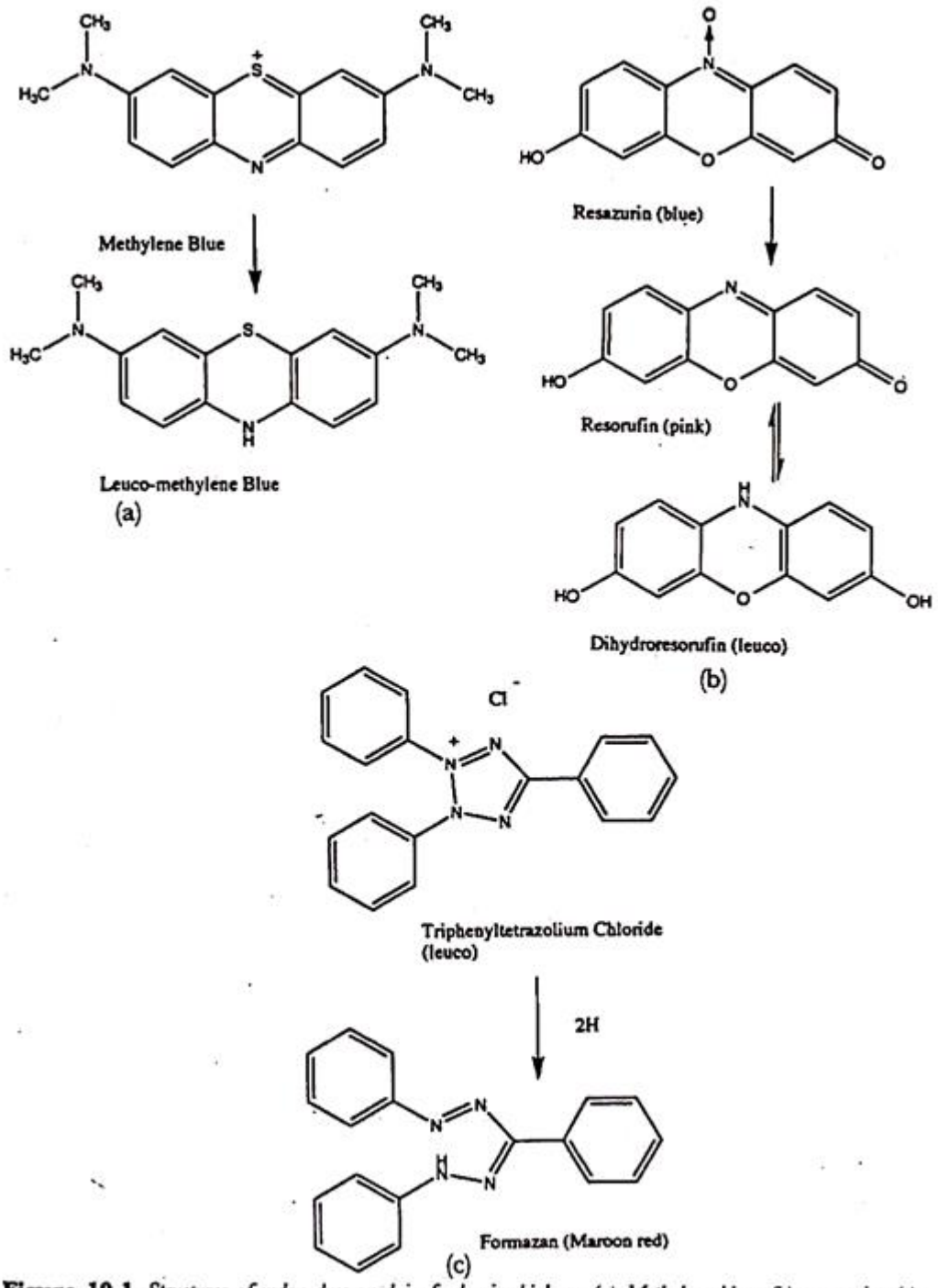


Figure 10.1 *Structures of redox dyes used in food microbiology. (a) Methylene blue, (b) resazurin, (c) triphenyltetrazolium chloride*

From 1937, and until relatively recently, the methylene blue test was a statutory test for grading the quality of milk in England and Wales. Changes in the technology of handling bulk milk, especially refrigeration, have made this test less reliable and it is no longer a statutory test because results show little correlation with the numbers of psychrotrophic bacteria.

Because the reduction of resazurin takes place in two stages, from blue to pink to colourless, there is a wider range of colour that can be scored using a comparator disc and the ten-minute resazurin test

is still useful for assessing the quality of raw milk at the farm or dairy before it is bulked with other milk.

Triphenyltetrazolium salts and their derivatives are initially colourless and become intensely coloured, and usually insoluble, after reduction to formazans. Triphenyltetrazolium chloride itself is most widely used as a component of diagnostic and selective agar media on which some bacterial colonies will become dark red to maroon as formazan becomes precipitated within the colony.

The crystals of the formazan produced from 2-(*p*-iodo-phenyl)-3-(*p*– nitro-phenyl)-5-phenyl-tetrazolium chloride (INT) are so intensely coloured that they are readily seen in individual microbial cells under the microscope and their presence may be used to assess the viability of cells. One possible development would be the incorporation of INT as part of the staining procedure in the DEFT analysis.

Electrical Methods:

When micro-organisms grow, their activity changes the chemical composition of the growth medium and this may also lead to changes in its electrical properties. Measuring this effect has become the basis of one of the most widely used alternative techniques of microbiological analysis.

The electrical properties most frequently monitored are conductance (G), capacitance (C) and impedance (Z), the latter being influenced by both capacitance and resistance (R) as well as the frequency of the alternating current applied (f). The conductance is simply the reciprocal of resistance, i.e.

$G = 1/R$ ……..(10.3)

The relationship between impedance, resistance and capacitance is given by:

$Z^2=X^2 + (1/2\pi fC)^2$ ……..(10.4)

It is possible to take frequent measurements of the electrical properties of a growth medium by growing organisms in cells supplied with two metal electrodes. By saving the data obtained for subsequent analysis on a computer, large numbers of samples can be monitored at the same time.

Central to the successful application of the method is the choice or design of a medium which will both support rapid growth of the micro-organisms to be monitored, and will change its electrical properties as a result of their growth.

With a suitable medium, the traces obtained resemble the bacterial growth curve, although this analogy can be misleading as the curves are not superimposable. In practice it requires quite a large number of bacteria to initiate a signal, usually about 10^6-10^7 cfu ml^{-1} in the cell.
The time it takes to reach this number and produce a signal (referred to as the detection time) will therefore include any lag phase plus a period of exponential growth and will depend on both the initial number and the growth rate.

Thus, for a particular organism/medium/temperature combination, the detection time will be inversely related to the logarithm of the number and activity of the organisms in the original sample.

Figure 10.2 shows typical traces of samples taken from a ten-fold dilution series of a pure culture. In the case of Escherichia coli growing in brain/heart infusion broth, incubated at 37 °C, it is possible to detect the presence of one or two viable cells in five or six hours.

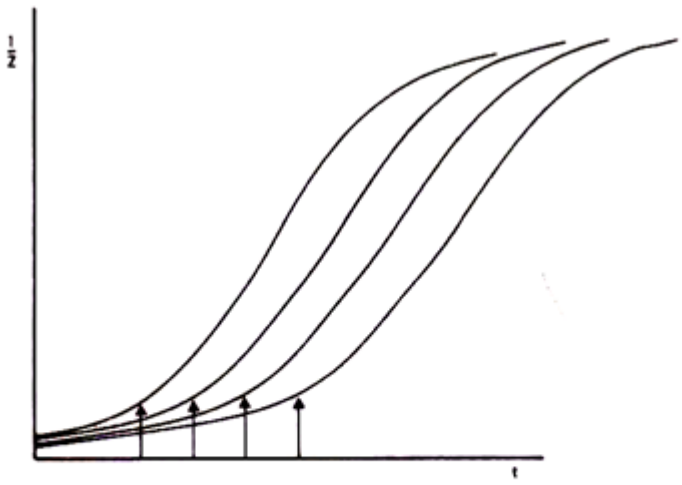


Figure 10.2 *Traces of 1/Z against time of a ten-fold dilution series of* Escherichia coli *growing in brain/heart infusion broth at 37 °C. The detection times are marked with arrows*

By obtaining detection times using samples where the microbial population is known, calibration curves relating detection time and microbial numbers can be drawn so that count data can be derived from detection times. One such example for Salmonella enteritidis is presented in Figure 10.3.

Some claim that the only value in converting detection times to counts is that the food microbiologist derives a sense of security from having data in a familiar form. Since electrical methods measure microbial activity directly, detection time may be a more appropriate measure of the potential to cause spoilage than a viable plate count.

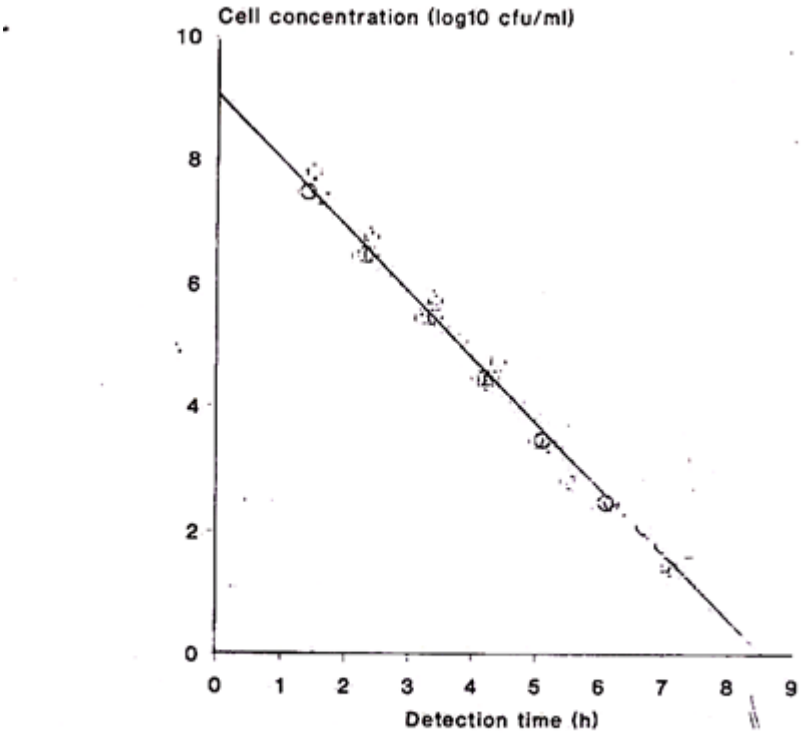


Figure10.3 *A calibration curve for* Salmonella enteritidis *using a Bactometer*

In the food industry the potential for simultaneously testing many samples makes electrical methods a useful means for assessing the quality of raw materials and products. They have the additional advantage that the worse the microbiological quality, the shorter is the detection time, and the sooner the manufacturer knows that there may be a problem.

In modern instruments, which can accommodate more than 500 samples, the results can be displayed using an unambiguous quality colour code of acceptable (green), marginally acceptable (orange), and unacceptable (red).

By carefully designing the medium to contain selective agents, and diagnostic compounds which will give a strong signal when they are metabolized, it is possible to use electrical methods to estimate the activity (and hence, by calibration, numbers) of specific groups of organisms.

Thus the incorporation of tri-methylamine oxide (TMAO) into a selective enrichment broth can be used as a pre-screening for the presence of Salmonella.

The presence of the enzyme TMAO reductase converts this neutral molecule into the strongly charged trimethyl-ammonium ion which has a considerable effect on the conductance of the medium. Absence

of a detection time in control sample cells inoculated with a Salmonella-specific phage act as further confirmation of the presence of Salmonella.

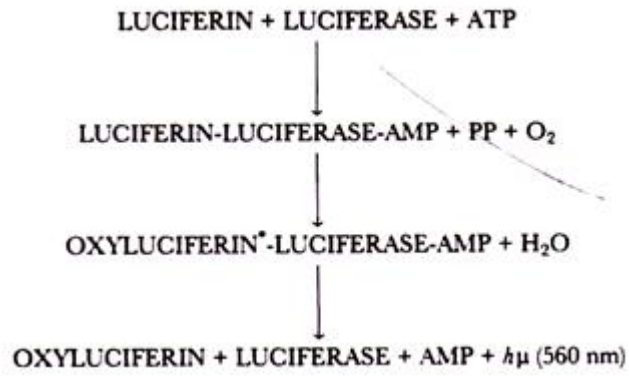
ATP Determination:

Adenosine triphosphate is found in all living cells and is the universal agent for the transfer of free energy from catabolic processes to anabolic processes. A number of quite different living organisms have evolved a mechanism for producing light by the activity of enzymes known as luciferases on substrates known as luciferins.

These reactions require the presence of ATP and magnesium ions and produce one photon of light at the expense of the hydrolysis of one molecule of ATP through a series of intermediates. An ATP molecule facilitates the formation of an enzyme- substrate complex which is oxidized by molecular oxygen to an electronically excited state.

The excited state of the molecule returns to the lower energy ground state with the release of a photon of light and dissociates to release the enzyme luciferase again.

These reactions are summarized below:



Because instruments are now available which can accurately measure low levels of light emission and pure luciferin and luciferase from, for example, fireflies can be manufactured, the reaction can be used as a very sensitive assay for ATP. Sensitive instruments using photomultiplier tubes can detect as little as 10^2-10^3 fg (fg = femtogram = 10^{-15} g) which corresponds to as few as 10^2—10^3 bacterial cells.

Although the method gives very good results with pure cultures, when applied to foods it is essential to ensure that non-microbial ATP, which will be present in foods in considerably larger quantities than the microbial ATP, has been destroyed or that the micro-organisms have been separated from interfering food components.

Non-microbial ATP can be selectively removed by treating with an ATPase after disrupting the somatic cells of animal and plant origin with a mild surfactant. The next stage is to destroy the ATPase activity and then extract the microbial ATP using a more powerful surfactant.

The alternative, of removing microbial cells from the food before the ATP assay, can be achieved by centrifugation or filtration of liquid foods but is very much more difficult from suspensions of solid foods. Even when they are successfully separated there are problems arising from the different amounts of ATP in different microbial cells.

Thus yeast cells may contain 100 times more ATP than bacterial cells and sub-lethally stressed micro-organisms may contain very low levels of ATP and yet be capable of recovering and growing on a food during long-term storage.

A method for removing specific groups of bacteria, which is being actively researched, is to use antibodies or lectins attached to magnetic beads. Lectins are a class of plant proteins which recognize and bind to specific carbohydrate residues which may be exposed on the outer surface of a micro-organism.

The bacteria adhere to the beads via the antibody-antigen, or lectin-carbohydrate, reaction and can be removed from the food suspension by a powerful magnet acting through the walls of the container. After the food materials have been poured away and the cells washed they can be released into suspension for assay by removing the magnet.

This need for often complex sample preparation has meant that, rapid and sensitive though it is, ATP measurement is not widely used for routine monitoring of microbial contamination of foods.

It is however being increasingly used to monitor hygiene in food-processing operation plant. Instruments are now available where a swab taken from equipment can be assayed directly for ATP giving a virtually immediate measure of surface contamination.

In these cases it is not necessary to distinguish between microbial and non-microbial ATP since the presence of either at high levels would indicate poor hygiene. This speed and simplicity make ATP determination the most overtly microbiological test that can be applied for the routine-monitoring of critical control points as part of the HACCP technique of quality assurance (see Section 11.6).

Method # 6. Rapid Methods for the Detection of Specific Organisms and Toxins:

Immunological Methods:

Because of the potential specificity of immunoassays using polyclonal or monoclonal antibodies, there has been considerable effort devoted to developing their application in food microbiology. Commercial immunoassay kits are now available for detecting a variety of foodborne micro-organisms and their toxins, including mycotoxins.

Raising antibodies to specific surface antigens of micro-organisms, or to macromolecules such as staphylococcal or botulinum toxins, is relatively straightforward and can be achieved directly. Mycotoxins, however, belong to a class of molecules known as haptens which can bind to an appropriate antibody but are of relatively low molecular weight and are not themselves immunogenic.

Haptens can be made immunogenic by binding them chemically to a carrier protein molecule, and antibodies have now been raised using this technique to a wide range of mycotoxins including the aflatoxins, trichothecenes, ochratoxin and fumonisins.

Although a number of different formats are used in immunoassays, their essential feature is the binding of antibody to antigen. A commonly used protocol is that of the sandwich ELISA (enzyme-linked immunosorbent assay) in which a capture antibody is immobilized on a solid surface of say a microtitre plate well.

The sample containing antigen is then added to the well, mixed and removed leaving any antigens present attached to the antibodies.

These are then detected lay adding a second antibody which is coupled to an enzyme such as horseradish peroxidase or alkaline phosphatase. This antibody will also bind to the antigen producing an antibody sandwich. Binding is detected by addition of a chromogenic substrate for the enzyme attached to the second antibody and measuring the colour developed (Figure 10.4).

Alternative detection systems are used, such as attachment of antibodies to latex and looking for agglutination in the presence of the antigen and fluorescence-labelled antibodies which can be used to detect target organisms using a fluorescence microscope or flow cytometry.

