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Food Spoilage: Microorganisms and their prevention

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ABSTRACT

Food spoilage can be defined as "any sensory change (tactile, visual, olfactory or flavour)" which the consumer considers to be unacceptable. Spoilage may occur at any stage along food chain. Spoilage may arise from insect damage, physical damage, indigenous enzyme activity in the animal or plant tissue or by microbial infections. Most natural foods have a limited life. Perishable foods such as fish, meat and bread have a short life span. Other food can be kept for a considerably longer time but decomposes eventually. Enzymes can bring about destruction of polymers in some foods while chemical reactions such as oxidation and rancidity decompose others but the main single cause of food spoilage is invasion by microorganisms such as moulds, yeast and bacteria. In case of mould spoilage a furry growth covers the food and it becomes soft and often smells bad. Bacterial contamination is more dangerous because very often food does not look bad even though severely infected, it may appear quite normal. The presence of highly dangerous toxins and bacterial spores is often not detected until after an outbreak of food poisoning, laboratory examination uncovers the infecting agent.

Key words: Food spoilage, Enzymes, Bacterial contamination, Food poisoning, Perishable foods.

INTRODUCTION

Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human consumption due to changes in sensory characteristics. Spoiled foods may be safe to eat, i.e. they may not cause illness because there are no pathogens or a toxin present, but changes in texture, smell, taste, or appearance cause them to be rejected. Some ecologists have suggested these noxious smells are produced by microbes to repulse large animals, thereby keeping the food resource for themselves [1]. Food loss, from farm to fork, causes considerable environmental and economic effects. The USDA Economic Research Service estimated that more than ninety-six billion pounds of food in the U.S. were lost by retailers, foodservice and consumers in 1995. Fresh produce and fluid milk each accounted for nearly 20% of this loss while lower percentages were accounted for by grain piroducts (15.2%), caloric sweeteners (12.4%), processed fruits and vegetables (8.6%), meat, poultry and fish (8.5%), and fat and oils (7.1%) [2]. Some of this food would have been considered still edible but was discarded because it was perishable, past its sell-by date, or in excess of needs. There are also environmental and resource costs associated with food spoilage and loss. If 20% of a crop is lost, then 20% of the fertilizer and irrigation water used to grow that crop was also lost. Shelf life of a food is the time during which it remains stable and retains its desired qualities.

The wide array of available dairy foods challenges the microbiologist, engineer, and technologist to find the best ways to prevent the entry of microorganisms, destroy those that do get in along with their enzymes, and prevent the growth and activities of those that escape processing treatments. Troublesome spoilage microorganisms include aerobic psychrotrophic Gram-negative bacteria, yeasts, molds, heterofermentative lactobacilli, and spore-forming bacteria. Psychrotrophic bacteria can produce large amounts of extracellular hydrolytic enzymes, and the extent of recontamination of pasteurized fluid milk products with these bacteria is a major determinant of their shelf life. Fungal spoilage of dairy foods is manifested by the presence of a wide variety of metabolic by-products, causing off-odors and flavors, in addition to visible changes in color or texture.

Coliforms, yeasts, heterofermentative lactic acid bacteria, and spore-forming bacteria can all cause gassing defects in cheeses. The rate of spoilage of many dairy foods is slowed by the application of one or more of the following treatments: reducing the pH by fermenting the lactose to lactic acid; adding acids or other approved preservatives; introducing desirable microflora that restricts the growth of undesirable microorganisms; adding sugar or salt to reduce the water activity (a_w); removing water; packaging to limit available oxygen; and freezing. The type of spoilage microorganisms differs widely among dairy foods because of the selective effects of practices followed in production, formulation, processing, packaging, storage, distribution, and handling [3].

Scenario of food spoilage worldwide

The issue of food losses is of high importance in the efforts to combat hunger, raise income and improve food security in the world's poorest countries. Food losses have an impact on food security for poor people, on food quality and safety, on economic development and on the environment. The exact causes of food losses vary throughout the world and are very much dependent on the specific conditions and local situation in a given country. In broad terms, food losses will be influenced by crop production choices and patterns, internal infrastructure and capacity, marketing chains and channels for distribution, and consumer purchasing and food use practices. Irrespective of the level of economic development and maturity of systems in a country, food losses should be kept to a minimum.

Food losses represent a waste of resources used in production such as land, water, energy and inputs. Producing food that will not be consumed leads to unnecessary CO_2 emissions in addition to loss of economic value of the food produced. Economically avoidable food losses have a direct and negative impact on the income of both farmers and consumers. Given that many smallholders live on the margins of food insecurity, a reduction in food losses could have an immediate and significant impact on their livelihoods. For poor consumers (food insecure or at-risk households), the priority is clearly to have access to food products that are nutritious, safe and affordable.

It is important to note that food insecurity is often more a question of access than a supply problem. Improving the efficiency of the food supply chain could help to bring down the cost of food to the consumer and thus increase access. Given the magnitude of food losses, making profitable investments in reducing losses could be one way of reducing the cost of food. But that would, of course, require that financial gains from reduced losses are not outweighed by their costs. How much food is lost and wasted in the world today and how can we prevent food losses? Those are questions impossible to give precise answers to, and there is not much ongoing research in the area. This is quite surprising as forecasts suggest that food production must increase significantly to meet future global demand [4].

Worldwide postharvest fruit and vegetables losses are as high as 30 to 40% and even much higher in some developing countries. Reducing postharvest losses is very important; ensuring that sufficient food, both in quantity and in quality is available to every inhabitant in our planet. The prospects are also that the world population will grow from 5.7 billion inhabitants in 1995 to 8.3 billion in 2025. World production of vegetables amounted to 486 million ton, while that of fruits reached 392 million ton. Reduction of post-harvest losses reduces cost of production, trade and distribution, lowers the price for the consumer and increases the farmer's income.

Fruits and vegetables are very important food commodities not only in India but all over the world. India, which is the second most populated country of the world, is still struggling to achieve self-sufficiency to feed about 800 million people. For this purpose, fruits and vegetables have got their specific importance to provide a balance and healthy diet to the people. India is the second largest producer of vegetables and fourth largest producer of fruits in the world. Though India is producing adequate quantities of fruits and vegetables, yet on account of losses in the field as well as in storage, they become inadequate. Generally, about 30 % fruits and vegetables are rendered unfit for consumption due to spoilage after harvesting. India annually produces fruits and vegetables of the value of about Rs. 7000 crores and wastage may be of the order of Rs. 2100 crores. This is a huge loss of valuable food even when the mini- mum food requirement of the population is not met. Therefore, it is important not only to grow more, but also to save what is grown at high cost.

Post harvest loss of fruits and vegetables has been defined as "that weight of wholesome edible product (exclusive of moisture content) that is normally consumed by human and that has been separated from the medium and sites of its immediate growth and production by deliberate human action with the intention of using it for human feeding but which for any reasons fails to be consumed by human." Not only quantity and quality but even the appearance of fruits and vegetables are affected and their market value is reduced. Most 'skin deep' injuries such as 'fly speck' in apples do not affect the edible part. Some infections are not harmful as they occur on inedible parts and can be trimmed off before.

Fresh fruits and vegetables are perishable and highly prone to these losses because they are composed of living tissues. These tissues must be kept alive and health throughout the process of marketing. These are composed of thousands of living cells which require care and maintenance.Therefore, the reduction of post-harvest loss of fruit and vegetables is a complementary means for increasing production. It may not be necessary to considerably step up the production of fruits and vegetables with the growing demand if the post-harvest loss is reduced to a great extent. The cost of preventing losses after harvest in general is less than preventing a similar additional amount of fruit and vegetable crop of the same quality. Attention to the concept of post-harvest food loss reduction, as a significant means to increase food availability, was drawn by the World Food Conference held in Rome in 1974.

The global dairy industry is impressive by large. In 2005, world milk production was estimated at 644 million tons, of which 541 million tons was cows' milk. The leading producers of milk were the European Union at 142 million tons, India at 88 million tons, the United States at 80 million tons (20.9 billion gallons), and Russia at 31 million tons. Cheese production amounted to 8.6 million tons in Western Europe and 4.8 million tons in the United States [5]. The vast array of products made from milk worldwide leads to an equally impressive array of spoilage microorganisms. A survey of dairy product consumption revealed that 6% of US consumers would eat more dairy products if they stayed fresher longer [6]. Products range from those that are readily spoiled by microorganisms to those that are shelf stable for many months, and the spoilage rate can be influenced by factors such as moisture content, pH, processing parameters, and temperature of storage.

Scenario of food spoilage in India

India is the second major producer of fruits and vegetables and ranks next to Brazil and China respectively, in the world. It contributes 10 percent of world fruit production and 14 per cent of world vegetable production. Fruits and vegetables are more prone to spoilage than cereals due to their nature and composition, and this spoilage occurs at the time of harvesting, handling transportation, storage, marketing and processing resulting in waste. Efficient management of these wastes can help in preserving vital nutrients of our foods and feeds, and bringing down the cost of production of processed foods, besides minimizing pollution hazards. According to India Agricultural Research Data Book 2004, the losses in fruits and vegetables are to the tune of 30 per cent. Taking estimated production of fruits and vegetables in India at 150 million tones, the total waste generated comes to 50 million tones per annum. The post-harvest technologies for perishable horticultural produce serve as an effective tool for getting better return to the produce and also help in avoiding wastage both at production site and distribution centers, which will help in regulating the market infrastructure.