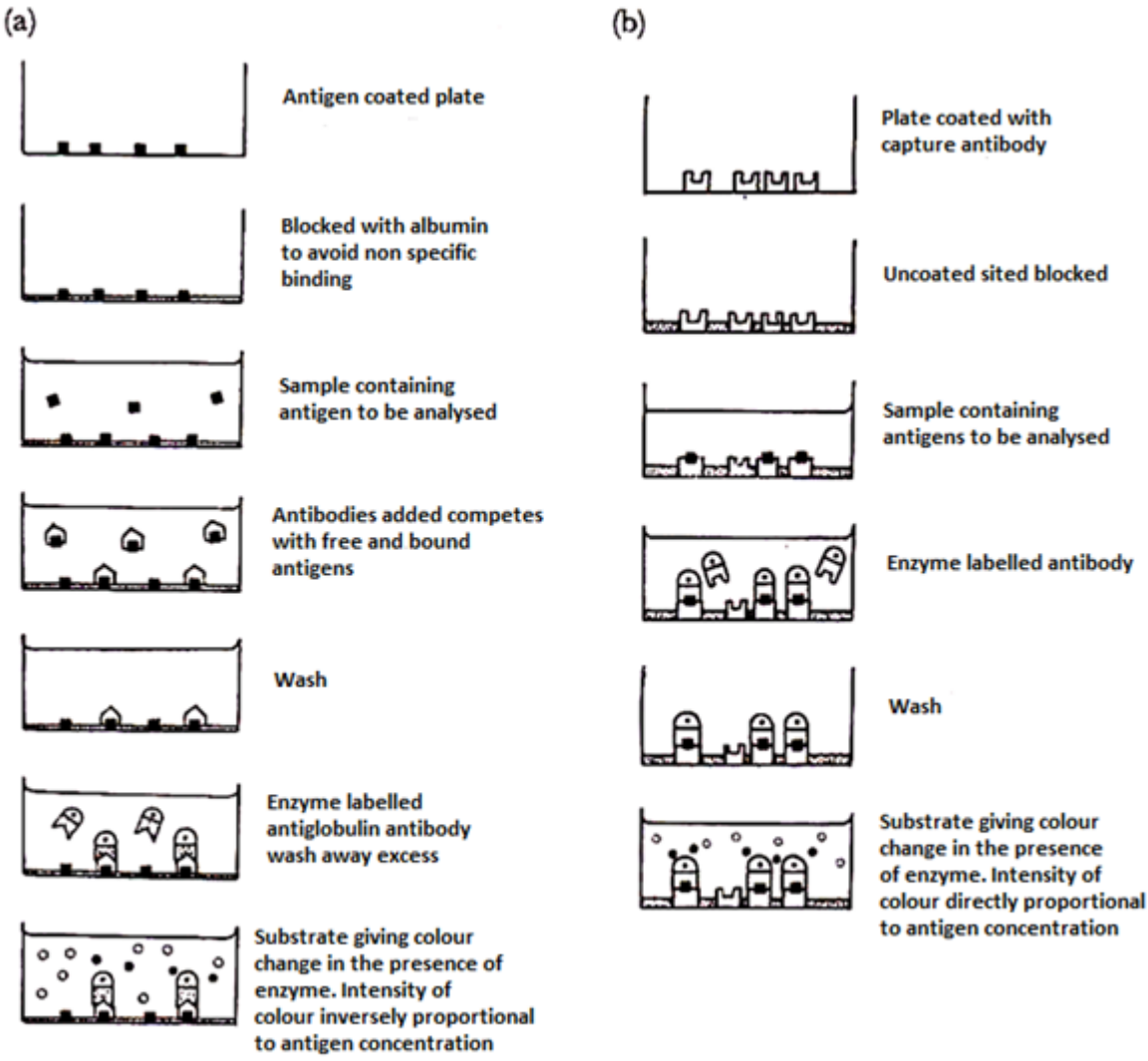


Figure 10.4 *Sandwich ELISAs. (a) Competitive sandwich ELISA, (b) direct sandwich ELISA*

Commercial ELISAs are available for such organisms as Salmonella and Listeria monocytogenes but they still require the presence of at least 10^5— 10^6 organisms.
Detection of smaller numbers therefore depends on some form of enrichment or concentration by one of the separation methods briefly mentioned above, so that although the immunoassay itself may be rapid the whole analytical protocol may take almost as long as conventional procedures.

Some advantage can be gained from the automation of the assay and a number of instruments are commercially available. There may also be some concern over the specificity of immunoassays.

While striving for antibodies that are sufficiently broad in their specificity to recognize all strains of the desired target organism, it is difficult to avoid the problem of cross reactivity with organisms other than those under investigation.

DNA/RNA Methodology:

All biochemical, immunological and other characteristics used in the detection of micro-organisms are governed directly or indirectly by the base sequences encoded in the organism's genome. The specificity of this information can now be mobilized to provide methods capable of identifying genera, species or even strains within a species.

Nucleic acid probes can be designed which recognize and bind (hybridize) to specified regions of either chromosomal or plasmid DNA or to RNA, and the region chosen to give the desired level of specificity. Thus, for example, ribosomal RNA contains both conserved and variable regions, the former being suitable for recognition at the genus level whereas the latter may be considerably more specific.

Although RNA is a more labile molecule than DNA, there are many more copies of ribosomal RNA in a cell than genomic DNA which should make methods based on this molecule more sensitive.

The nucleic acids have to be released from the cells by some form of lysis and, in the case of double-stranded DNA, it has also to be denatured, usually by heat treatment, to the single-stranded form. The denatured nucleic acid is then adsorbed onto a membrane, fixed to it by heat or alkali treatment, and the membrane is treated with some form of blocking agent to prevent nonspecific binding of the probe.

After incubating with the labelled probe and washing off un-adsorbed probe, the presence of the hybridization product is measured using the label attached to the probe. In the earliest stages of the development of this methodology probes were directly labelled with radioactive isotopes such as ^{32}P or ^{35}S and hybridization was detected by autoradiography.
This is a very sensitive method but the routine use of radioactive compounds in a food-associated environment is not usually acceptable. Probes can be labelled with an enzyme and detected with a chromogenic substrate or they can be labelled with a small molecular weight hapten for which an enzyme-linked monoclonal antibody is available.

Such probes are available for the enterotoxin gene of Staphylococcus aureus, the haemolysin gene and rRNA of Listeria monocytogenes, 23S rRNA of Salmonella, as well as several other systems. One interesting example is a ribosomal RNA probe to detect Listeria monocytogenes which uses a chemiluminescent label.

The single-stranded DNA probe has a chemiluminescent molecule bound to it. When the probe binds to its RNA target, the chemiluminescent molecule is protected from degradation in a subsequent step so that successful hybridization is indicated by light emission measured in a luminometer (Figure 10.5).

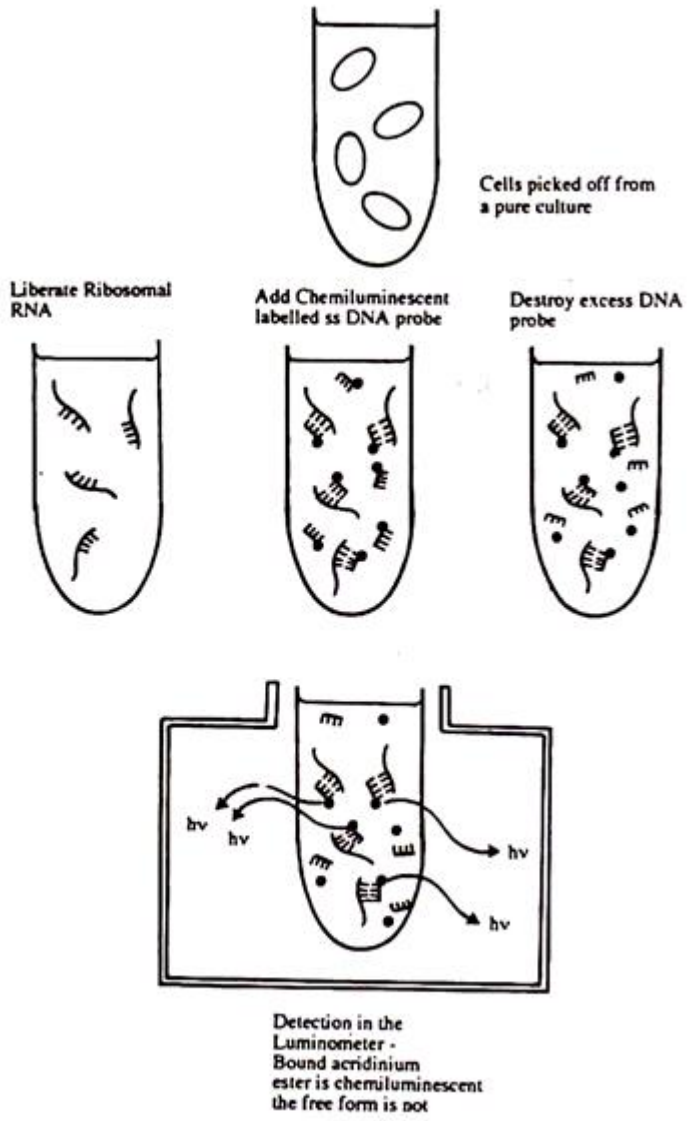


Figure 10.5 *Hybridization protection assay*

Like the ELISA methods, nucleic acid methods also require some enrichment of the target to produce sufficient nucleic acid to reach the threshold of sensitivity of about 10^6 copies of the target sequence. They are particularly well suited for rapid confirmation of isolated colonies on an agar plate.

The polymerase chain reaction (PCR) provides a method for amplifying specific fragments of DNA and in principle could allow detection of a single copy of the target sequence.

The method depends on the availability of two short oligonucleotide primer sequences (usually about 20 nucleotides long) which will hybridize to opposite strands of heat-denatured DNA at either end of the region which will eventually be probed (Figure 10.6).

A DNA polymerase then catalyses extension of the primers to produce two double-stranded copies of the region of interest. The whole process is recycled and the two copies become four, eight, sixteen and so on. Thus by cycling 25 times approximately 10^6 copies can be generated from the original.

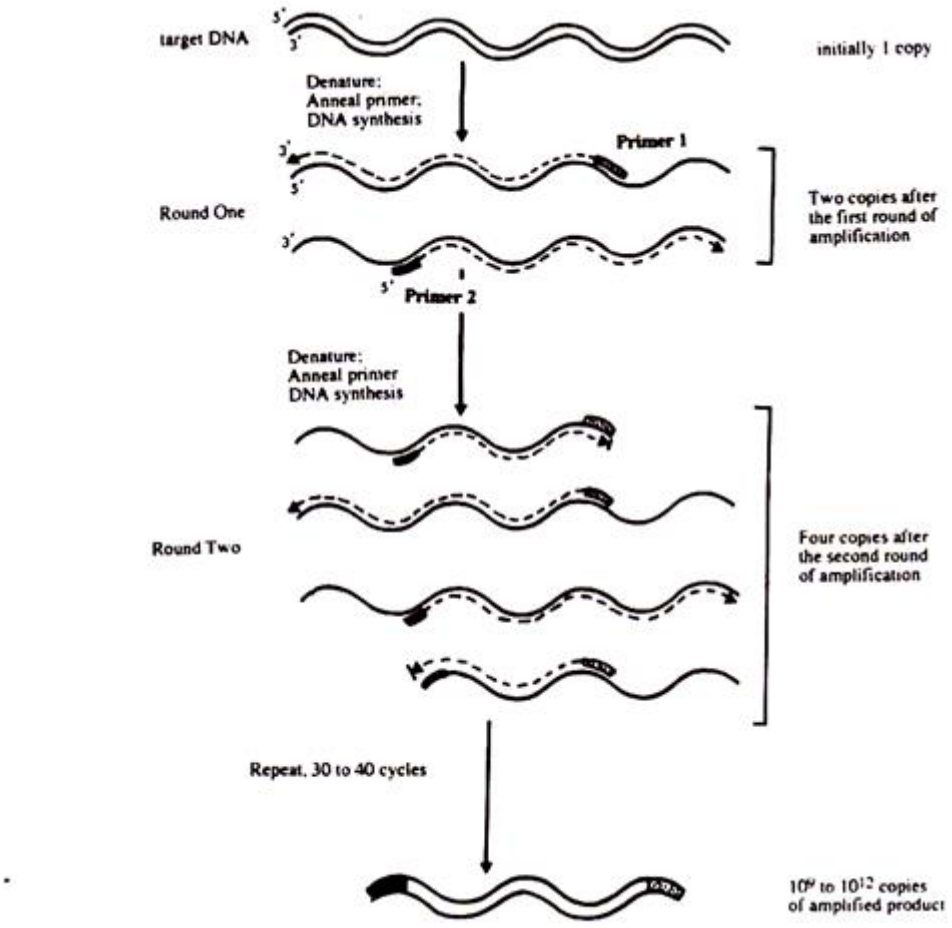


Figure 10.6 *The polymerase chain reaction*

Equipment is commercially available for the precision automated thermal cycling necessary for the routine use of this method. Also essential to the success of the procedure is a heat stable DNA polymerase which will survive the DNA denaturation step to catalyse subsequent extension of the primers. This, known as Taq polymerase, is obtained from the very thermophilic bacterium Thermus aquaticus.

The method is not however without disadvantages such as the sensitivity of the PCR reaction to inhibition by food components. Because it does not depend on enrichment or isolation of colonies, it

does not distinguish between living or dead cells and organisms killed by a heat process, for example, could still be detected.

Nevertheless this, and other nucleic acid-based amplification methods, offer enormous potential for detecting the presence of small numbers of significant microorganisms in a few hours and research continues to refine them.

Method # 7. Laboratory Accreditation:

From what has already been said it should be clear that there can be a number of different ways of detecting the same organism in a food matrix. The choice of method used can be governed by several factors and the relative merits of different methods is a topic of constant investigation and debate.

This can however lead to the situation where differences in a result reported by two laboratories simply reflect the different method used.

In addition to problems arising from intrinsic differences in the performance of different methods, the same method in different laboratories can be subject to variation introduced by factors such as differences in procedures, equipment and its calibration.

Some possible examples would include autoclave temperature profile when sterilizing media, time and temperature of incubation, sources of medium components and, of course, competence and experience of laboratory personnel.

A number of approaches are adopted to avoid such potential problems. Several national and international bodies approve standard methods for conducting certain analyses and one of these should be adopted for routine work and strictly adhered to wherever possible.

Testing laboratories also often participate in quality assurance schemes where a central body distributes standard samples for analysis, often specifying the precise time this should be conducted and the method to be used.

Results are reported back, collated and a report circulated to participating laboratories which can then judge their performance against that of others. Finally, laboratories can seek some form of independent recognition.

There are quality systems such as the Good Laboratory Practice scheme and standards such as BS 5750 (ISO 9000) which are concerned with the quality of management within the organization but which do not set a particular level of quality or competence to be achieved.

There are also schemes of laboratory accreditation more concerned with the quality of performance in specific tests. In the UK this accreditation is usually sought through the National Measurement Accreditation Service (NAMAS) which accredits laboratories over a whole range of activities not just microbiological testing.

Most countries have their own equivalent organization such as NATA (Australia), DANAK (Denmark), ILAB (Ireland), and STERLAB (The Netherlands). The accrediting body inspects the laboratory and its procedures to ensure that tests are carried out consistently and correctly using approved methods with suitable quality control measures in place.

Among the features investigated are the training and qualifications of staff, the suitability of equipment and procedures for its calibration and maintenance, participation in a proficiency testing scheme and the presence of full documentation prescribing the laboratory's operating procedures.

Obtaining laboratory accreditation can be a costly exercise but, if achieved, provides independent testimony to a laboratory's proficiency and will give increased confidence to potential customers.

Reference:

https://www.biologydiscussion.com/food-microbiology/microbiological-examination-of-foods-7-methods/59581

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – IV – Food Biotechnology – SBB2203

1. FERMENTED MILK AND OTHER PRODUCTS

Fermented Milk Products

Overview

The primary function of fermenting milk was, originally, to extend its shelf life. With this came numerous advantages, such as an improved taste and enhanced digestibility of the milk, as well as the manufacture of a wide variety of products. Historically the fermentation of milk can be traced back to around 10,000 B.C. It is likely that fermentation initially arose spontaneously from indigenous microflora found in milk. Fortunately, the bacteria were lactococci and lactobacilli which typically suppress spoilage and pathogenic organisms effectively. The evolution of these products likely came as a result of the climate of the region in which they were produced: thermophilic lactic acid fermentation favours the heat of the sub-tropics; mesophilic lactic acid fermentation occurs at cooler temperatures. Today the fermentations are controlled with specific starter cultures and conditions. Some of the many fermented milk products are: acidophilus milk, crème fraîche, cultured buttermilk, kefir, koumiss, filmjölk, sour cream, and viili. Yogurt and cheese are also fermented milk products. More detail on yogurt and cheese can be found under their specific ingredient profiles.

Fermented milk products can be classified into 3 categories:

- viscous products
- beverage products
- carbonated products

Within these categories, the fermented milk products may be fresh, or have an extended shelf life. The fresh products contain live starter culture bacteria, including probiotics, while the extended shelf life products contain no live microorganisms.

Foods from Microorganisms

Microorganisms are widely used in the food industry to produce various types of foods that are both nutritious and preserved from spoilage because of their acid content.

Dairy foods. In the dairy industry, many products result from fermentation by microorganisms in milk and the products of milk. For example, **buttermilk** results from the souring of low-fat milk by lactic acid. The flavor is due to substances such as diacetyl and acetaldehyde, which are produced by species of *Streptococcus*, *Leuconostoc*, and *Lactobacillus* as they grow.

A fermented milk product with a puddinglike consistency is **yogurt.** Two bacteria, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, are essential to its production. After the milk has been heated to achieve evaporation, the bacteria are added, and the condensed milk is set aside at a warm temperature to produce the yogurt. **Sour cream** is produced in **a** similar way, using cream as **a** starter material.

The protein portion of the milk, the casein, is used to produce cheese and cheese products. Precipitated from the milk, the protein **curd** is **an unripened cheese**such as cottage cheese. The leftover liquid, the whey, can be used to make cheese foods.

When the cheese is allowed to ripen through the activity of various microorganisms, various cheeses are produced. **Soft cheeses**, such as Camembert, do not spoil rapidly. Camembert cheese is a product of the growth of the fungus *Penicillium camemberti*. **Hard cheeses** have less water and are ripened with bacteria or fungi. Swiss cheese is ripened by various bacteria, including species of *Propionibacterium*, which produces gas holes in the cheese. Bleu cheese is produced by *Penicillium roqueforti*, which produces veins within the cheese as it grows.

Other fermented foods. Other fermented foods are also the product of microbial action. **Sauerkraut**, for example is produced by *Leuconostoc* and *Lactobacillus* species growing within shredded cabbage. Cucumbers are fermented by these same microorganisms to produce **pickles.**

Bread. Bread is still another product of microbial action. Flour, water, salt, and yeast are used to make the dough. The **yeast** most often used is *Saccharomyces cerevisiae*. This organism ferments the carbohydrates in the dough and produces carbon dioxide, which causes the dough to rise and creates the soft texture of bread. Unleavened bread is bread that contains no yeast. Sourdough bread can be made by using lactic acid bacteria to contribute a sour flavor to the dough.