Like any other food, fruits and vegetables are also prone to microbial spoilage caused by fungi, bacteria, yeast and moulds. A significant portion of losses of fruits and vegetables during post-harvest period is attributed to diseases caused by fungi and bacteria. The succulent nature of fruits and vegetables makes them easily invaded by these organisms. Besides attacking fresh fruits and vegetables, these organisms also cause damage to canned and processed products. Many serious post-harvest diseases occur rapidly and cause extensive break down of the commodity, sometimes spoiling the entire package. It is estimated that 36 % of the vegetable decay is caused by soft rot bacteria. Similarly fruit rot in aonla and other soft fruits caused by fungi is also very destructive. As far as vegetables are concerned, naturally the source of infection is from the field, water used for cleaning the surface, contact with equipment and storage environment. The most common pathogens causing rots in vegetables and fruits are fungi such as *Alternaria*, *Botrytis*, *Diplodia*, *Monilinia*, *Phomopsis*, *Rhizopus*, *Pencillium*, *Fusarium*, etc. Among bacteria *Ervinia*, *Pseudomonas*, etc. cause extensive damage.

High temperature and relative humidity favour the development of post-harvest decay organisms. More acidic tissue is generally attacked by fungi, while fruits and vegetables having pH above 4.5 are more commonly attacked by bacteria, ego bacterial soft rot of potato caused by *Ceratocystis*, *fimbriata*, water soft rot of carrot by *Sclerotinia sclerotiorum* etc.

In India, there is a vast scope for growing fruit and vegetable throughout the yearin one or other part of the country because the climatic conditions are highlysuitable for growing various types of fruits and vegetables. Fruit and vegetable are highly perishable but most important commodity for human diet due to their high nutritional value. They are the cheapest and other source of protective food supplied in fresh or processed or preserved form throughout the year for human consumption. Hence the national picture will improve significantly.

Fruit and vegetable are available in surplus only in certain seasons and availability in different regions. In peak season due to improper handling practices, marketing, storage problems around 20-25% fruit and vegetable are spoilt in various stages. Fruit and vegetable are living commodities as they respire. Hence, proper post harvest management handling and processing is required in horticulture crops. A variety of fresh fruit and vegetable in India can be made available in plenty due to favourable agro-climatic situations. Hence there is no derth for raw material for processing. Product profile being developed in India at present is limited to few fruit and vegetable like mango, pineapple, grapes etc. But there is a wider potentiality for processing of papaya, banana, jack, guava, aonla, carambola and other minor fruits. Similarly there is a greater scope for processing cauliflower, carrot, bitter-gourd onion, garlic, watermelon, muskmelon etc.

Proper handling, packaging, transportation and storage reduce the post-harvest losses of fruit and vegetables. For every one percent reduction in loss will save 5 million tons of fruit and vegetable per year. Processing and preservation technology helps. There are about 4000 small and large scale processing units in the country which process only about 2.5% of the total fruit and vegetable as against 40-85% in developed countries.

Food spoilage microorganisms

Chemical reactions that cause offensive sensory changes in foods are mediated by a variety of microbes that use food as a carbon and energy source. These organisms include prokaryotes (bacteria), single-celled organisms lacking defined nuclei and other organelles, and eukaryotes, single-celled (yeasts) and multicellular (molds) organisms with nuclei and other organelles. Some microbes are commonly found in many types of spoiled foods while others are more selective in the foods they consume; multiple species are often identified in a single spoiled food item but there may be one species (a specific spoilage organism, SSO) primarily responsible for production of the compounds causing offodors and flavors. Within a spoiling food, there is often a succession of different populations that rise and fall as different nutrients become available or are exhausted. Some microbes, such as lactic acid bacteria and molds, secrete compounds that inhibit competitors [7].

Spoilage microbes are often common inhabitants of soil, water, or the intestinal tracts of animals and may be dispersed through the air and water and by the activities of small animals, particularly insects. It should be noted that with the development of new molecular typing methods, the scientific names of some spoilage organisms, particularly the bacteria, have changed in recent years and some older names are no longer in use. Many insects and small mammals also cause deterioration of food but these will not be considered here.

Yeasts

Yeasts are a subset of a large group of organisms called fungi that also includes molds and mushrooms. They are generally single-celled organisms that are adapted for life in specialized, usually liquid, environments and, unlike some molds and mushrooms, do not produce toxic secondary metabolites. Yeasts can grow with or without oxygen (facultative) and are well known for their beneficial fermentations that produce bread and alcoholic drinks. They often colonize foods with a high sugar or salt content and contribute to spoilage of maple syrup, pickles, and sauerkraut. Fruits and juices with a low pH are another target, and there are some yeasts that grow on the surfaces of meat and cheese.

There are four main groups of spoilage yeasts: *Zygosaccharomyces* and related genera tolerate high sugar and high salt concentrations and are the usual spoilage organisms in foods such as honey, dried fruit, jams and soy sauce. They usually grow slowly, producing off-odors and flavors and carbon dioxide that may cause food containers to swell and burst. *Debaryomyces hansenii* can grow at salt concentrations as high as 24%, accounting for its frequent isolation from salt brines used for cured meats, cheeses, and olives. This group also includes the most important spoilage organisms in salad dressings [8]. *Saccharomyces* spp. are best known for their role in production of bread and wine but some strains also spoil wines and other alcoholic beverages by producing gassiness, turbidity and off-flavors associated with hydrogen sulfide and acetic acid. Some species grow on fruits, including yogurt containing fruit, and some are resistant to heat processing [9].

Candida and related genera are a heterogeneous group of yeasts, some of which also cause human infections. They are involved in spoilage of fruits, some vegetables and dairy products [10]. *Dekkera/Brettanomyces* are principally involved in spoilage of fermented foods, including alcoholic beverages and some dairy products. They can produce volatile phenolic compounds responsible for off-flavors [11].

Molds

Molds are filamentous fungi that do not produce large fruiting bodies like mushrooms. Molds are very important for recycling dead plant and animal remains in nature but also attack a wide variety of foods and other materials useful to humans. They are well adapted for growth on and through solid substrates, generally produce airborne spores, and require oxygen for their metabolic processes.

Most molds grow at a pH range of 3 to 8 and some can grow at very low water activity levels (0.7–0.8) on dried foods. Spores can tolerate harsh environmental conditions but most are sensitive to heat treatment. An exception is *Byssochlammys,* whose spores have a D value of 1–12 minutes at 90ºC. Different mold species have different optimal growth temperatures, with some able to grow in refrigerators. They have a diverse secondary metabolism producing a number of toxic and carcinogenic mycotoxins. Some spoilage molds are toxigenic while others are not [12].

Spoilage molds can be categorized into four main groups: Zygomycetes are considered relatively primitive fungi but are widespread in nature, growing rapidly on simple carbon sources in soil and plant debris, and their spores are commonly present in indoor air. Generally they require high water activities for growth and are notorious for causing rots in a variety of stored fruits and vegetables, including strawberries and sweet potatoes. Some common bread molds also are zygomycetes. Some zygomycetes are also utilized for production of fermented soy products, enzymes, and organic chemicals. The most common spoilage species are *Mucor* and *Rhizopus*. Zygomycetes are not known for producing mycotoxins but there are some reports of toxic compounds produced by a few species.

Penicillium and related genera are present in soils and plant debris from both tropical and Antarctic conditions but tend to dominate spoilage in temperate regions. They are distinguished by their reproductive structures that produce chains of conidia. Although they can be useful to humans in producing antibiotics and blue cheese, many species are important spoilage organisms, and some produce potent mycotoxins (patulin, ochratoxin, citreoviridin, penitrem). *Penicillium* spp. cause visible rots on citrus, pear, and apple fruits and cause enormous losses in these crops. They also spoil other fruits and vegetables, including cereals. Some species can attack refrigerated and processed foods such as jams and margarine. A related genus, *Byssochlamys*, is the most important organism causing spoilage of pasteurized juices because of the high heat resistance of its spores.

Aspergillus and related molds generally grow faster and are more resistant to high temperatures and low water activity than *Penicillium* spp. and tend to dominate spoilage in warmer climates. Many aspergilla produce mycotoxins: aflatoxins, ochratoxin, territrems, cyclopiazonic acid. Aspergilli spoil a wide variety of food and non-food items (paper, leather, etc.) but are probably best known for spoilage of grains, dried beans, peanuts, tree nuts, and some spices.

Other molds, belonging to several genera, have been isolated from spoiled food. These generally are not major causes of spoilage but can be a problem for some foods. *Fusarium* spp. cause plant diseases and produce several important mycotoxins but are not important spoilage organisms. However, their mycotoxins may be present in harvested grains and pose a health risk.

Bacteria

Spore-forming bacteria are usually associated with spoilage of heat-treated foods because their spores can survive high processing temperatures. These Gram-positive bacteria may be strict anaerobes or facultative (capable of growth with or without oxygen). Some spore-formers are thermophilic, preferring growth at high temperatures (as high as 55ºC). Some anaerobic thermophiles produce hydrogen sulphide (*Desulfotomaculum*) and others produce hydrogen and carbon dioxide (*Thermoanaerobacterium*) during growth on canned/ hermetically sealed foods kept at high temperatures, for example, soups sold in vending machines.

Other thermophiles (*Bacillus* and *Geobacillus* spp.) cause a flat sour spoilage of high or low pH canned foods with little or no gas production, and one species causes ropiness in bread held at high ambient temperatures [13]. Mesophilic anaerobes, growing at ambient temperatures, cause several types of spoilage of vegetables (*Bacillus* spp.); putrefaction of canned products, early blowing of cheeses, and butyric acid production in canned vegetables and fruits (*Clostridium* spp.); and "medicinal" flavors in canned low-acid foods (*Alicyclobacillus*) (Chang & Kang, 2003). Psychrotolerant sporeformers produce gas and sickly odors in chilled meats and brine-cured hams (*Clostridium* spp.) while others produce off-odors and gas in vacuum-packed, chilled foods and milk (*Bacillus* spp.).