Many different types of cultured milk products can be found around the world including milk, cheese, yogurt, other cultured dairy foods, ice cream and more

Product	Alternative names	Typical milkfat content	Typical shelf life at 4 °C	Fermentation agent	Description
Cheese		1-75%	varies	a variety of bacteria or mold	Any number of solid fermented milk products.
Crème fraîche	creme fraiche	30-40%	10 days	naturally occurring lactic acid bacteria in cream	Mesophilic fermented cream, originally from France; higher-fat variant of sour cream
Cultured sour cream	sour cream	14–40%	4 weeks	*Lactococcus lactis* subsp. *lactis*	Mesophilic fermented pasteurized cream with an acidity of at least 0.5%. Rennet extract may be added to make a thicker product. Lower fat variant of crème fraîche
Filmjölk	fil	0.1-4.5%	10–14 days	*Lactococcus lactis* and *Leuconostoc*	Mesophilic fermented milk, originally from Scandinavia
Yogurt	yoghurt, yogourt, yoghourt	0.5–4%	35–40 days	*Lactobacillus bulgaricus* and *Streptococcus thermophilus*	Thermophilic fermented milk, cultured with *Lactobacillus bulgaricus* and *Streptococcus thermophilus*
Kefir	kephir, kewra, talai, mudu kekiya, milkkefir, búlgaros	0-4%	10–14 days	Kefir grains, a mixture of bacteria and yeasts	A fermented beverage, originally from the Caucasus region, made with kefir grains; can be made with any sugary liquid, such as milk from mammals, soy milk, or fruit juices
Kumis	koumiss, kumiss, kymys, kymyz, airag, chigee	4%	10–14 days	Lactobacilli and yeasts	A carbonated fermented milk beverage traditionally made from horse milk
Viili	filbunke	0.1-3.5%	14 days	*Lactococcus lactis subsp. cremoris, Lactococcus lactis* biovar. diacetylactis, Leuconostoc mesenteroides subsp. cremoris* and *Geotrichum candidum*	Mesophilic fermented milk that may or may not contain fungus on the surface; originally from Sweden; a Finnish specialty

Product	Alternative names	Typical milkfat content	Typical shelf life at 4 °C	Fermentation agent	Description
Cultured buttermilk		1–2%	10 days	*Lactococcus lactis (Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis biovar. diacetylactis* and *Leuconostoc mesenteroides subsp. cremoris)*	Mesophilic fermented pasteurized milk
Acidophilus milk	acidophilus cultured milk	0.5-2%	2 weeks	*Lactobacillus acidophilus*	Thermophilic fermented milk, often lowfat (2%, 1.5%) or nonfat (0.5%), cultured with *Lactobacillus acidophilus*

FERMENTED FOODS

1 Introduction

Traditional fermented food is not only the staple food for most of developing countries but also the key healthy food for developed countries. As the healthy function of these foods are gradually discovered, more and more high throughput biotechnologies are being used to promote the old and new industry. As a result, the microflora, manufacturing processes and product healthy function of these foods were pushed forward. The application and progress of the high throughput biotechnologies into traditional fermented food industries like fermented milk products (yogurt, cheese), fermented sausages, fermented vegetables (kimchi, sauerkraut), fermented cereals (sourdough) and fermented beans (tempeh, natto) are gaining momentum. Given the further promotion by high throughput biotechnologies, the middle and/or down-stream process of traditional fermented foods would be optimized and the process of industrialization of local traditional fermented food having many functional factors but in small quantity would be accelerated. Fermented foods may be defined as foods which are processed through the activities of microorganisms but in which the weight of the microorganisms in the food is usually small. The influence of microbial activity on the nature of the food, especially in terms of flavor and other organoleptic properties is profound.

2 Fermented Vegetables

Like the fermentation of other foods, vegetables have been preserved by fermentation from time immemorial by lactic bacterial action. A wide range of vegetables and fruits including cabbages, olives, cucumber, onions, peppers, green tomatoes, carrots, okra, celery, and cauliflower have been preserved.

Only sauerkraut and cucumbers will be discussed, as the same general principles apply to the fermentation of all vegetables and fruits. In general they are fermented in brine, which eliminates other organisms and encourages the lactic acid bacteria.

2.1 Sauerkraut

Sauerkraut is produced by the fermentation of cabbages, *Brassica oleracea*, and has been known for a long time. Specially selected varieties which are mild-flavored are used. The cabbage is sliced into thin pieces known as slaw and preserved in salt water or brine containing about 2.5% salt. The slaw must be completely immersed in brine to prevent it from darkening. Kraut fermentation is initiated by *Leuconostoc mesenteroides*, a heterofermentative lactic acid bacterium (i.e., it produces lactic acid as well as acetic acid and CO_2). It grows over a wide range of pH and temperature conditions. CO_2 creates anaerobic conditions and eliminates organisms which might produce enzymes which can cause the softening of the slaw and also encourages the growth of other lactic acid bacteria. Gram negative coliforms and pseudomonads soon disappear, and give way to a rapid proliferation of other lactic acid bacteria, including *Lactobacillus brevis*, which is heterofermentative, and the homofermentative *Lactobacillus plantarum*; sometimes *Pediococcus cerevisiae* also occurs. Compounds which contribute to the flavor of sauerkraut begin to appear with the increasing growth of the lactics. These compounds include lactic and acetic acids, ethanol, and volatile compounds such as diacetyl, acetaldehyde, acetal, isoamyl alcohol, n-hexanol, ethyl lactate, ethyl butarate, and isoamyl acetate. Besides the 2.5% salt, it is important that a temperature of about 15°C be used. Higher temperatures cause a deterioration of the kraut.

2.2 Cucumbers (pickling)

Cucumber (*Cucumis sativus*) is eaten raw as well after fermentation or pickling. Cucumbers for pickling are best harvested before they are mature. Mature cucumbers are too large, ripen easily and are full of mature seeds. Cucumbers may be pickled by dry salting or by brine salting .

Dry salting is also generally used for cauliflower, peppers, okra, and carrots. It consists of adding 10 to 12% salt to the water before the cucumbers are placed in the tank. This prevents bruising or other damage to the vegetables.

Brine salting is more widely used. A lower amount of salt is added, between 5 and 8% salt being used. Higher amounts were previously used to prevent spoilage. During the primary fermentation lasting two or three days, most of the unwanted bacteria disappear allowing the lactics and yeasts to proliferate. In the final stages, after 10 to 14 days, *Lactobacillus plantarum* and *L. brevis*, followed by *Pediococcus*, are the major organisms.

3 Fermented Foods From Cereals and Beans

3.1 Idli

Idli is a popular fermented breakfast and hospital food which has been eaten in South India for many years. It is prepared from rice grains and the seeds of the leguminous mung grain, *Phaeseolus mungo*, or from black gram (*udad* dhal), *Vigna mungo*, which are also known as dal. When the material contains Bengal grain, *Circer orientium*, the product is known as khaman. It has a spongy texture and a pleasant sour taste due to the lactic acid in the food. It is often embellished with flavoring ingredients such as cashew nuts, pepper and ginger.

3.1.1 Production of idli

The seeds of the dahl (black gram) are soaked in water for 1-3 hours to soften them and to facilitate decortication, after which the seeds are mixed and pounded with rice in a proportion of three parts of the beans and one of rice (Figure 31.1). The mixture is allowed to ferment overnight (20-22 hours). In the traditional system the fermentation is spontaneous and the mixture is leavened up to approximately 2 or 3 times. The organisms involved in the acidification have been identified as *Streptococcus faecalis*, and *Pediococcus* spp. The leavening is brought about by *Leuconostoc mesenteroides*, although the yeasts, *Torulopsis candida* and *Trichosporon pulluloma* have also been found in traditional Idli. The fermented batter is steamed and served hot. Idli is highly nutritious, being rich in nicotinic acid, thiamine, riboflavin, and methionine.

3.2 Production of beer

Barley beers can be divided into two broad groups: top-fermented beers and bottom fermented beers. This distinction is based on whether the yeast remains at the top of brew (top-fermented beers) or sediments to the bottom (bottom-fermented beers) at the end of the fermentation.

3.2.1 Raw materials for brewing

The raw materials used in brewing are: barley, malt, adjuncts, yeasts, hops, and water.

a) Brewer's yeasts

Yeasts in general will produce alcohol from sugars under anaerobic conditions, but not all yeasts are necessarily suitable for brewing. Brewing yeasts besides producing alcohol, are able to produce from wort sugars and proteins in a balanced proportion of esters, acids, higher alcohols, and ketones which contribute to the peculiar flavor of beer.

b) Brewery Processes

The processes involved in the conversion of barley malt to beer may be divided into the following:

1. Malting
2. Cleaning and milling of the malt
3. Mashing
4. Mash operation
5. Wort boiling treatment
6. Fermentation
7. Storage or lagering
8. Packaging

3.2.2 Malting

The purpose of malting is to develop amylases and proteases in the grain. These enzymes are produced by the germinated barley to enable it to break down the carbohydrates and proteins in the grain to nourish the germinated seedling before its photosynthetic systems are developed enough to support the plant.

a. Cleaning and milling of malt

The purpose of milling is to expose particles of the malt to the hydrolytic effects of malt enzymes during the mashing process. The finer the particles, greater the extract from the malt.

b. Mashing

Mashing is the central part of brewing. It determines the nature of the wort, hence the nature of the nutrients available to the yeasts and therefore the type of beer produced. The purpose of mashing is to extract as much as possible the soluble portion of the malt and to enzymatically hydrolyze insoluble portions of the malt and adjuncts. The aqueous solution resulting from mashing is known as wort. The wort is boiled for 1-1½ hours in a stainless steel kettle. When corn syrup or sucrose is used as an adjunct it is added at the beginning of the boiling. Hops are also added, some before and some at the end of the boiling. Hops are the dried cone-shaped female flower of hop-plant *Humulus lupulus.* The

importance of hops in brewing lies in its resins which provide the precursors of the bitter principles in beer and the essential (volatile) oils which provide the hop aroma.

The purpose of boiling is as follows.

(a) To *concentrate* the wort,

(b) To *sterilize* the wort

(c) To *inactivate* any enzymes

(d) To *extract* soluble materials from the hops

(e) To precipitate protein, which forms large flocs because of heat denaturation and complexing with tannins extracted from the hops and malt husks. Unprecipitated proteins form hazes in the beer, but too little protein leads to poor foam head formation.

(f) To develop color in the beer; some of the color in beer comes from malting but the bulk develops during wort boiling. Color is formed by several chemical reactions including caramelization of sugars, oxidation of phenolic compounds, and reactions between amino acids and reducing sugars.

(g) Removal of volatile compounds: volatile compounds such as fatty acids which could lead to rancidity in the beer are removed.

c. Fermentation

The cooled wort is pumped or allowed to flow by gravity into fermentation tanks and yeast is inoculated or 'pitched in' at a rate of 7-15 x 10^6 yeast cells/ml, usually collected from a previous brew. The progress of fermentation is followed by wort specific gravity. During fermentation the gravity of the wort gradually decreases because yeasts are using up the extract. However, alcohol is also being formed. As alcohol has a lower gravity than wort the reading of the special hydrometer (known as a saccharometer) is even lower. °Brix is used in the sugar industry, whereas Balling (United States) and °Plato (continental Europe) are used in the brewing industry.

d. Lagering

During lagering secondary fermentation occurs. Yeasts are sometimes added to induce this secondary fermentation, utilizing some sugars in the green beer. The secondary fermentation saturates the beer with CO_2.

3.2.3 Packaging

The beer is transferred to pressure tanks from where it is distributed to cans, bottles and other containers. The beer is not allowed to come in contact with oxygen during this operation; it is also

not allowed to lose CO_2 or to become contaminated with microorganisms. To achieve these objectives, the beer is added to the tanks under a CO_2, atmosphere, bottled under a counter pressure of CO_2 and all the equipment is cleaned and disinfected regularly.

3.2.4 Beer defects

The most important beer defect is the presence of haze or turbidity, which can be of biological or physico-chemical origin. Biological turbidities are caused by spoilage organisms and arise because of poor brewery hygiene (i.e. poorly washed pipes) and poor pasteurization. Spoilage organisms in beer must be able to survive the following stringent conditions found in beer: low pH, the antiseptic substances in hops, pasteurization of beer, and anaerobic conditions.

Yeasts and certain bacteria are responsible for biological spoilage because they can withstand these. Wild or unwanted yeasts which have been identified in beer spoilage are spread into many genera including *Kloeckera*, *Hansenula,* and *Brettanomyces,* but *Saccharomyces* spp appear to be commonest, particularly in top-fermented beers. These include *Sacch. cerevisiae* var. *turblidans,* and *Sacch. diastaticus.* The latter is important because of its ability to grow on dextrins in beer, thereby causing hazes and off flavors. Among the bacteria, *Acetobacter,* and the lactic acid bacteria, *Lactobacillus* and *Streptococcus* are the most important. The latter are tolerant of low pH and hop antiseptics and are microaerophilic hence they grow well in beer. *Acetobacter* is an acetic acid bacterium and produces acetic acid from alcohol thereby giving rise to sourness in beer. *Lactobacillus pastorianus* is the typical beer spoiling lactobacilli, in top-fermented beers, where it produces sourness and a silky type of turbidity. *Streptococcus damnosus* (*Pediococcus damnosus, Pediococcus cerevisiae*) is known as 'beer sarcina' and gives rise to 'sarcina sickness' of beer which is characterized by a honey-like odor.

3.3 Wines

Wine is by common usage defined as a product of the "normal alcoholic fermentation of the juice of sound ripe grapes". Nevertheless any fruit with a good proportion of sugar may be used for wine production. If the term is not qualified then it is regarded as being derived from grapes, *Vitis vinifera.* The production of wine is simpler than that of beer in that no need exists for malting since sugars are already present in the fruit juice being used. This however exposes wine making to greater contamination hazards

3.3.1 Processes in wine making

a. Crushing of grapes

Selected ripe grapes are crushed to release the juice which is known as 'must', after the stalks which support the fruits have been removed. These stalks contain tannins which would give the wine a harsh taste if left in the must. The skin contains most of the materials which give wine its aroma and color. Grape juice has an acidity of 0.60-0.65% and a pH of 3.0-4.0 due mainly to malic and tartaric acids with a little citric acid.

b. Fermentation

(i) **Yeast used:** The grapes themselves harbor a natural flora of microorganisms (the bloom) which in previous times brought about the fermentation and contributed to the special characters of various wines. Yeasts are then inoculated into the must. The yeast which is used is *Saccaromyces cerevisiae* var, *ellipsoideus* (synonyms: *Sacch. cerevisiae, Sacch. ellipsoideus, Sacch, vini.).*
Wine yeasts have the following characteristics: (a) growth at the relatively high acidity (i.e., low pH) of grape juice; (b) resistance to high alcohol content (higher than 10%); (c) resistance to sulfite.

c. Control of fermentation

(a) **Temperature:** Heat is released during the fermentations. It has been calculated the temperature of a must containing 22% sugar would rise 52°F (11°C) if all the heat were stopped from escaping. If the initial temperature were 60°F (16°C) the temperature would be 100°F (38°C) and fermentation would halt while only 5% alcohol has been accumulated. For this reason the fermentation is cooled and the temperature is maintained at around 24°C with cooling coils mounted in the fermentor.

(b) **Yeast nutrition:** Yeasts normally ferment the glucose preferentially although some yeasts e.g. *Sacch. elegans* prefer fructose. Most nutrients including macro- and micro-nutrients are usually abundant in must; occasionally, however, nitrogeneous compounds are limiting. They are then made adequate with small amounts of $(NH_4)_2 SO_4$.

(c) **Oxygen:** As with beer, oxygen is required in the earlier stage of fermentation when yeast multiplication is occurring. In the second stage when alcohol is produced the growth is anaerobic and this forces the yeasts to utilize such intermediate products as acetaldehydes as hydrogen acceptors and hence encourage alcohol production.

(d) **Flavor development:** Although some flavor materials come from the grape most of it come from yeast action and has been shown to be due to alcohols, esters, fatty acids, and carbonyl compounds,

the esters being the most important. Diacetyl, acetoin, fuel oils, volatile esters, and hydrogen sulfide have received special attention.

d. Ageing and Storage

The fermentation is usually over in three to five days. At this time 'pomace' formed from grape skins (in red wines) will have risen to the top of the brew. At the end of this fermentation the wine is allowed to flow through a perforated bottom if pomace had been allowed. When the pomace has been separated from wine and the fermentation is complete or stopped, the next stage is 'racking'. The wine is allowed to stand until a major portion of the yeast cells and other fine suspended materials have collected at the bottom of the container as sediment or 'lees'. It is then 'racked', during which process the clear wine is carefully pumped. The wine is then transferred to wooden casks (100-1,000 gallons), barrels (about 50 gallons) or tanks (several thousand gallons). The wood allows the wine only slow access to oxygen. Water and ethanol evaporate slowly leading to air pockets which permit the growth of aerobic wine spoilers e.g. acetic acid bacteria and some yeasts. The casks are, therefore regularly topped up to prevent the pockets. In modern tanks made of stainless steel the problem of air pockets is tackled by filling the airspace with an inert gas such as carbon dioxide or nitrogen. During ageing desirable changes occur in the wine. These changes are due to a number of factors:

e. Clarification

The wine is allowed to age in a period ranging from two years to five years, depending on the type of wine. At the end of the period some will have cleared naturally. For others artificial clarification may be necessary. The addition of a fining agent is often practiced to help clarification. Fining agents react with the tannin, acid, protein or with some added substance to give heavy quick-settling coagulums. The usual fining agents for wine are gelatin, casein, tannin, egg albumin, and bentonite.

f. Packaging

Before packing in bottles the wine from various sources is sometimes blended and then pasteurized. In some wineries, the wine is not pasteurized, rather it is sterilized by filtration. In many countries the wine is packaged and distributed in casks.

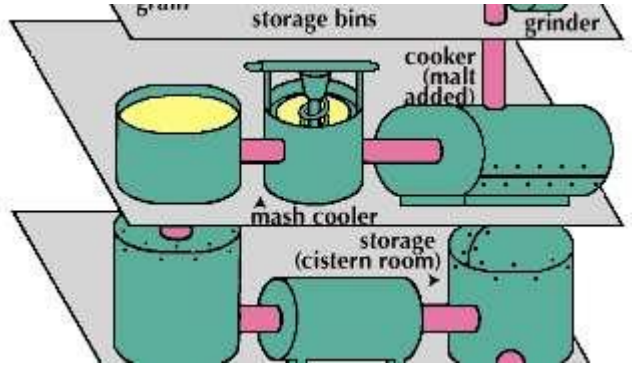
g. Wine defects

The most important cause of wine spoilage is microbial; less important defects are acidity and cloudiness. Factors which influence spoilage by bacteria and yeasts include the following (a) wine composition, specifically the sugar, alcohol, and sulfur dioxide content; (b) storage conditions e.g.

high temperature and the amount of air space in the container; (c) the extent of the initial contamination by microorganism during the bottling process. When proper hygiene is practiced bacterial spoilage is rare. When it does occur the microorganisms concerned are acetic acid bacteria which cause sourness in the wine. Lactic acid bacteria especially *Leuconostoc*, and sometimes *Lactobacillus* also spoil wines. Various spoilage yeasts may also grow in wine. The most prevalent is *Brettanomyces*, slow growing yeasts which grow in wine causing turbidities and off-flavors. Other wine spoilage yeasts are *Saccharomyces oviformis*, which may use up residual sugars in a sweet wine and *Saccharomyces bayanus* which may cause turbidity and sedimentation in dry wines with some residual sugar. *Pichia membanaefaciens* is an aerobic yeast which grows especially in young wines with sufficient oxygen. Other defects of wine include cloudiness and acidity.

Distilled spirit (alcoholic beverage)

Distilled spirit, also called **distilled liquor**, alcoholic beverage (such as brandy, whisky, rum, or arrack) that is obtained by distillation from wine or other fermented fruit or plant juice or from a starchy material (such as various grains) that has first been brewed. The alcoholic content of distilled liquor is higher than that of beer or wine.



The fermentation and distillation process for producing whiskey. The production of whiskey begins with grinding grain into a meal, which is cooked. Malt is introduced to the meal, which results in mash that is cooled and pumped into a fermenter, where yeast is added. The fermented mixture is heated in a still, where the heat vaporizes the alcohol. The alcohol vapours are caught, cooled, condensed, and drawn off as clean, new whiskey. This liquid is stored in a cistern room, and water is added to lower the proof (absolute alcohol content) before the whiskey is placed in new charred oak barrels for aging and later bottling.

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The production of distilled spirits is based upon fermentation, the natural process of decomposition of organic materials containing carbohydrates. It occurs in nature whenever the two necessary ingredients, carbohydrate and yeast, are available. Yeast is a vegetative microorganism that lives and multiplies in media containing carbohydrates—particularly simple sugars. It has been found throughout the world, including frozen areas and deserts.

Distilled spirits are all alcoholic beverages in which the concentration of ethyl alcohol has been increased above that of the original fermented mixture by a method called distillation. The principle of alcoholic distillation is based upon the different boiling points of alcohol (78.5 °C, or 173.3 °F) and water (100 °C, or 212 °F). If a liquid containing ethyl alcohol is heated to a temperature above 78.5 °C but below 100 °C and the vapour coming off the liquid is condensed, the condensate will have a higher alcohol concentration, or strength.

History Of Distilling

Because the two ingredients necessary to alcoholic fermentation are widely spread and always appear together, civilizations in almost every part of the world developed some form of alcoholic beverage very early in their history. The Chinese were distilling a beverage from rice beer by 800 BCE, and

arrack was distilled in the East Indies from sugarcane and rice. The Arabs developed a distillation method that was used to produce a distilled beverage from wine. Greek philosophers reported a crude distillation method. The Romans apparently produced distilled beverages, although no references concerning them are found in writings before 100 CE. Production of distilled spirits was reported in Britain before the Roman conquest. Spain, France, and the rest of western Europe probably produced distilled spirits at an earlier date, but production was apparently limited until the 8th century, after contact with the Arabs.

The first distilled spirits were made from sugar-based materials, primarily grapes and honey to make grape brandy and distilled mead, respectively. The earliest use of starchy grains to produce distilled spirits is not known, but their use certainly dates from the Middle Ages. Some government control dates from the 17th century. As production methods improved and volume increased, the distilled spirits industry became an important source of revenue. Rigid controls were often imposed on both production and sale of the liquor.

The earliest stills were composed simply of a heated closed container, a condenser, and a receptacle to receive the condensate. These evolved into the pot still, which is still in use, particularly for making malt whiskeys and some gins. The next refinement was heating the alcohol-containing liquid in a column made up of a series of vaporization chambers stacked on top of one another. By the early 19th century large-scale continuous stills, very similar to those used in the industry today, were operating in France and England. In 1831 the Irishman Aeneas Coffey designed such a still, which consisted of two columns in series.

Since distillation requires that the liquid portion of a fermentation mixture be vaporized, considerable heat must be applied to the process. The fuel used in distilling spirits has always been that which has been most readily available at the particular time and place. Peat, coal, and wood were the fuels used historically, while the fuels of choice today are coal, natural gas, and oil. The high steam requirement for continuous-still operation inhibited the development of rectifying columns for production of spirits until after the Industrial Revolution.

Many of the minor components of distilled spirits, which are present only in parts per million, are detectable by the senses of taste and smell, but efforts to identify and quantify these compounds chemically have often been hampered by the lower limits of detection by analytical methods. Classes of compounds such as aldehydes, organic acids, esters, and alcohols were easily identified by conventional methods, but many of them could not be determined until after

the development of chromatography. The Russian botanist Mikhail Tsvet was an early pioneer of this measurement technique, reporting his first work in 1903. Refinements in both technique and equipment, made during the first half of the 20th century, allowed numerous flavour components in distilled spirits to be identified by gas chromatography.

Producing The Mash

Raw materials

The raw materials used for making a distilled spirit are of two basic types: (1) those containing a high concentration of natural sugars or (2) those containing other carbohydrates that can easily be converted to sugars by enzymes. Enzymes are proteins that act as catalysts to promote chemical reactions. Very small amounts of an enzyme can cause a fundamental change in a large amount of material. Most enzymes are specific in their action, so that a system of several enzymes is necessary, for example, to convert starch into sugar and ultimately into ethyl alcohol. The amylases are enzymes that convert starches into sugars; sprouting grains—especially barley—are natural sources of these enzymes. Yeast has a complex enzyme system that converts sugar into carbon dioxide and a multiplicity of other products, including ethyl alcohol.

Reduced activity of any enzyme in the system distorts the results, often forming unwanted products. Enzymes are easily poisoned by certain compounds; they are also sensitive to temperature variations and to the degree of acidity of the medium.

Sugary materials

Grapes, cultivated in most of the subtropic and warm temperate zones of the world, are the major fruit employed as the raw material of distilled spirits, and the final product of their fermentation is brandy. Other natural fruits, such as apples and peaches, are used to a lesser extent, and many fruits are limited to local importance.

Sugary vegetables include sugarcane, sugar beets, and *Agave tequilana* (a type of cactus). Sugarcane and its products, including cane juices, molasses, and sugar, are the most important of the vegetable group. Grown throughout the tropics and semitropics, sugarcane is used in making rum and an alcohol derived from rum. Sugarcane juice can be pressed from the cane for use as the base raw

material for fermentation, or the juice may be concentrated for sugar production, with the molasses residue from the sugar crystallization used as a base for fermentation. This process is also applied to sugar beets.

Starchy materials

For many centuries, it was only feasible to employ local grain crops for liquor production, and, in this way, the basic characteristics of the local distilled beverage were established. Improved transportation removed this restriction, and today economic considerations frequently determine grain selection, with the principal grain used being the one available at the lowest price per unit of fermentable materials.

Corn (maize) is the most important cereal grain employed; it is produced worldwide. Rye grain, though less efficient in fermentation than corn, is used extensively in whiskey production, primarily for the flavour characteristics it imparts to the final product. It is particularly employed in Canada and the United States. Rice, a widely grown cereal, has limited use in distilled spirits production outside of Asia from India to Japan. Barley grain, probably the first cereal employed for distillation in large quantities, was formerly a major crop throughout Ireland and western Europe. Wheat, because of its high cost, is used only where corn is in short supply and is then limited to production of grain alcohol for blending or in production of liqueurs. Potatoes have been used in distilled spirits production primarily in central Europe; in the tropics, other starchy roots are employed.

Preparing the mash

Milling and pressing

The purpose of milling and pressing is to make the starch or sugar more available for enzyme action. Crushing and pressing (grapes and other fruits), milling (cereal grains), or a combination of milling and pressing (sugarcane) are used.

In milling, grains are reduced to a meal to allow wetting of their starch cells. Various types of mills are used. Roller mills, where the grain passes through a series of corrugated rollers, was long the most common type. The grinding action of the rollers is mainly a shearing action. More efficient and economical impact-type mills (such as hammer mills) are now gaining in importance.

After the Industrial Revolution, steam replaced water as the power source for milling. Since the mid-20th century, electricity has been almost the exclusive power source in milling.

Mashing.

The purpose of the mashing operation is to (1) mix the proper proportions of grains, (2) increase the availability of the starch for enzyme action, and (3) convert the starches into fermentable sugars.

Mashing is done in a vessel called a mash tun, which is equipped with a means of agitation for mixing and is either jacketed or contains coils for heating and cooling. In mashing, the starch cells of the grain, enclosed in their own protective coatings, are broken to allow wetting and liquefaction of the entire starch mass. The process usually begins with the grain most difficult to treat. When corn is used, the ground meal is wetted at a temperature of approximately 66 °C (150 °F), and the temperature is then raised to boiling or sometimes higher while under pressure. The temperature is reduced when the starch cells are broken. The grain ranking second in cell resistance (usually rye) is added next. Other starchy substances, such as potatoes, are usually crushed and heated, exploding the starch cells. The temperature of the mash is reduced before ground malt meal, either in dry form or as a water slurry (insoluble mixture), is added. The amylase enzymes in the malt then produce a mixture in which the starches have been converted to fermentable sugars, suitable for utilization by the yeast. The sugars, principally dextrose and maltose, vary in concentration among producers but, generally, are sufficiently concentrated to make a final product ranging from 7 to 9 percent alcohol.

Any germinating cereal grain can be used for malt. In rare cases, rye malt is used in making rye whiskey, but, because the enzyme activity of malted barley is the highest, barley is used almost exclusively in the distilling industry. Barley malt contains sufficient enzymes to convert approximately 10 times its weight in other unmalted grains. Of the two enzymes—α-amylase and β-amylase—the former is the more important for conversion of other grains. In addition to converting starches from other carbohydrates to sugars, barley malt contains soluble proteins (amino acids), contributing flavour to the distillate secured from fermentation and distillation of grain-malt mixtures.

Fermenting And Distilling

Fermentation

Yeast and yeast culture

As mentioned above, yeasts are found throughout the world; more than 8,000 strains of this vegetative microorganism have been classified. Approximately nine or 10 pure strains, with their subclassifications, are used for fermentation of grain mashes; these all belong to the type *Saccharomyces cerevisiae.* Each strain has its own characteristics, imparting its special properties to the distillate derived from its fermentation. A limited number of yeasts are used in the fermentation of wines, from which brandy is distilled. Strains used in the fermentation of grain mashes are also used in fermentation for rum, tequila, and beer production.

In grain-based products, yeast cells are grown in grain mixtures. The preparation of a cooked mash of rye and barley malt is most common. The mash is sterilized, then inoculated with lactic-acid bacteria to increase acidity. (Yeast is more tolerant of higher acidity than many commonly occurring bacteria.) When the desired acidity is reached, the mixture is again sterilized and a pure yeast culture is added. The yeast is grown under controlled conditions until it reaches the optimum point for mixing with the grain mash. In liquid fermentation, as from fruits and sugarcane, the yeast is generally grown in a mixture similar to the one it will be used to ferment; for example, a yeast culture to be used for molasses fermentation is usually grown in molasses.

Fermenting methods

In the fermentation process, simple sugars, including dextrose and maltose, are converted to ethyl alcohol by the action of yeast enzymes. Several intermediate compounds are formed during this complex chemical process before the final ethyl alcohol is obtained.

Yeast functions best in a slightly acid medium, and the prepared grain mash, fruit juice, molasses, or other mixture must be checked for adequate acidity (pH value). If acidity is insufficient, acid or acid-bearing material is added to achieve the necessary adjustment. The previously prepared yeast is then added, and final dilution of the mixture is made. The final concentration of sugars is adjusted so that the yeast fermentation will produce a finished fermented mixture containing between 7 and 9 percent alcohol.

Commercial fermentation is carried on in large vats. In the past these were open and made of wood, usually cypress. Most plants now use closed stainless steel vats for easier cleaning, and many are

equipped with jackets or cooling coils for better temperature control. The time required for completion of fermentation is mainly dependent upon the temperature of the fermenting mash. Normal yeast is most effective in breaking down all of the fermentable sugars at temperatures ranging from 24 to 29 °C (75 to 85 °F), and, in this range, completion of fermentation requires from 48 to 96 hours. Fermentation at lower temperatures requires longer periods. The mash is ready for distillation upon completion of fermentation. If fermentation is allowed to continue past this period, it will be adversely affected by bacterial action. The ethyl alcohol content will be reduced, and the flavour and aroma of the finished product will be tainted.

Distillation

As mentioned above, the difference in the boiling points of alcohol and water is utilized in distillation to separate these liquids from each other. Basic distillation apparatus consists of three parts: the still or retort, for heating the liquid; the condenser, for cooling the vapours; and the receiver, for collecting the distillate.

The pot still

The simple pot still is a large enclosed vessel, heated either by direct firing on the bottom or by steam coils within the vessel, with a cylindrical bulb at its top leading to a partially cooled vapour line. The bulb and vapour line separate entrained liquid particles from the vapour on its way to the final condenser. The usual pot-still operation involves a series of two or three pot stills. Any vapour falling below a predetermined alcoholic content is fed into a second still, and condensed vapour from the second still falling below the required alcoholic content is fed to the third. The condensed vapours of the desired alcoholic content from all three stills are then commingled in a single receiving container.

The pot still, used primarily in Scotland and Ireland for whiskey production and in France for brandies, has had only brief use in distilled spirits production elsewhere and is gradually becoming obsolete. Even in countries in which the pot still has long been used, it has been replaced by continuous distillation for the major portion of alcoholic-liquor production, and its current use is limited to production of flavouring whiskeys and other flavouring ingredients.

The flavour profile of a pot-still product is more complex than that of a continuous-still product of the same alcohol content. This is a result of the different distillation methods. At a given temperature

and pressure, vapours over a boiling mixture have a composition that is a function of the vapour pressures of the components of the mixture. In a pot still, the temperature of the fermentation mixture rises as the lower-boiling-temperature alcohol vaporizes. Meanwhile, the alcohol content of the distillate drops as the rising temperature vaporizes more water along with the alcohol. Distillation is allowed to continue until the alcohol content of the distillate falls to a predetemined level. Because of the rising temperature encountered in distilling a single batch, the composition of the first part of the condensate to leave the pot is different from that of the last part. The composition of the final product is the average of the composition of the vapours condensed during the entire run. By contrast, the temperature of the continuous still is held approximately constant throughout the run. This results in a flavour profile that is more uniform.

The continuous still

The continuous still, which came into use in the early 19th century, consists of a tall cylindrical column filled with perforated plates onto which water-rich vapours condense while alcohol-enriched vapours pass through. These plates thus serve as a series of small pot stills, one on top of the other. Live steam, used as the heat source, is fed into the bottom of the still, and the liquid to be distilled is fed near the top. Steam pressure holds the liquid on the plates, and, with any overflow caught by the plate below, the liquid level on each plate is maintained. Use of a sufficient number of plates assures that the concentration of alcohol in the vapour leaving the top of the still will be appropriate for the desired product and that the liquid leaving the bottom has been stripped of any alcohol.

Many distillation operations combine column and pot stills. The condensed distillate from the column still is fed to the doubler, a type of pot still heated by closed steam coils, and redistilled.

The rectification still

Rectification is the process of purifying alcohol by repeatedly or fractionally distilling it to remove water and undesirable compounds. As mentioned above, a fermentation mixture primarily contains water and ethyl alcohol and distillation involves increasing the percentage of ethyl alcohol in the mixture. Water vaporizes very easily, however, and, unless care is taken, the distillate of a fermentation mixture will contain unacceptably large quantities of water. The fermentation mixture furthermore contains small quantities of complex constituents that can contribute to the flavour of the product even if they are present only in parts per million. It is important to retain those components

that make a positive contribution to the product and to remove those that are unwanted, primarily some organic aldehydes, acids, esters, and higher alcohols. The ones that remain in the product are called congeners, and the congener level is controlled by the particular rectification system and by the system's method of operation.

The multicolumn rectifying system usually consists of three to five columns. The first column is always a preliminary separation column called the beer still, or analyzer. It usually consists of a series of metal plates with holes punched in them and baffles to control the liquid levels on the plates. The product coming from this column is between 55 and 80 percent ethyl alcohol. A 95 percent product can be produced on a two-column system consisting of a beer column and a rectifying column. The bulk of congener removal is accomplished in the rectifier—esters and higher alcohols, for example, being drawn off as side streams. However, a multicolumn system of several specialized rectifiers allows better control of the finished product. An aldehyde column, or purifier, is frequently used to separate these highly volatile low-boiling components, and sometimes ethyl alcohol is recovered in an extractive column and returned to the rectifier.

Three characteristics determine the elimination or retention of flavouring compounds: (1) their boiling points, (2) their solubilities in ethyl alcohol and water, and (3) their specific gravities. Some higher alcohols, for example, are removed on the basis of their solubility and specific gravity. These higher alcohols have limited solubility in water, and their specific gravities are less than that of water. Also, their boiling points are higher than that of ethyl alcohol and lower than that of water. Since they tend to accumulate in the rectifying column at the region where their boiling points cause them to condense, they can be drawn off as a liquid side stream. This side stream also contains a considerable amount of water. The limited solubility in water, plus the lower specific gravities, cause the higher alcohols to float to the top of the alcohol–water mixture, from which they can be removed.

Maturation, Blending, And Packaging

Aging

Learn how to make vodka

Overview of how vodka is made.

*Contunico © ZDF Enterprises GmbH, Mainz*See all videos for this article

One method of classifying distilled liquors is as aged or unaged. Vodka, neutral spirits for use in a variety of products, most gins, and some rums and brandies are unaged. Aged products are predominantly whiskeys and most rums and brandies.