Lactic acid bacteria (LAB) are a group of Gram-positive bacteria, including species of *Lactobacillus*, *Pediococcus*, *Leuconostoc* and *Oenococcus*, some of which are useful in producing fermented foods such as yogurt and pickles. However, under low oxygen, low temperature, and acidic conditions, these bacteria become the predominant spoilage organisms on a variety of foods. Undesirable changes caused by LAB include greening of meat and gas formation in cheeses (blowing), pickles (bloater damage), and canned or packaged meat and vegetables. Off-flavors described as mousy, cheesy, malty, acidic, buttery or liver-like may be detected in wine, meats, milk, or juices spoiled by these bacteria. LAB may also produce large amounts of an exopolysaccharide that causes slime on meats and ropy spoilage in some beverages.

Pseudomonas and related genera are aerobic, gram-negative soil bacteria, some of which can degrade a wide variety of unusual compounds. They generally require a high water activity for growth (0.95 or higher) and are inhibited by pH values less than 5.4. Some species grow at refrigeration temperatures (psychrophilic) while other are adapted for growth at warmer, ambient temperatures. Four species of *Pseudomonas* (*P. fluorescens, P. fragi, P. lundensis,* and *P. viridiflava*), *Shewanella putrefaciens,* and *Xanthomonas campestris* are the main food spoilage organisms in this group. Soft rots of plant-derived foods occur when pectins that hold adjacent plant cells together are degraded by pectic lyase enzymes secreted by *X. campestris, P. fluorescens* and *P. viridiflava*. These two species of *Pseudomonas* comprise up to 40% of the naturally occurring bacteria on the surface of fruits and vegetables and cause nearly half of post-harvest rot of fresh produce stored at cold temperatures. *P. fluorescens, P. fragi, P. lundensis*, and *S. putrefacien*s cause spoilage of animal-derived foods (meat, fish, milk) by secreting lipases and proteases that cause formation of sulfides and trimethylamine (off-odors) and by forming biofilms (slime) on surfaces (*55;73*). Some strains are adapted for growth at cold temperatures and spoil these foods in the refrigerator.

Enterobacteriaceae are gram-negative, facultatively anaerobic bacteria that include a number of human pathogens (*Salmonella, E. coli, Shigella, Yersinia*) and also a large number of spoilage organisms. These bacteria are widespread in nature in soil, on plant surfaces and in digestive tracts of animals and are therefore present in many foods. *Erwinia carotovora* is one of the most important bacteria causing soft rot of vegetables in the field or stored at ambient temperatures.

Biogenic amines are produced in meat and fish by several members of this group while others produce off-odors or colors in beer (*Obesumbacterium*), bacon and other cured meats (*Proteus, Serratia*), cheeses (several genera), cole slaw (*Klebsiella*), and shell eggs (*Proteus, Enterobacter, Serratia*). Temperature, salt concentration, and pH are the most important factors determining which, if any, of these microbes spoil foods.

Many Gram-negative bacteria, including pseudomonads and enterobacteriaceae, secrete acyl homoserine lactones (AHLs) to regulate the expression of certain genes, such as virulence factors, as a function of cell density. These AHL quorum-sensing signals may regulate proteolytic enzyme production and iron chelation during spoilage of some foods [14] although the role of these signals in other spoilage systems is not clear [15].

Other bacteria are associated with spoilage of chilled, high protein foods such as meat, fish, and dairy products. They may not be the predominant spoilage organisms but contribute to the breakdown of food components and may produce off-odors.Most species are aerobic although some grow at lowoxygen levels and may survive vacuum packaging,and one (*Brochothrix*) is a facultative anaerobe. Some examples include: *Acinetobacter* and *Psychrobacter*, which are predominant bacteria on poultry carcasses on the processing line and have been isolated from a variety of spoiled meat and fish. *Acinetobacter* grows at a pH as low as 3.3 and has been detected in spoiled soft drinks. These two genera do not produce extracellular lipases, hydrogen sulfide, or trimethylamine (fishy odor) and so are considered to have a low spoilage potential.

Alcaligenes is a potential contaminant of dairy products and meat and has been isolated from rancid butter and milk with an off-odor. These bacteria occur naturally in the digestive tract of some animals and also in soil and water. *Flavobacterium* is found widely in the environment and in chilled foods, particularly dairy products, fish, and meat. It uses both lipases and proteases to produce disagreeable odors in butter, margarine, cheese, cream, and other products with dairy ingredients. *Moraxella* and *Photobacterium* are important constituents of the microflora on the surface of fish. *Photobacterium* can grow and produce trimethylamine in ice-stored, vacuum-packaged fish. *Brochothrix* has been isolated from meat, fish, dairy products and frozen vegetables. During spoilage, it produces odors described as sour, musty, and sweaty [16].