The term *age* refers to the actual duration of storage, while *maturity* expresses the degree to which chemical changes occur during storage. The maturation of whiskeys falls into two categories, according to whether storage is in new or reused cooperage. New charred, white-oak containers are required by law in the United States for the maturation of products to be called straight bourbon or rye whiskey. These containers, each containing 50 to 55 gallons, are stored in warehouses sometimes having controlled temperature and humidity. Older warehouses are called rick

houses because the barrels are stored on stationary frames called ricks. In many newer houses, barrels are stacked on pallets.

White oak is one of the few woods that can hold liquids while allowing the process of breathing through the pores of the wood. The pore size of the wood is such that small molecules such as water move through the wood more easily than larger molecules such as alcohol. This breathing process is caused by temperature and humidity differences between the liquid in the barrel and the air in the warehouse. Charring the wood makes some of the wood compounds more soluble. As the liquid in the container moves back and forth through the wood, ingredients are extracted and carried back into the container's contents. Maturation also results from the contact of oxygen from the outside air with ingredients in the alcohol mixture. Therefore, maturation during aging consists of the interaction of the original compounds of the distillate, of oxidation reactions, and of the extraction of flavouring compounds from the wood. These factors must be well balanced in the properly matured product. The lower the level of the original congeners, the less wood extract required to achieve a good balance.

Outside the United States, reused cooperage is common. Since used containers have already yielded their initial oak extracts, the resulting product is low in extracted flavouring ingredients, which is desirable in some beverages. This maturation method, typified by Scotch and Irish whiskeys, can be carried on in casks holding up to 132 gallons. These casks have usually had previous use for storage or maturation of other whiskeys or wines and may be reused for many maturation cycles. Maturation in dry warehousing increases the alcoholic content of the liquid in the container, but the more common practice for Scotch and Irish whiskeys of maturation in high humidity warehouses reduces the alcoholic concentration.

The maturation procedure for brandies is similar to that of some whiskeys, but the brandies are usually matured in fairly large casks or oak containers. Most brandies are matured for three to five years, but some remain for as long as 20 to 40 years or even longer.

Rum is usually matured in reused oak containers; high concentrations of oak extracts are not considered desirable. Normal maturation time is two to three years, but rum, generally a blended product, may contain a percentage of older rums.

Most governments specify storage time for various products. The United States requires a two-year storage period for most whiskeys but has no requirement for any pure alcohol or neutral spirits (close

to 100 percent alcohol) added to such whiskeys in the production of blended whiskey. Canada requires storage of two years for all distilled spirits. Scotland and England require a three-year storage and Ireland, five years for all products classified as whiskey; there are no requirements for vodka and gin.

Blending

Blending is another method of obtaining a balanced product with precise flavour characteristics. Blended products are composed of one or more highly flavoured components, a high-proof component with a low congener content, a colour adjustment ingredient, and perhaps an additional flavouring material. An example is a blended whiskey, which may contain several whiskeys, a grain spirit distilled at 90 to 95 percent alcohol, caramel colouring, and perhaps a small amount of a flavouring blender (part of which may be sherry or port wine). A blended Scotch consists of several highly flavoured malt whiskeys produced in pot stills and a base whiskey produced from grain in a continuous distillation system.

Packaging

Bottling

Distilled spirits react upon exposure to many substances, extracting materials from the container that tend to destroy the liquor aroma and flavour. For this reason, glass, being nonreactive, has been the universal container for packaging alcoholic liquors. (A few products are now packaged in plastic bottles, but these are primarily 50-millilitre miniatures, the light weight of which is particularly suited for use by airlines.) Packaging economics require containers that are standardized in size and shape and that lend themselves to automatic processes.

Early hand methods of filling, labeling, corking, and other operations have been replaced by highly mechanized bottling lines, with bottles cleaned, filled, capped, sealed, labeled, and placed in a shipping container at a rate as high as 400 bottles per minute. This progress became possible with the development of high-strength glass, plastic closures with inert liners, and high-speed machines. Even specialized packaging, long a hand operation, has been replaced by standardization of containers, allowing production on automatic lines.

Spirit strength may be designated in several ways—weight per gallon, percentage by weight, or percentage by volume, all these having reference to absolute (i.e., pure) alcohol and water. There are other standards in common use—e.g., U.S. proof spirit, which is 50 percent alcohol by volume. Each degree of U.S. proof represents 0.5 percent alcohol, so that a liquor having 50 percent alcohol is termed 100 proof. British proof is based on a specific concentration of alcohol, a 50 percent alcoholic content being equivalent to 114.12 U.S. proof. British proof is expressed as degrees over or under proof (that is, over or under 50 percent alcohol), while U.S. proof is expressed in direct proof figures. The metric Gay-Lussac system simply states the percentage by volume of alcohol in a distilled liquor.

Reference:
https://www.britannica.com/topic/distilled-spirit

Part-A

1. Comment on Yoghurt.
2. Write a note on Fermentation.
3. Explain the fermentation process of Idli and Dosa.
4. Define Kefir.
5. Discuss the Cheese.
6. Investigate the importance of Probiotic foods.
7. Briefly write about Milk products.
8. List out the health benefits of *Lactobacillus*.
9. Write short notes on Fermentation technology.

Part-B

1. Compare and contrast between Milk products and other fermented foods.
2. Demonstrate Milk processing with a flow chart.

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DEPARTMENT OF BIOTECHNOLOGY

UNIT – V – Food Biotechnology – SBB2203

Food Preservation

Food preservation methods are intended to keep microorganisms out of foods, remove microorganisms from contaminated foods, and hinder the growth and activity of microorganisms already in foods.

To keep microorganisms out of food, contamination is minimized during the entire food preparation process by sterilizing equipment, sanitizing it, and sealing products in wrapping materials. Microorganisms may be removed from liquid foods by **filtering** and sedimenting them or by washing and trimming them. **Washing** is particularly valuable for vegetables and fruits, and **trimming** is useful for meats and poultry products.

Heat. When **heat** is used to preserve foods, the number of microorganisms present, the **microbial load**, is an important consideration. Various types of microorganisms must also be considered because different levels of resistance exist. For example, bacterial spores are much more difficult to kill than vegetative bacilli. In addition, increasing acidity enhances the killing process in food preservation.

Three basic heat treatments are used in food preservation: **pasteurization**, in which foods are treated at about 62°C for 30 minutes or 72°C for 15 to 17 seconds; **hot filling**, in which liquid foods and juices are boiled before being placed into containers; and **steam treatment** under pressure, such as used in the canning method. Each food preserved must be studied to determine how long it takes to kill the most resistant organisms present. The heat resistance of microorganisms is usually expressed as the **thermal death time**, the time necessary at a certain temperature to kill a stated number of particular microorganisms under specified conditions.

In the **canning** process, the product is washed to remove soil. It is then blanched by a short period of exposure to hot water to deactivate enzymes in the food. Diseased sections in the food are removed, and the food is placed into cans by a filling machine. Sealed cans are then placed into a sterilizing machine called a **retort**, and the food is processed for a designated time and temperature.

Low temperatures. Low temperatures are used to preserve food by lowering microbial activity through the reduction of microbial enzymes. However, psychrophilic bacteria are known to grow even at cold **refrigerator** temperatures. These bacteria include members of the genera *Pseudomonas*, *Alcaligenes*, *Micrococcus*, and *Flavobacterium*. Fungi also grow at refrigeration temperatures.

Slow freezing and quick freezing are used for long-term preservation. **Freezing** reduces the number of microorganisms in foods but does not kill them all. In microorganisms, cell proteins undergo denaturation due to increasing concentrations of solutes in the unfrozen water in foods, and damage is caused by ice crystals.

Chemicals. Several kinds of chemicals can be used for food preservation, including **propionic acid, sorbic acid, benzoic acid**, and **sulfur dioxide.** These acids are acceptable because they can be metabolized by the human body. Some **antibiotics** can also be used, depending upon local laws and ordinances. Tetracycline, for example, is often used to preserve meats. Storage and cooking normally eliminates the last remnants of antibiotic.

In many foods, the **natural acids** act as preservatives. In sauerkraut, for example, lactic acid and acetic acid prevent contamination, while in fermented milks (yogurt, sour cream), acids perform the same function. For centuries, foods were prepared in this manner as a way of preventing microbial spoilage.

Drying. Drying is used to preserve food by placing foods in the **sun** and permitting the water to evaporate. **Belt, tunnel**, and **cabinet dryers** are used in industry for such things as instant coffee and cocoa. **Freeze-drying**, a process called **lyophilization**, is also valuable for producing a product free of moisture and very light.

Radiations. Ultraviolet radiation is valuable for reducing surface contamination on several foods. This short-wavelength light has been used in the cold storage units of meat processing plants. Ionizing radiations such as **gamma rays** can be used to preserve certain types of vegetables, fruits, and spices, according to state and U.S. federal regulations.

FOOD PRESERVATION METHODS

1. **Introduction**
2. **Traditional preservation methods**

2.1. **Fermenting**

2.2. **Drying**

2.3. **Salt curing**

2.4. **Smoking**

2.5. **Refrigeration**

1. Introduction

Food preservation usually involves preventing the growth of bacteria, fungi (such as yeasts), or other micro-organisms, as well as retarding the oxidation of fats that cause rancidity. Food preservation may also include processes that inhibit visual deterioration, such as the enzymatic browning reaction in apples after they are cut during food preparation.

Many processes designed to preserve food will involve a number of food preservation methods. Preserving fruit by turning it into jam, for example, involves boiling (to reduce the fruit's moisture content and to kill bacteria, etc.), sugaring (to prevent their re-growth) and sealing within an airtight jar (to prevent recontamination). Some traditional methods of preserving food have been shown to have a lower energy input and carbon footprint, when compared to modern methods.
Maintaining or creating nutritional value, texture and flavor is an important aspect of food preservation, although, historically, some methods drastically altered the character of the food being preserved. In many cases these changes have come to be seen as desirable qualities – cheese, yogurt and pickled onions being common examples.

Demand for minimally processed foods has resulted in the development of innovative, non-thermal food preservation methods, such as high-pressure sonication, ozone, and UV treatment. This book presents a summary of these novel food processing techniques. It also covers new methods used to monitor microbial activity, including spectroscopic methods (FT-IR and Raman), molecular and electronic noses, and DNA-based methods.

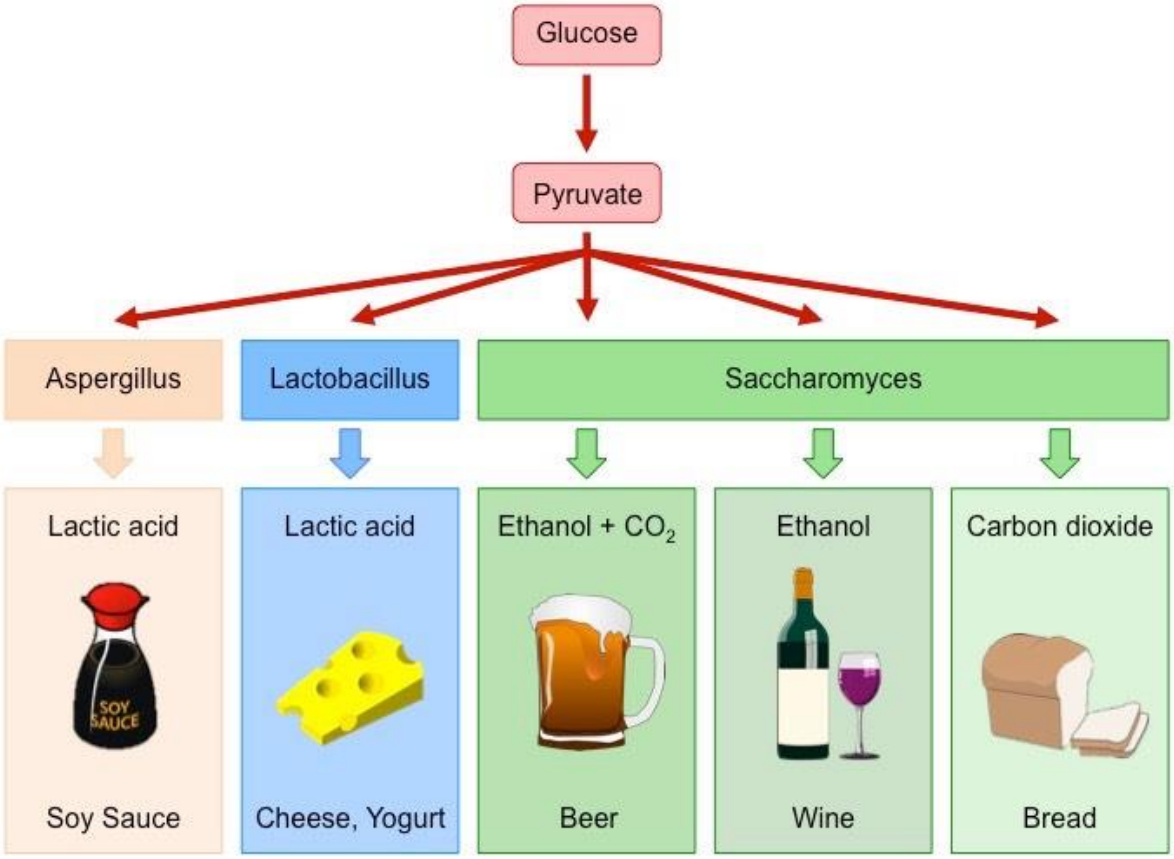
2. Traditional preservation methods

2.1. Fermenting

Fermentation is the chemical transformation of complex organic substances into simpler compounds by enzymes produced by bacteria, molds, and yeasts. It's a kind of "pre-digestion," performed by microorganisms long before humans were around to witness it

It depends on what you're trying to ferment and you can ferment just about anything, so the methods are incredibly diverse. Some foods, like raw dairy, will ferment all on their own because they contain an abundance of living lactobacilli, while others, like pasteurized dairy, require the addition of a starter agent because all the lactobacilli have been killed. The pasteurized dairy will still pick up bacteria and "change" without human interference, but it won't be a desirable change without lactobacilli present to hold off the unwanted bacteria. Still others, like cabbage, come with enough lactobacilli bacteria to start fermentation, but you have to squeeze the natural juices out to kick start the process and then add enough salt to limit the growth of putrefactive bacteria. But in the end, fermentation always comes down to enzymatic actions taken by molds, yeasts, and/or bacteria upon foods.

The fermentation products acetic acid, lactic acid, and alcohol act as natural preservatives for food and its nutrients while creating exciting, complex flavors. In a world without refrigeration, this was essential if you wanted to store enough food for leaner times without it spoiling or bleeding vitamins. Also, because the food is "pre-digested" by microorganisms, it's easier to digest and you get more energy out of it. Fermentation can also create new nutrients, especially B-vitamins, and fermented food can populate our guts with helpful bacteria (or pass along helpful genetic data to existing bacteria). Obviously, traditional cultures didn't know all these things, but they knew fermented food lasted longer, tasted better, and made them feel better.

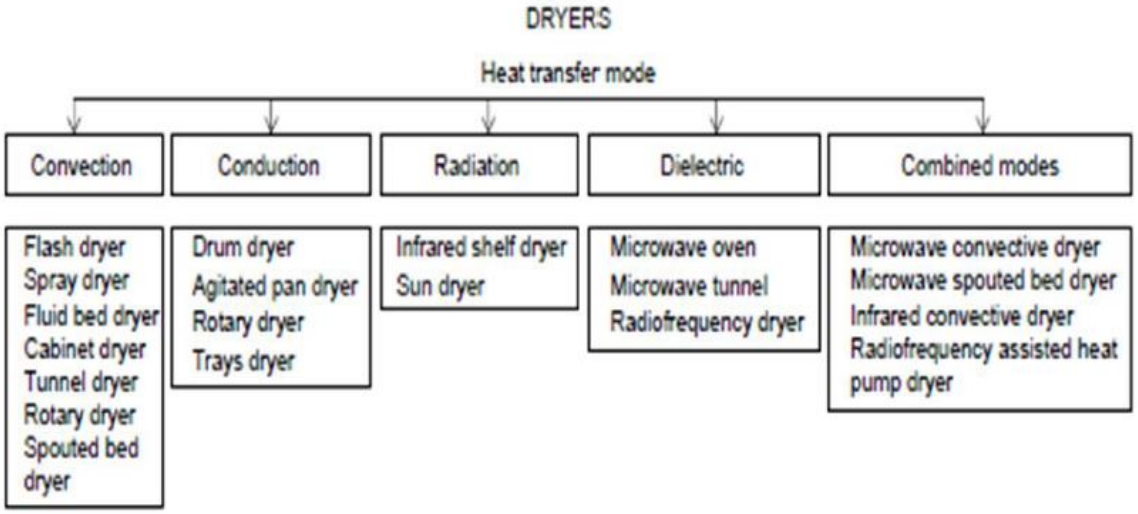


2.2 Drying

Removing moisture is probably the oldest food preservation technique around. And once man had control of fire, which hominids had 1 million years ago and Neanderthals enjoyed at least 400,000 years ago, he could start drying his food quicker and more completely.

Before food dehydrators were around, people used the wind, the sun, the open air, and of course fire to dehydrate foods. Key factors to consider: maximizing air exposure to ensure even air circulation, uniform thickness throughout, temperature fluctuations (constant temperatures are best), Humidity (lower is generally better). If drying meat or fish, salt will aid in preservation and flavor while keeping away insects. If using fire, those factors become less crucial.

If you remove enough moisture from a food, it will be protected from bacterial contamination. The rats and mice and neighbors might still get it, but at least the ones you can't see with the naked eye will not. A water activity of 0.76 or lower should do the trick. Dried meat, when combined with rendered fat and maybe some berries, could keep a person alive and thriving for months upon months.



2.3. Salt curing

Long ago, people realized that applying copious amounts of salt to a slab of animal or fish preserves it and prevents degradation. Via osmosis, the salt actually draws water out of microbial cells, thereby killing microorganisms and preventing spoilage. The meat itself loses moisture, thereby preventing future bacterial colonization.

All that was really used was salt, time, and a place to store everything. Adding sugar allowed the proliferation of lactobacilli (which feed on sugar), which altered the meat further. Modern curing often uses nitrates, which preserve the pink color.

It's a low-tech way to store precious meat for long periods of time. Also, the salt penetrates the tissue and, over time, denatures the proteins. This produces glutamate, which tastes really good, and concentrates the meaty flavor. The slow fermentation on account of the lacto bacteria can also create some really interesting, complex flavors and improve the preservability of the meat. It might even make certain

meats healthier, too. As shown in this (admittedly limited) study, patients had better reactions to traditionally cured pastured pork than to fresh pastured pork.

2.4. Smoking

Where there's fire, there's smoke. And since we've been cooking meat, we've been exposing it to smoke. Ever go to an all you can eat Korean BBQ joint? You come out smelling like pure meat. Dogs will love you and vegetarians will scream at your approach. See, when you're in a confined space, like a restaurant or a Paleolithic shelter, and a fire's raging, you will be exposed to smoke. Same goes for meat. Again, there's no absolute "proof" that our Paleolithic ancestors were smoking meat to preserve it, but it seems like a natural development to me.

Traditionally, Native Americans would expose strips of fish and meat to the air and to large amounts of smoke concurrently. This would both dehydrate and smoke the food, without technically cooking it (as the fire contributed only smoke, not heat). "Hot smoking" uses smoke and heat to cook and flavor the meat.

Smoking does a couple things. It dries out the meat or fish, whether by direct cooking or indirect heat. Reducing the moisture content dissuades bacterial colonization, thereby preserving the food. But smoke also contains phenolic compounds that bind to the surface of the food and act as antioxidants. Phenolics with antioxidant capabilities, as you probably already know from previous discussions, can prevent oxidation and rancidity. A study even proposes that these phenolic compounds derived from smoking (with alder wood) act not only as preservatives, but also as potential health benefactors. If the smoking does not fully dehydrate the meat, however, only the surface will be protected.

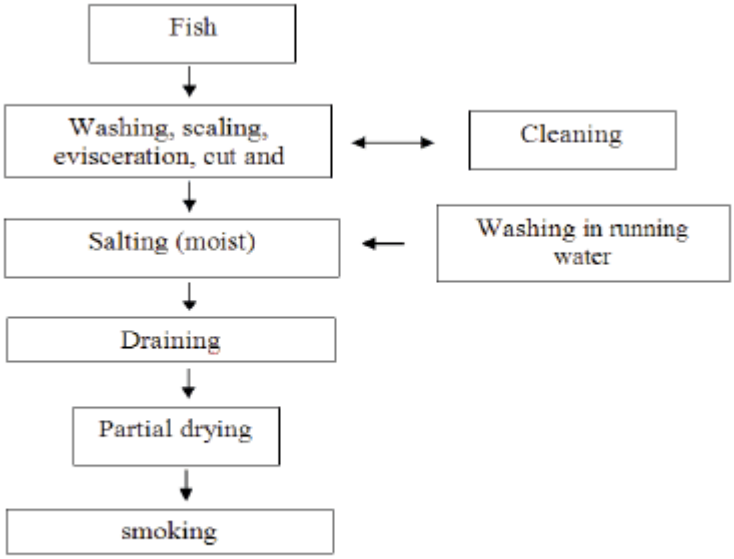


Figure 1 - Flow chart of smoking process of matrinxã.

2.5. Refrigeration

Refrigeration is a process in which work is done to move heat from one location to another. The work of heat transport is traditionally driven by mechanical work, but can also be driven by heat, magnetism, electricity, laser, or other means. Refrigeration has many applications, including, but not limited to:

household refrigerators, industrial freezers, cryogenics, and air conditioning. Heat pumps may use the heat output of the refrigeration process, and also may be designed to be reversible, but are otherwise similar to refrigeration units.

The use of refrigerators in kitchens for storing fruits and vegetables has allowed adding fresh salads to the modern diet year round, and storing fish and meats safely for long periods. Optimum temperature range for perishable food storage is 3 to 5°C (37 to 41 °F)

Dairy products are constantly in need of refrigeration, and it was only discovered in the past few decades that eggs needed to be refrigerated during shipment rather than waiting to be refrigerated after arrival at the grocery store. Meats, poultry and fish all must be kept in climate-controlled environments before being sold. Refrigeration also helps keep fruits and vegetables edible longer.

2.6. Sugaring

Sugaring is a food preservation method similar to pickling. Sugaring is the process of desiccating a food by first dehydrating it, then packing it with pure sugar. This sugar can be crystalline in the form of table or raw sugar, or it can be a high sugar density liquid such as honey, syrup or molasses.
The purpose of sugaring is to create an environment hostile to microbial life and prevent food spoilage. Sugaring is commonly used to preserve fruits as well as vegetables such as ginger. From time to time sugaring has also been used for non-food preservations. For example, honey was used as part of the mummification process in some ancient Egyptian rites.

A risk in sugaring is that sugar itself attracts moisture. Once a sufficient moisture level is reached, native yeast in the environment will come out of dormancy and begin to ferment the sugars into alcohol and carbon dioxide. This leads to the process of fermentation. Although fermentation can be used as a food preservation method, it must be intentionally controlled, or the results will tend to be unpleasant

2.7.Pickling

Pickling is the process of preserving food by anaerobic fermentation in brine or vinegar. The resulting food is called a pickle. This procedure gives the food an interesting twist in flavor. In East Asia, vinaigrette (vegetable oil and vinegar) is used as the pickling medium.

Another distinguishing characteristic is a pH 4.6 or lower, which is sufficient to kill most bacteria. Pickling can preserve perishable foods for months. Antimicrobial herbs and spices, such as mustard seed, garlic, cinnamon or cloves, are often added. If the food contains sufficient moisture, a pickling brine may be produced simply by adding dry salt. For example, German sauerkraut and Korean kimchi are produced by salting the vegetables to draw out excess water. Natural fermentation at room temperature, by lactic acid bacteria, produces the required acidity. Other pickles are made by placing vegetables in vinegar. Unlike the canning process, pickling (which includes fermentation) does not require that the food be completely sterile before it is sealed. The acidity or salinity of the solution, the temperature of fermentation, and the exclusion of oxygen determine which microorganisms dominate, and determine the flavor of the end product.

When both salt concentration and temperature are low, Leuconostocmesenteroides dominates, producing a mix of acids, alcohol, and aroma compounds. At higher temperatures Lactobacillus plantarum dominates, which produces primarily lactic acid. Many pickles start with Leuconostoc, and change to Lactobacillus with higher acidity.
Traditionally manufactured pickles are source of healthy probiotic microbes, which occur by natural fermentation in brine, but pickles produced using vinegar are not probiotic.

2.8. Canning

Canning is a method of preserving food in which the food contents are processed and sealed in an airtight container. Canning provides a shelf life typically ranging from one to five years, although under specific circumstances it can be much longer. A freeze-dried canned product, such as canned dried lentils, could last as long as 30 years in an edible state.

The packaging prevents microorganisms from entering and proliferating inside.
To prevent the food from being spoiled before and during containment, a number of methods are used: pasteurisation, boiling (and other applications of high temperature over a period of time), refrigeration, freezing, drying, vacuum treatment, antimicrobial agents that are natural to the recipe of the foods being preserved, a sufficient dose of ionizing radiation, submersion in a strong saline solution, acid, base, osmotically extreme (for example very sugary) or other microbially-challenging environments.

Other than sterilization, no method is perfectly dependable as a preservative. For example, the microorganism Clostridium botulinum (which causes botulism), can only be eliminated at temperatures above the boiling point.

From a public safety point of view, foods with low acidity (a pH more than 4.6) need sterilization under high temperature (116-130 °C). To achieve temperatures above the boiling point requires the use of a pressure canner. Foods that must be pressure canned include most vegetables, meat, seafood, poultry, and dairy products. The only foods that may be safely canned in an ordinary boiling water bath are highly acidic ones with a pH below 4.6,[3] such as fruits, pickled vegetables, or other foods to which acidic additives have been added

3. Navel food preservation methods

3.1. Membrane filtration

A membrane or, more properly, a semipermeable membrane, is a thin layer of materialcapable of separating substances when a driving force is applied across the membrane.Once considered a viable technology only for desalination, membrane processes are increasinglyemployed for removal of bacteria and other microorganisms, particulate material, andnatural organic material, which can impart color, tastes, and odors to the water and reactwith disinfectants to form disinfection byproducts (DBP). As advancements are made inmembrane production and module design, capital and operating costs continue to decline.

Membrane processes have become more attractive for potable water production in recent years due to the increased stringency of drinking water regulations. Membrane processes have excellent separation capabilities and show promise for meeting many of the existing and anticipated drinking water standards

The pressure-driven membrane processes discussed here are microfiltration(MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO).

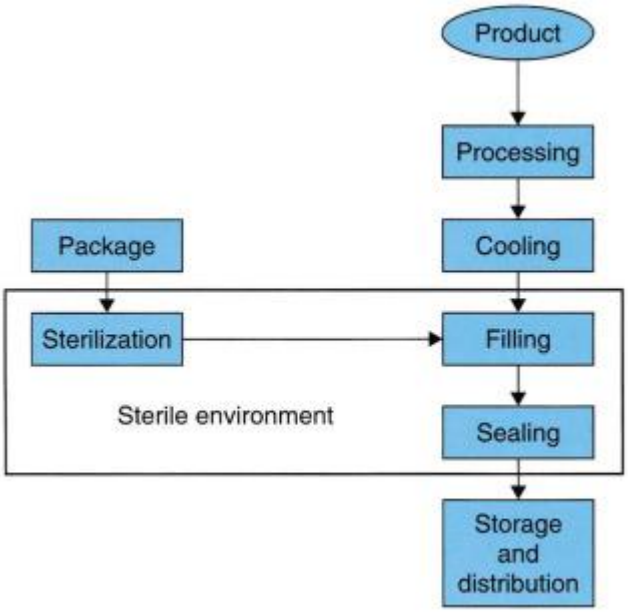
3.2 Microfiltration (MF)
MF is loosely defined as a membrane separation process using membranes with a pore size of approximately 0.03 to 10 microns, a MWCO of greater than 100,000 daltons, and a relatively low feedwater operating pressure of approximately 100 to 400 kPa (15 to 60 psi). Representative materials removed by MF include sand, silt, clays, Giardia lamblia and Cryptosporidiumcysts, algae, and some bacterial species.

MF is not an absolutebarrier to viruses; however, when used incombination with disinfection, MF appearsto control these microorganisms in water

By physically removing the pathogens, membrane filtration can significantly reduce chemical addition, such as chlorination. Another application for the technology is for removal of natural or synthetic organic matter to reduce fouling potential. In its normal operation,
MF removes little or no organic matter; however, when pretreatment is applied, increased removal of organic material, as well as a retardation of membrane fouling can be realized. Two other applications involve using MF as a pretreatment to RO or NF to reduce fouling potential. Both RO and NF have been traditionally employed to desalt or remove hardness from groundwater

3.3 Ultrafiltration (UF)
UF involves the pressure-driven separation of materials from water using a membrane pore size of approximately 0.002 to 0.1 microns, an MWCO of approximately 10,000 to 100,000 daltons, and an operating pressure of approximately 200 to 700 kPa (30 to 100 psi). UF will remove all microbiological species removed by MF (partial removal of bacteria), as well as some viruses (but not an absolute barrier to viruses) and humic materials.
Disinfection can provide a second barrier to contamination and is therefore recommended..

UF is designed to remove suspended and dissolved macromolecular solids from fluids.
The commercially available modules are therefore designed to accept feedwaters that carry high loads of solids. Because of the many uses for UF membranes, pilot studies are normally conducted to test how suitable a given stream is for direct UF.



3.4. Nano-filtration (NF)
NF membranes have a nominal pore size of approximately 0.001 microns and an MWCO of 1,000 to 100,000 daltons. Pushing water through these smaller membrane pores requires a higher operating pressure than either MF or UF. Operating pressures are usually near 600 kPa (90 psi) and can be as high as 1,000 kPa (150 psi). These systems can remove virtually all cysts, bacteria, viruses, and humic materials.
They provide excellent protection from DBP formation if the disinfectant residual is added after the membrane filtration step. Because NF membranes also remove alkalinity, the product water can be

corrosive, and measures, such as blending raw water and product water or adding alkalinity, may be needed to reduce corrosivity. NF also removes hardness from water, which accounts for NF membranes sometimes being called “softening membranes.” Hard water treated by NF will need pretreatment to avoid precipitation of hardness ions on the membrane.
More energy is required for NF than MF or UF, which has hindered its advancement as a treatment alternative.

3.5. Reverse Osmosis (RO)

RO systems are compact, simple to operate, and require minimal labor, making them suitable for small systems. They are also suitable for systems where there is a high degree of seasonal fluctuation in water demand.
RO can effectively remove nearly all inorganic contaminants from water. RO can also effectively remove radium, natural organic substances, pesticides, cysts, bacteria, and viruses. RO is particularly effective when used in series. Water passing through multiple units can achieve near zero effluent contaminant concentrations. Disinfection is also recommended to ensure the safety of water.

Some of the advantages of RO are:

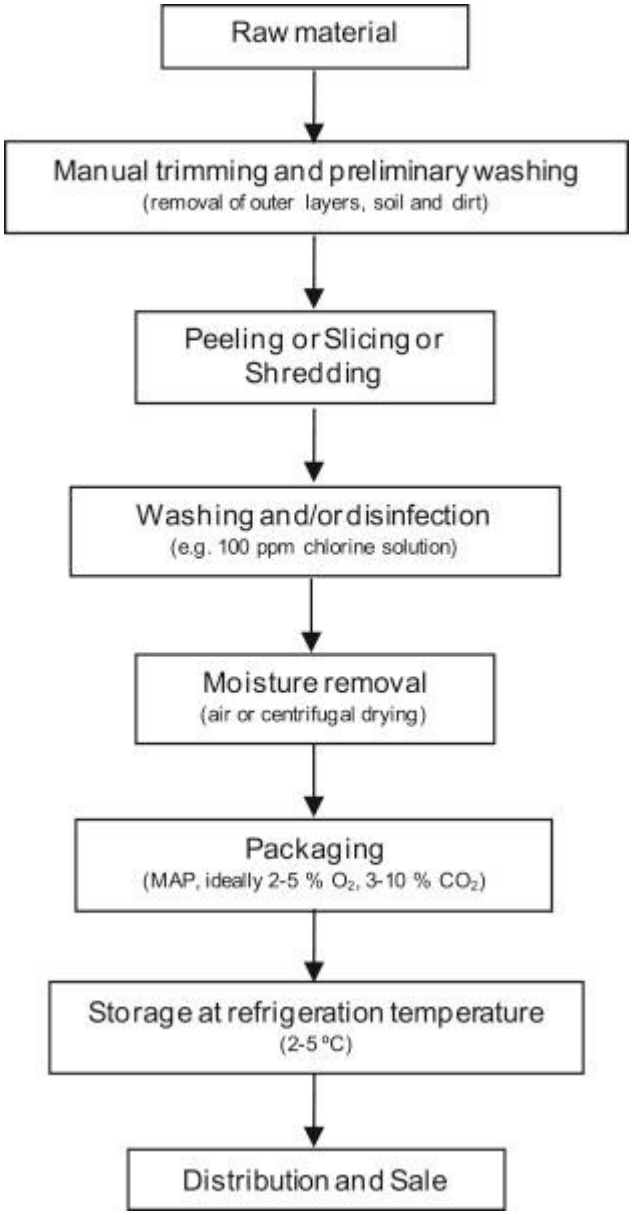
• Removes nearly all contaminant ions and most dissolved non-ions,
• Relatively insensitive to flow and total dissolved solids (TDS) level, and thus suitable for small systems with a high degree of seasonal fluctuation in water demand,
• RO operates immediately, without any minimum break-in period,
• Low effluent concentration possible,
• Bacteria and particles are also removed, and
• Operational simplicity and automation allow for less operator attention and make RO suitable for small system applications.

Some of the limitations of RO are:

• High capital and operating costs,
• Managing the wastewater (brine solution) is a potential problem,
• High level of pretreatment is required in some cases,

3.6. Modified atmospheric packaging

The modified atmosphere concept for packaged goods consists of modifying the atmosphere surrounding a food product by vacuum, gas flushing or controlled permeability of the pack thus controlling the biochemical, enzymatic and microbial actions so as to avoid or decrease the main degradations that might occur. This allows the preservation of the fresh state of the food product without the temperature or chemical treatments used by competitive preservation techniques, such as canning, freezing, dehydration and other processes.
MAP is the replacement of air in a pack with a single gas or mixture of gases; the proportion of each component is fixed when the mixture is introduced. No further control is exerted over the initial composition, and the gas composition is likely to change with time owing to the diffusion of gases into and out of the product, the permeation of gases in to and out of the pack, and the effects of product and microbial metabolism.
The normal composition of air is 21% oxygen, 78% nitrogen and less than 0.1% carbon dioxide. Modification of the atmosphere within the package by reducing the oxygen content while increasing the levels of carbon dioxide and/or nitrogen has been shown to significantly extend the shelf-life of perishable foods at chill temperatures.



Advantages of MAP:

a) Increased shelf-life allowing less frequent loading of retail display shelves;
b) Reduction in retail waste
c) Improved presentation-clear view of product and all round visibility;
d) Hygienic stackable pack, sealed and free from product drip and odour;
e) Easy separation of sliced products;
f) Little or no need for chemical preservatives;
g) Increased distribution area and reduced transport costs due to less frequent deliveries;
h) Centralised packaging and portion control;
i) Reduction in production and storage costs due to better utilisation of labour, space and equipment.

Disadvantages of MAP:

a) Capital cost of gas packaging machinery;
b) Cost of gases and packaging materials;
c) Cost of analytical equipment to ensure that correct gas mixtures are being used;
d) Cost of quality assurance systems to prevent the distribution of leakers, etc.
e) Increased pack volume which will adversely affect transport costs and retail display space;

f) Potential growth of food-borne pathogens due to temperature abuse by retailers and consumers;
g) Benefits of MAP are lost once the pack is opened or leaks.

Gases Used in MAP

The basic concept of the MAP of fresh foods is he replacement of the air surrounding the food in the package with a mixture of atmospheric gases different in proportion from that of air.