Spoilage of fruits and vegetables

The main sources of microorganisms in vegetables are soil, water, air, and other environmental sources, and can include some plant pathogens. Fresh vegetables are fairly rich in carbohydrates (5% or more), low in proteins (about 1 to 2%), and, except for tomatoes, have high pH. Microorganisms grow more rapidly in damaged or cut vegetables. The presence of air, high humidity, and higher temperature during storage increases the chances of spoilage. The common spoilage defects are caused by molds belonging to genera *Penicillium, Phytophthora, Alternaria, Botrytis,* and *Aspergillus*. Among the bacterial genera, species from *Pseudomonas, Erwinia, Bacillus,* and *Clostridium* are important. Microbial vegetable spoilage is generally described by the common term rot, along with the changes in the appearance, such as black rot, gray rot, pink rot, soft rot, stem-end rot [17].

Vegetables are another tempting source of nutrients for spoilage organisms because of their near neutral pH and high water activity. Although vegetables are exposed to a multitude of soil microbes, not all of these can attack plants and some spoilage microbes are not common in soil, for example, lactic acid bacteria. Most spoilage losses are not due to microorganisms that cause plant diseases but rather to bacteria and molds that take advantage of mechanical and chilling damage to plant surfaces.

Some microbes are found in only a few types of vegetables while others are widespread. *Erwinia carotovora* is the most common spoilage bacterium and has been detected in virtually every kind of vegetable. It can even grow at refrigeration temperatures [18]. Bacterial spoilage first causes softening of tissues as pectins are degraded and the whole vegetable may eventually degenerate into a slimy mass. Starches and sugars are metabolized next and unpleasant odors and flavors develop along with lactic acid and ethanol. Besides *E. carotovora*, several *Pseudomonas* spp. and lactic acid bacteria are important spoilage bacteria.Molds belonging to several genera, including *Rhizopus, Alternaria* and *Botrytis*, cause a number of vegetable rots described by their color, texture, or acidic products. The higher moisture content of vegetables as compared to grains allows different fungi to proliferate, but some species of *Aspergillus* attack onions.

Intact, healthy fruits have many microbes on their surfaces but can usually inhibit their growth until after harvest. Ripening weakens cell walls and decreases the amounts of antifungal chemicals in fruits, and physical damage during harvesting causes breaks in outer protective layers of fruits that spoilage organisms can exploit. Molds are tolerant of acidic conditions and low water activity and are involved in spoilage of citrus fruits, apples, pears, and other fruits. *Penicillium, Botrytis,* and *Rhizopus* are frequently isolated from spoiled fruits [19]. Yeasts and some bacteria, including *Erwinia* and *Xanthomonas*, can also spoil some fruits and these may particularly be a problem for fresh cut packaged fruits [20].

Fruits juices generally have relatively high levels of sugar and a low pH and this favors growth of yeasts, molds and some acid-tolerant bacteria. Spoilage may be manifested as surface pellicles or fibrous mats of molds, cloudiness, and off-flavors. Lack of oxygen in bottled and canned drinks limits mold growth. *Saccharomyces* and *Zygosaccharomyces* are resistant to thermal processing and are found in some spoiled juices [21]. *Alicyclobacillus* spp., an acidophilic and thermophilic spore-forming bacteria, has emerged as an important spoilage microbe, causing a smoky taint and other off-flavors in pasteurized juices [22] *Propionibacterium cyclohexanicum*, an acid-tolerant non-sporeforming bacterium also survives heating and grows in a variety of fruit juices [23]. Lactic acid bacteria can spoil orange and tomato juices, and some pseudomonads and enterobacteriaceae also spoil juices. These bacteria are not as heat tolerant but may be post-pasteurization contaminants colonization by many, but not all, microbes and are the most important first step in delaying the spoilage process. Microbes require certain conditions for growth, and therefore management of the environment of foods can change these factors and delay spoilage:

Many, but not all, microbes grow slowly or not at all at low temperatures, and refrigeration can prolong the lag phase and decrease growth rate of microbes. Many microbes require a high water activity and therefore keeping foods such as grains and cereal products dry will help to preserve them.

Some microbes require oxygen, others are killed by oxygen, and still others are facultative. Managing the atmosphere during storage in packaging can retard or prevent the growth of some microbes. Several types of modified atmosphere packaging (MAP) have been developed to retard growth of pathogenic and spoilage organisms. However, microbes are endlessly innovative and eventually seem to circumvent the barriers set against them. Therefore further strategies and multiple hurdles are utilized to extend shelf life. These procedures must be assessed for compatibility with different foods so that there are no significant organoleptic changes in the foods caused by the treatment or preservative. These methods for food preservation will not be covered in depth here.

Spoilage of dairy products

Milk is an excellent medium for growth for a variety of bacteria [24]. Spoilage bacteria may originate on the farm from the environment or milking equipment or in processing plants from equipment, employees, or the air. LAB are usually the predominant microbes in raw milk and proliferate if milk is not cooled adequately. When populations reach about 106 cfu/ml, off-flavors develop in milk due to production of lactic acid and other compounds. Refrigeration suppresses growth of LAB and within one day psychrophilic bacteria (*Pseudomonas, Enterobacter, Alcaligenes* and some spore-formers) grow and can eventually produce rancid odors through the action of lipases and bitter peptides from protease action [25].