Gaseous composition of dry air at sea level

Gas	Percentage
Nitrogen (N2)	78.03
Oxygen (O2)	20.99
Argon (Ar)	0.94
Carbon dioxide (CO2)	0.03
Hydrogen (H2)	0.01

Packaging Materials

There are six main characteristics to consider when selecting packaging material for MAP foods:

1) Resistance to puncture
2) Sealing reliability
3) Antifogging properties
4) Carbon dioxide permeability
5) Oxygen permeability
6) Water transmission rate
7)

Although an increasing choice of packaging materials is available to the MAP industry, most packs are still constructed from four basic polymers: polyvinyl chloride (PVC), polyethylene terephthalate (PET), polyproylene (PP) and polyethylene (PE)

Machine Systems for MAP

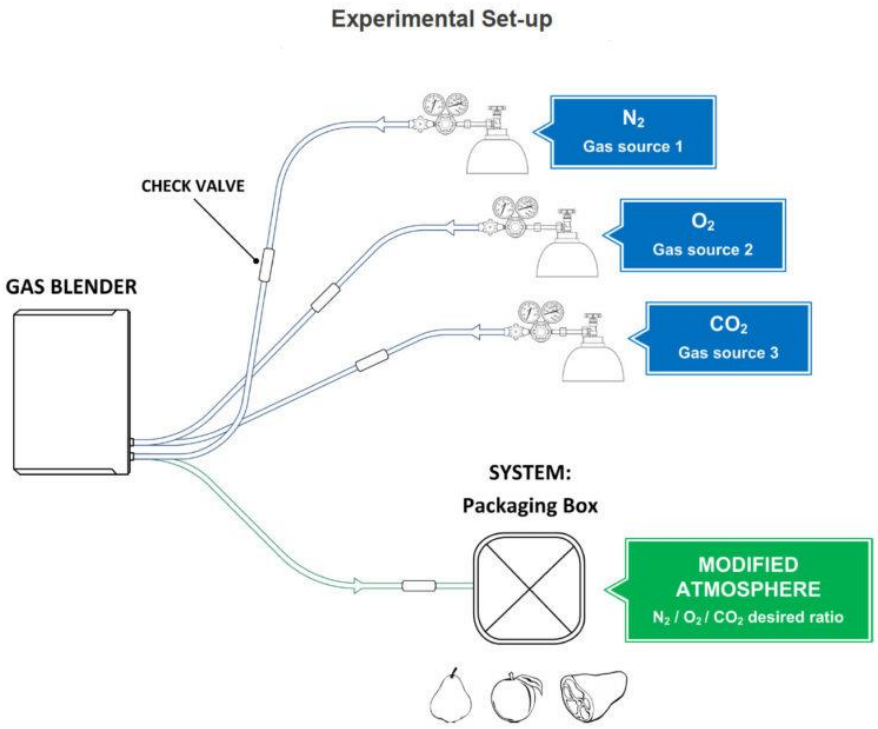
The first element for optimum gas packaging is appropriate equipment. There are two different techniques to replace the air:

1) **Gas flushing**

   The gas flush technique is normally accomplished on a form fill-seal machine. The replacement of air inside a package is performed by a continuous gas stream. This gas stream dilutes the air in the atmosphere surrounding the food product. The package is then sealed.

2) **Compensated vacuum.**

   The compensated vacuum technique removes the air inside by pulling a vacuum on the atmosphere inside the package and then breaking the vacuum with the desired gas mixtures. Since the replacement of the air is accomplished in a two-step process, the speed of operation of the equipment is slower than the gas flush technique.



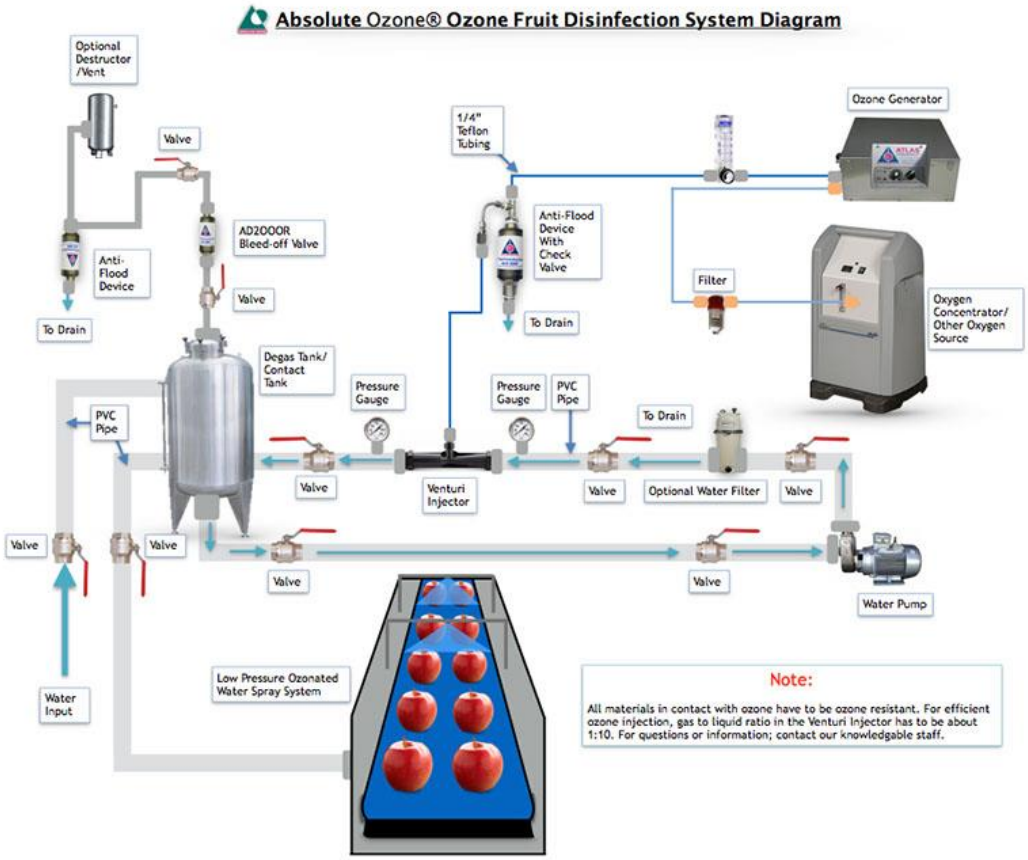
3.6.1. Ozone

-Most powerful oxidizer available, instantly destroys microbes
-Kills Ecoli and Salmonella instantly
-Longer shelf life
-Environmentally friendly
- No chemical storage
-3,000 times more germicidal than chlorine
-No harmful chemical residual
-Stops mold and spores

Ozone can reduce contamination of food and increase storage life. The age-enhancing hormone ethylene is instantly destroyed. This reduces spoiling and keeps food looking fresh. Ozone can be used in water and air to reduce bacteria levels in excess of 2-log reduction.

Because ozone is a safe, powerful disinfectant, it can be used to control biological growth of unwanted organisms in products and equipment used in the food processing industries. Ozone is particularly suited to the food industry because of its ability to disinfect microorganisms without adding chemical by-products to the food being treated, or to the food processing water or atmosphere in which food are stored.

In aqueous solutions, ozone can be used to disinfect equipment, process water, and some foodstuff. In gaseous form, ozone can act as a preservative for certain foods products and can also sanitize food packaging materials. Some products currently being preserved with ozone include eggs during cold storage, fresh fruits and vegetables, and fresh fish.

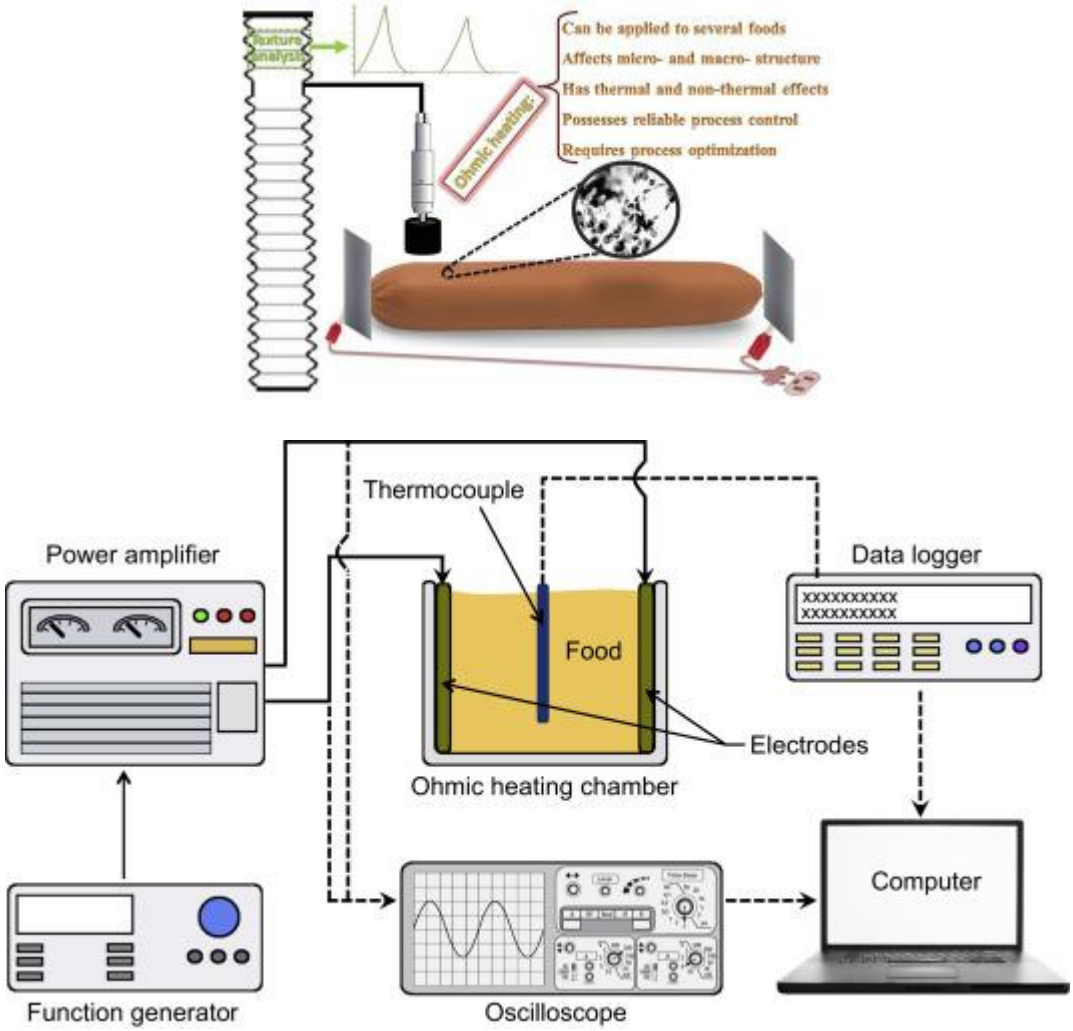


3.7. Ohmic heating

Ohmic heating is a thermal method that minimizes energy input and thus reduces thermal damage to food. If an electric current is passing through a conductive medium, in this case the food, the medium warms up as a result of the movement of ions.

The conductive electric resistance heating ohmic heating utilises the effect of the electrical resistance within a conductive liquid or solid material. In this manner a direct conversion of electric energy into heat takes place. In production plants the product is continuously pumped through a columnequipped with several electrodes.

The advantage of ohmic heating is its ability to heat materials rapidly and uniformly, including products containing particulates. The principal mechanisms of microbial inactivation in ohmic heating are thermal while some evidence exists for non-thermal effects of ohmic heating as well. A large number of potential future applications exist for ohmic heating, including its use in blanching, evaporation, dehydration, fermentation and extraction. Ohmic heating is employed in pasteurising and sterilising of liquid and particulate foods, especially of ready-to-serve meals, fruits, vegetables, meat, poultry or fish, and is an alternative to sterilisation of foods by means of conventional heat exchangers or autoclaves. The applicability is limited to foods with sufficient conductivity.



3.8. High intensity pulsed electric field (PEF or HELP)

High intensity pulsed electric field (PEF or HELP) processing involves the application of pulses of high voltage (typically 20–80 kV/cm) to foods placed between two electrodes. HELP may be applied in the form of exponentially decaying, square wave, bipolar, or oscillatory pulses and at ambient, sub-ambient, or slightly above-ambient temperature for less than 1 s.

Energy loss due to heating of foods is minimised, reducing the detrimental changes of the sensory and physical properties of food. Microbial inactivation by HELP has been explained by several theories. The most studied possibilities are electrical breakdown and electroporation.

Electric high-voltage impulses generate a trans-membrane potential across the cell membrane of, for example, a bacterial cell which overlays the natural membrane potential. If the difference between outer and inner membrane potential rises above a critical value of about 1 V, polarisation and in the end breakdown of the membrane is induced. At sufficient high field-strength (above 10 kV/cm) and duration of the pulses (usually between nano-and microseconds) vegetative micro-organisms in liquid media are inactivated due to irreversible membrane destruction.

Bacterial spores, however, are not inactivated. Factors that affect the microbial inactivation with HELP are process factors (electric field intensity, pulse width, treatment time and temperature and pulse wave

shapes), microbial entity factors (type, concentration and growth stage of micro-organism) and media factors (pH, antimicrobials and ionic compounds, conductivity and medium ionic strength).
HELP has been applied mainly to improve the quality of foods. Application of HELP is restricted to food products that can withstand high electric fields, i.e. have low electrical conductivity, and do not contain or form bubbles. The particle size of the liquid food in both static and flow treatment modes is also a limitation. Although HELP has potential as a technology for food preservation, existing HELP systems and experimental conditions are diverse, and conclusions about the effects of critical process factors on pathogens of concern and kinetics of inactivation need to be further studied.
Based on practical experience from pilot plants employment of HELP will mainly be in the sparing pasteurisation of liquid foods e.g. juices, milk or liquid whole egg.

3.10. Light pulses

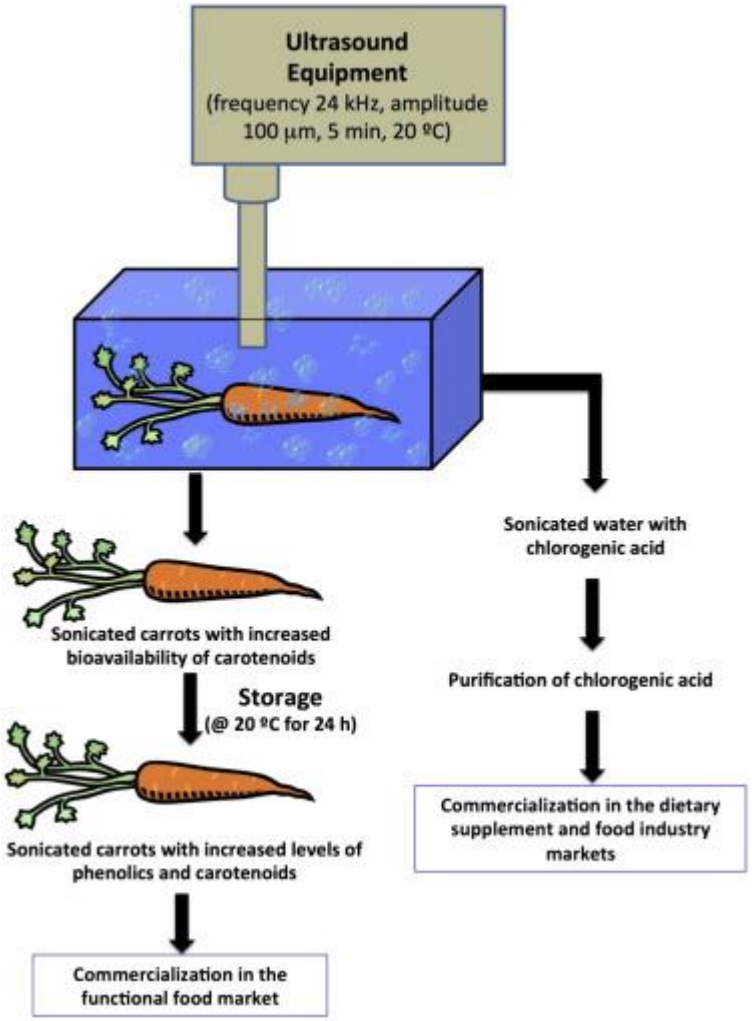
Pulsed light is a method of food preservation that involves the use of intense and short-duration pulses of broad spectrum ‘‘white light’’ (ultraviolet to the near infrared region). For most applications, a few flashes applied in a fraction of a second provide a high level of microbial inactivation. This technology is applicable mainly in sterilising or reducing the microbial population on packaging or food surfaces. It could be shown that light-impulses are able to extend the durability of bread, cakes and pastries, sea food or meat.
As light pulses penetrate certain packaging materials, wrapped items also can be treated. Still there is a need of independent research on the inactivation kinetics under a full spectrum of representative variables of food systems and surfaces.

3.11. Oscillating magnetic fields

Experiments have shown, that strong static (SMF) or oscillating (OMF) magnetic fields potential to inactivate vegetative micro-organisms. The impulse duration is between 10 ms and several miiliseconds. The frequencies are maximally 500 MHz, because above that value the items begin to warm up noticeably. Preservation of foods with OMF involves sealing food in a plastic bag and subjecting it to 1–100 pulses in an OMF at temperature of 0 to 50 C for a total exposure time ranging from 25 to 100 ms.
The effects of magnetic fields on microbial populations have produced controversial. Before considering this technology for food preservation purposes consistent results concerning the efficacy of the method are needed.

3.12. Ultrasound

Ultrasonic waves (energy generated by sound waves of 20,000 Hz or more) generate gas bubbles in liquid mediathat produce a high temperature and pressure increase when they immediately burst. The bactericidal effect of ultrasound is attributed to intracellular cavitation, that is, micro-mechanical shocks that disrupt cellular structural and functional components up to the point of cell lysis. Critical processing factors are the nature of the ultrasonic waves, the exposure time with the microorganisms, the type of micro-organism, the volume of food to be processed, the composition of the food, and the temperature.
The effects, however, are not severe enough for a sufficient reduction of micro-organisms so most applications use combinations with other preservation methods.
Because of thecomplexity and sometimes protective nature of the foodthe singular use of ultrasound as a preservation methodis impracticable. Although ultrasound technology has awide range of current and future applications in thefood industry, including inactivation of micro-organismsand enzymes, presently, most developments forfood applications are non-microbial.



3.12.High pressure processing

The technology of high pressure processing (HPP),also referred to as ultra high pressure UHP) or highhydrostatic pressure (HHP) is used to delay microbial spoilage ofmilk.

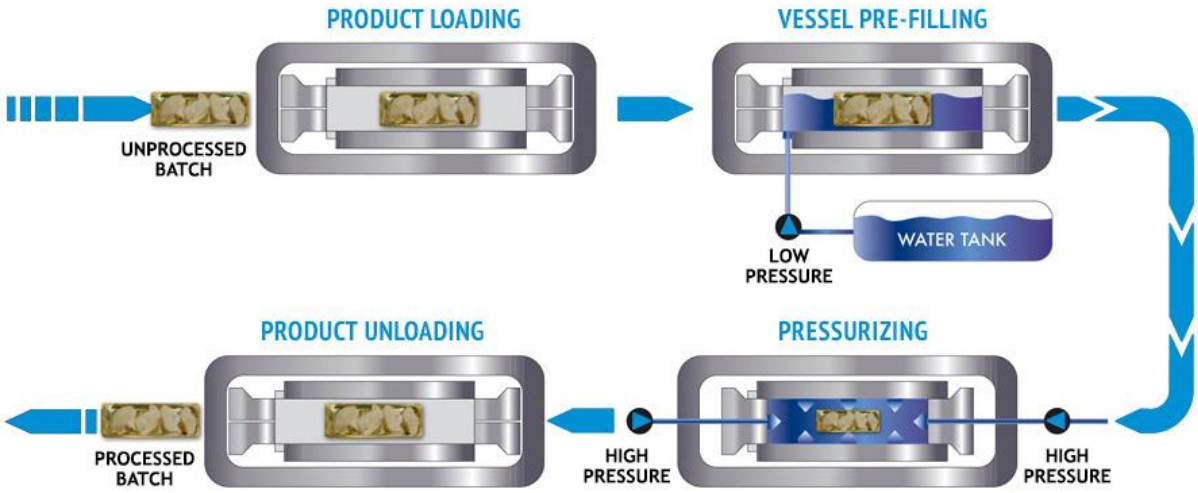
HPP subjects liquid and solid foods, with orwithout packaging, to pressures between 100 and 800MPa. Process temperature during pressure treatmentcan be from below 0 ^{o}C to above 100 ^{o}C. Exposure times can range from a few seconds to over 20 min.

Food treated in this way has been shown to keep itsoriginal freshness, colour, flavour and taste. HPP actsinstantaneously and uniformly throughout a mass offood independent of size, shape and food composition.

Water activity and pH are critical process factors in theinactivation of microbes by HPP. An increase in foodtemperature above room temperature and to a lesserextent a decrease below room temperature in some casesincreases the inactivation rate of micro-organisms duringHPP treatment.

Temperatures ranging from 90 to 110C in conjunction with pressures of500–700 MPa have been used to inactivate spore-formingbacteria such as Clostridium botulinum. Currentpressure processes include batch and semi-continuoussystems.

Besides destruction of micro-organisms there are further influences of pressure on foodmaterials to be expected: protein denaturation or modification,enzyme activation or inactivation, changes inenzyme–substrate interactions, changes in the properties of polymer carbohydrates and fats.



3.12.1. High pressure carbon dioxide (HPCD)

High pressure carbon dioxide (HPCD) is another upcoming treatment that is beingmextensively used as a non-thermal technique for food pasteurization. The process is not only environmentally friendly due to the non-toxic nature of carbon dioxide but also involves application of lower CO2 pressure as compared to those employed for HPP.

The use of lower pressures makes this technique an energy-saving process. The major factor involved in the destruction is CO2 although pressure helps in greater penetration of CO2 in the cells.

Lethality imparted by pressurized CO2 is a result of disassociation of CO2 (in foods with high water content) into reactive ions such as carbonates (CO32-), bicarbonates (HCO3-) and hydrogen (H+). These reactive ionic species can then have an effect on the permeability of the cell membrane and properties of cell constituents. In addition, generation of carbonic acid (H2CO3) in the water present in food products further results in a reduction in the pH of the food products enhancing the penetration of CO2.

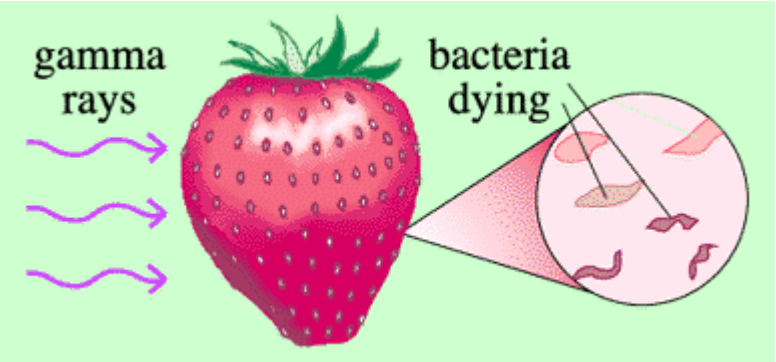
3.13. Irradiation

The use of ionizing radiation as a means of food preservation is being extensively researched and is approved in many countries. The use of radiation dose up to 7 kilo Gray (kGy) has been sanctionedby WHO as safe. The critical target of ionizing radiation is the bacterial DNA.

Gamma rays, X-rays and electron beam are the most common types of ionizing radiation.

Gamma radiation is generated using radioactive isotopes such as cobalt-60 or Cesium-137 whereas for electron beam high speed electrons are generated using electricity. Generation of X-rays involves interposition of a metal target between the food and the electron beam. The choice of use between e-beam and X-ray is typically made as an exchange between efficiency and product penetration depth. Unlike gamma radiation, the processing time using electron beam is very short and the technique does not produce radioactive waste.

The effect of both techniques on the quality is minimal as no heat is generated during the process. However, electron beam can penetrate only up to 8 cm in foods which is its major limitation. Nonetheless both these techniques are being studied for eliminating Salmonella.

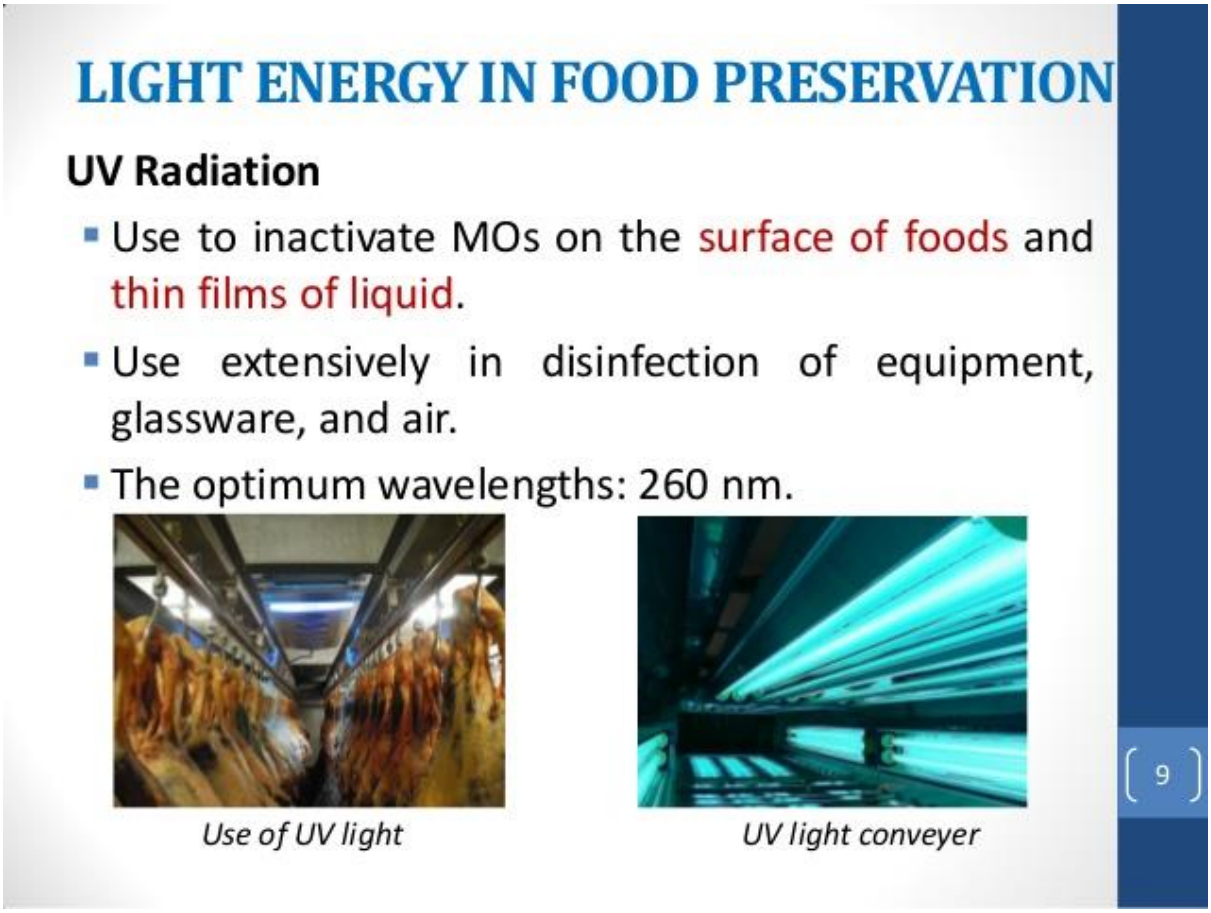


However, several adverse effects (lipid oxidation, textural degradation) caused by ionizing radiation have prevented this technology from being extended. Especially, lipid oxidation of meat products. The negative effects of gamma radiation on the appearance and color of chicken breasts, pork loin and beef loin.
Although irradiation has a high potential to be used for food preservation, its use is limited by an uncorroborated view that irradiated foods are not well accepted by the public as safe and desirable.

3.13.1. Ultra-violet radiation

Irradiation using non-ionizing rays, especially ultraviolet (UV)-C (wavelengths of 220–300 nm with 90% emission at 253.7 nm) has been approved as a non-thermal method.
This technique has been used extensively to decontaminate foodsurfaces directly or other materials which come in contact with food surfaces. The main industrial application of UV is its use in disinfection of drinking water. The mechanism of action of UV light involves the interruption of bacterial replication due to the formation of thymine dimers in the bacterial chromosome either killing them or making them unable to reproduce.

The main drawback of UV irradiation is that it is a surface sterilization method. The efficiency of the treatment will strongly depend on the actual location of the bacterial contaminant as well as the composition, surface topography and transmissivity of the food
Moreover, the penetration of UV in liquid foods will strongly depend on the characteristics of the liquid product. The presence of solid particles and other components can seriously hinder the penetration. In addition the actual physical arrangement, power and wavelength of the UV source will also play a significant role.
Besides, care has to be taken while using short wave UV regarding the damage that it can cause to human eyes in addition to being a cause of skin cancers and burns in humans upon excessive exposure



Use of UV light

UV light conveyer

4. Hurdle technology or synergism

Hurdle approach or the process of using multiple technologies is an effective approach to improve microbial decontamination in comparison to that of a single technology alone.
Deliberate and intelligent combination of preservative treatments can help in maintaining the quality of food and delivering almost similar levels of microbial destruction as conventional methods alone. At the same time it warranties to counteract the negative effect of individual technologies on food quality. The choice of hurdles will strongly depend onthe type of food it is being applied to in addition to the mode of inactivation. Potential synergistic effects among different technologies have been reported to be more effective than individual technologies applied alone. The outer membrane of gram negative cells prevents the entry of hydrophobic compounds. A combined treatment of heat and irradiation can result in sub-lethal injury to the cells. The sublethally injured cells can be more vulnerable to attack by antimicrobial compounds thereby reducing the dose of each individual technique.

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CHEMICAL METHODS OF FOOD PRESERVATION

1 Introduction

Preservative for food may be defined as any chemical compound and/or process, when applied to food, retard alterations caused by the growth of microorganisms or enable the physical properties, chemical composition and nutritive value to remain unaffected by microbial growth. Some chemicals have been used traditionally since several decades as direct or indirect inhibitors of microbial growth and are still widely used despite their limitations

The majority of food preservation operations used today also employ some kind of chemical additive to reduce spoilage. Of the many dozens of chemical additives available, all are

designed either to kill or retard the growth of pathogens or to prevent or retard chemical reactions that result in the oxidation of foods.

Some familiar examples of the former class of food additives are sodium benzoate and benzoic acid; calcium, sodium propionate, and propionic acid; calcium, potassium, sodium sorbate, and sorbic acid; and sodium and potassium sulfite. Examples of the latter class of additives include calcium, sodium ascorbate, and ascorbic acid (vitamin C); butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT); lecithin; and sodium and potassium sulfite and sulphur dioxide.

2 Classification of Preservatives

According to FSSA rules → class I and class II preservatives

Class I preservatives

a. Common salt
b. Sugar
c. Dextrose
d. Glucose
e. Spices
f. Vinegar or acetic acid
g. Honey
h. Edible vegetable oil

Addition of class I preservatives in any food is not restricted, unless otherwise provide in the rule.

Class II preservatives

a. Benzoic acid including salts their of
b. Sulphurous acid including salts theirof
c. [Nitrates of] nitrites of sodium or potassium
d. Sorbic acid including its sodium, potassium and calcium salts
e. Nicin
f. Propionic acid including salts theirof
g. Methyl or propyl para-hydroxy benzoate
h. Sodium diacetate
i. Sodium, potassium and calcium salts of lactic acid

Use of class II preservatives is restricted. They shall be added to only specified product and at a concentration not exceeding the proportion specified for the product

Use of more than one class II preservative is prohibited. No person shall use in or upon a food more than one class II preservative

2.1 Benzoic acid and its salt

Widely use as an antimicrobial agent. **Benzoate is more effective against yeasts and bacteria** than molds. Antimicrobial activity is achieved by inhibition in enzymatic system of microbial cells, affecting acetic acid metabolism, citric acid cycle and oxidative phosphorylation. Antimicrobial activity is affected by pH of medium. The maximum inhibition occurs at pH value of 2.5 to 4.0 and it decreases when pH rises above 4.5.

The food products preserved with the benzoate include fruit juices and drinks, salads, jams and jellies, pickles, dried fruits and preserves, ketch up and sauce, syrup, carbonated beverages, bakery items, salad dressings, margarine and other fat spreads, spices.

2.2 Sulphur dioxide and sulfites

Sulphur dioxide (SO_2) gas is one of the oldest antimicrobial agents. It is a colourless, nonflammable gaseous compound or liquid under pressure with a suffocating pungent odour. When dissolved in water of foods, it yields sulphurous acid and its ions, owing to its solubility in water.

Sulphite salts such as sodium sulphite, sodium bisulphite, potassium sulphite, potassium bisulphite, sodium metabisulphite, potassium metabisulphite used as preservatives. When dissolved in water, form sulphurous acid, bisulphite and ions. **Sulphurous acid formed from these compounds is an active antimicrobial substance.** The effectiveness of sulphurous acid is enhanced at low pH values. Antimicrobial activity of sulfites against yeasts, molds and bacteria is selective, with certain species being more sensitive to inhibition than others. **Bacteria are generally more sensitive** to inhibition than yeasts and molds. In addition to antimicrobial action, they are also used, to prevent enzymatic and non enzymatic changes as well as discoloration in some foods. Sulphur dioxide and sulphites are used in fruit products such as fruit juice concentrate, squashes, pickles and chutneys.

2.3 Sorbic acid and its salts

Sorbic acid and its salts (calcium, potassium or sodium salts) are **effective antimicrobial agents against yeast and molds,** as well as bacteria. **They are less effective against bacteria**. Sorbate has

an upper pH limit for activity around 6.0-6.5. The food products preserved with sorbates are carbonated beverages, salad dressings, tomato products, jams, jellies, syrup, candy and chocolate syrup, cheese, sausages, smoked fish, fruit juices, grains, breads and cakes.

2.4 Propionic acid and its salts

Propionic acid & its salts (Ca & Na) are used most extensively in the prevention of mold growth and rope development in baked goods and for mold inhibition in many cheese foods and spreads. **They are more effective against molds** as compared to yeasts and bacteria. Propionates has an upper pH limit for activity around 5 to 6.

2.5 Lactic acid and its salts

Lactic acid is formed during fermentation of lactose by lactic acid bacteria. Lactic acid & its salts are not very common & not easily available. It can be used in pickles (with acetic acid), fermented dough crispy biscuits, some beverages, dairy products & meat & meat products. Calcium lactate is used as a firming agent in pickles, fruits & vegetables. Na & K lactate are also recommended with sodium diacetate for control of food poisoning & other bacteria in meat product.

2.6 Acetic acid

Acetic acid has antimicrobial properties. **The action tends to be static** rather than cidal. **It is more effective against bacteria & yeast** than molds. A 5 to 10% solution of acetic acid is known as Vinegar. Acetic acid in the form of vinegar is used in mayonnaise, pickles, sauce, pickled sausage etc.

2.7 Sodium chloride (common salt)

Antimicrobial action of NaCl arises from its lowering water activity (aw) of the food product. This reduces available water in food to the extent which renders condition unfavorable for microbial growth. At higher concentration it has a pronounced bacteriostatic action. The 10% NaCl inhibits the growth of most bacteria. Delaying action upon microorganisms- Creates dehydration of microbial cell—by osmosis—altering results into plasmosis of the cell. Reduction in solubility of oxygen in water decreases oxygen level in food—reduce growth of aerobic microorganisms. **It is more effective against bacteria & mold** compare to yeast. One of the traditional method of food preservation. Mainly used to preserve pickles, meat and fish. Fish is usually salted by immersing in brine or by mixing with dry salt. High important as a preservative for cheese & table butter.

Depending upon type of cheese salt content varied from 1 to 5 %. In table butter salt is added at a max concentration as 3%.

2.8 Sucrose (sugar)

More effective against bacteria & mold compared to yeast. Antimicrobial action of sucrose arises from, lowering water activity (aw) of the food product—reduce the available water in food to the extent which renders condition unfavourable for microbial growth. This creates dehydration of microbial cell—by osmosis results into plasmosis of the cells. The food products preserved with sugar are fruit products (jam, jellies, squash etc.), dairy products (sweetened condensed milk, sweets).