Pasteurization kills the psychrophiles and mesophilic bacteria (LAB), but heat-tolerant species (*Alcaligenes, Microbacterium,* and the sporeformers *Bacillus* and *Clostridium*) survive and may later cause spoilage in milk or other dairy products. Immediately following pasteurization, bacterial counts are usually <1000 cfu/ml. However, post-pasteurization contamination of milk, particularly with *Pseudomonas* and some Gram-positive psychrophiles does occur [26, 27]. Spoilage problems in cheese can sometimes be traced to low quality milk but may also result from unhygienic conditions in the processing plant. Hard and semi-hard cheeses have a low moisture content (<50%) and a pH ~5.0, which limits the growth of some microbes. Some coliforms and *Clostridium* spp. that cause late gas blowing can grow under these conditions as can several species of molds. Other psychrotrophs produce biogenic amines, particularly tyramine, during storage of cheese (Novicka *et al.*, 2003). Soft cheeses with a higher pH of 5.0–6.5 and a moisture content of 50–80% may be spoiled by *Pseudomonas*, *Alcaligenes*, and *Flavobacterium*. *Clostridium sporogenes* has been found in spoiled processed cheese, where it produces gas holes and off-flavors [28].Yeasts and molds are the main spoilage organisms found in cultured milks (yogurt, sour cream and buttermilk) because the higher acidity in these products inhibits many bacteria [29]. *Pseudomonas*, yeasts and molds can spoil butter and "light" butters. Since the light butters have higher moisture content than butter, they can support more microbial growth. Cream may become rancid when populations of *Pseudomonas* and *Enterobacter* proliferate.

Prevention from food spoilage microorganism

Many food products are perishable by nature and require protection from spoilage during their preparation, storage and distribution to give them desired shelf-life. Because food products are now often sold in areas of the world far distant from their production sites, the need for extended safe shelf-life for these products has also expanded. The development of food preservation processes has been driven by the need to extend the shelf-life of foods. Food preservation is a continuous fight against microorganisms spoiling the food or making it unsafe. Several food preservation systems such as heating, refrigeration and addition of antimicrobial compounds can be used to reduce the risk of outbreaks of food poisoning; however, these techniques frequently have associated adverse changes in organoleptic characteristics and loss of nutrients.

Within the disposable arsenal of preservation techniques, the food industry investigates more and more the replacement of traditional food preservation techniques by new preservation techniques due to the increased consumer demand for tasty, nutritious, natural and easy-to-handle food products. Improvements in the cold distribution chain have made international trade of perishable foods possible, but refrigeration alone cannot assure the quality and safety of all perishable foods.

The most common classical preservative agents are the weak organic acids, for example acetic, lactic, benzoic and sorbic acid. These molecules inhibit the outgrowth of both bacterial and fungal cells and sorbic acid is also reported to inhibit the germination and outgrowth of bacterial spores. In the production of food it is crucial that proper measures are taken to ensure the safety and stability of the product during its whole shelf-life. In particular, modern consumer trends and food legislation have made the successful attainment of this objective much more of a challenge to the food industry. Firstly, consumers require more high quality, preservative-free, safe but mildly processed foods with extended shelf-life. For example, this may mean that foods have to be preserved at higher pH values and have to be treated at mild-pasteurization rather than sterilization temperatures. As acidity and sterilization treatments are two crucial factors in the control of outgrowth of pathogenic spore-forming bacteria, such as *Clostridium botulinum*, addressing this consumer need calls for innovative approaches to ensure preservation of products. Secondly, legislation has restricted the use and permitted levels of some currently accepted preservatives in different foods. This has created problems for the industry because the susceptibility of some microorganisms to most currently used preservatives is falling.

An increasing number of consumers prefer minimally processed foods, prepared without chemical preservatives. Many of these ready-to-eat and novel food types represent new food systems with respect to health risks and spoilage association. Against this background, and relying on improved understanding and knowledge of the complexity of microbial interactions, recent approaches are increasingly directed towards possibilities offered by biological preservation [30].

The high salt concentration in the serum-in-lipid emulsion of butter limits the growth of contaminating bacteria to the small amount of nutrients trapped within the droplets that contain the microbes. However, psychrotrophic bacteria can grow and produce lipases in refrigerated salted butter if the moisture and salt are not evenly distributed [31]. When used in the bulk form, concentrated (condensed) milk must be kept refrigerated until used. It can be preserved by addition of about 44% sucrose and/or glucose to lower the water activity below that at which viable spores will germinate (a_w 0.95) [32]. Lactose, which constitutes about 53% of the non fat milk solids, contributes to the lowered water activity. When canned as evaporated milk or sweetened condensed milk, these products are commercially sterilized in the cans, and spoilage seldom occurs. Microbial growth and enzyme activity are prevented by freezing. Therefore, microbial degradation of frozen desserts occurs only in the ingredients used or in the mixes prior to freezing.

Chemical preservatives

Chemical preservatives are substances which are added to food just to retard, inhibit or arrest the activity of microorganisms such as fermentation, putrefaction and decomposition of the food. Commonly used preservatives include, common salt, sugar, dextrose, spices, vinegar, ascorbic acid, benzoic acid and its salt, SO_2 and the salts of sulphuric acid, nitrates, sorbic acid and its salts, propionic acid and its salts, lactic acid and its salts. The features of potassium metabisulphate are: (1) It release the SO_2 and it is unstable; (2) It is used for the fruit which have non water solvent pigment (colourless); (3) It can not be used in naturally coloured juices such as phalsa, jamun because they have the anthocynin pigment; (4) It can not be used in the product which are packed in container because it acts on the tin containers and oil; (5) Hydrogen Sulphide (H_2S) which has an unpleasant smell and also form a black compound with the base plate of containers; (6) Best to control moulds than bacteria; (7) 350 ppm KMS is mostly used in fruit juice products. Features of sodium benzoate are: (1) It is salt of benzoic acid and soluble in water; (2) It delays the fermentation in the juices; (3) It is commonly used in the product which are having natural colour such as anthocynin pigment; (4) It is more effective against the yeast; (5) 750 ppm Sodium benzoate is mostly used in fruit juices, squashes and cordials.

Use of food additives

Food additives are substances or mixture of substances other than basic foodstuffs, which are present in the foods as reagent of any aspects of production, processing, storage, packaging etc. Food additives are (i) sugar, (ii) salt, (iii) acids, (iv) spices. In case of sugar and salts, they exerts osmotic pressure by water is diffuses from theproduct through a semi-permeable membrane until the concentration reached equilibrium. They kills the microorganisms or do not allow them to multiplication.

(i) Sugar: The concentration of 68-70% is used for preparation of jam, jelly, marmalades etc. sugar act as a preservative by osmosis and not as a true poison for micro organisms. It absorbs most of the available water, so little water available for the growth of micro organisms.

(ii) Salt: 15-20% concentration is used for the preparation such as pickles. Salt inhibits enzymatic browning and discolouration and also acts as an anti-oxidant. It exerts its preservative action by: a. Causing high osmotic pressure resulting in the plasmolysis of microbial cells; b. Dehydrating food and micro organisms by tying up the moisture; c. Ionizing to yield the chloride ion which is harmful to micro organisms, and d. Reducing the solubility of oxygen in water, sensitizing the cells against CO_2.

(iii) Acids: Many processed foods and beverages needs the addition of acids to impart their characteristic flavour and taste in the final product because acids provides desired flavour and taste. They adjust the sugar and acid ratio in the food. They give proper balance flavour of the food. They also play the role for controlling the pectin-gel formation. Acetic acid (Vinegar), Citric acid (Lime juice), Lactic acid (Lactose) etc. are used. Acetic acid is commonly used for pickles, chutney, sauce and ketchup, just to inhibit the growth of microorganisms. Citric acid is used for preparation of jam, jelly, squash, nectar *etc.* just to increase the acidity. Lactic acid: It is used for the formation of curd from milk, raw flavour, specific to pickles. Spices are plant products, are used in flavouring the foods and beverages to enhance the food flavour, colour and palatability, act as antibacterial and antifungal activity.

Weak carboxylic acids, such as acetic, sorbic and benzoicacids, are generally regarded as safe anti-microbial additives, and have wide application as preservatives in foods and beverages [33]. However, many yeasts are able to survive, adapt and even grow in the presence of the maximum levels of these preservatives permitted for use in foods. When compared with other fungi and bacteria, yeast are more resistant to weak carboxylic acids.

The elucidation of the cytotoxic effects induced by weak acids in yeast, which may compromise cell viability and ultimately result in cell death, can provide further insights into the mechanisms that determine different susceptibilities of yeast to weak carboxylic acids compared to other microorganisms, and will allow the improvement or design of new strategies for food and beverage preservation.

Zygosaccharomyces bailii is a food and beverage spoilage yeast that is characterised by a high tolerance to weak carboxylic acids at low pH [34], where *Saccharomyces cerevisiae* cannot survive. Thus, these two yeast species have frequently been selected as models for the study of yeast response to acid stress. In *S. cerevisiae* [35] and in *Z. bailii* [36] acetic acid induced cell death. However, and as is widely known, in *Z. bailii* this effect was observed at much higher concentrations of the acid. Although individual cells of *S. cerevisiae* and *Z. bailii* exhibited different short term intracellular pH responses to acetic acid , in both species the induction of cell death was related with an intracellular acidic action [37]. In *Z. bailii*, the mechanism of death due to exposure to weak acids remains to be clarified.

Acetic acid in concentrations between 20 and 120 mM induces in exponentially growing *S. cerevisiae* cells a programmed cell death (PCD) process that displays the most common apoptotic hallmarks, such as chromatin condensation along the nuclear envelope, exposure of phosphatidylserine on the surface of the cytoplasmic membrane, and occurrence of internucleosomal DNA [32].

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