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Microbial and biochemical spoilage of foods: an overview

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Abstract

During harvesting, processing and handling operations food may become contaminated with a wide range of microorganisms. Subsequently, during distribution and storage only a small fraction of these will develop and cause serious deteriorations. Which microorganisms will develop or what (bio)chemical reactions occur is dependant upon food derived or environmental factors. This paper will describe the main mechanisms involved in the loss of food quality for the most important food commodities. Food spoilage may be caused by a wide range of reactions including some that are mainly physical or chemical, others due to action of enzymes or microorganisms. The primary factors associated with food spoilage are associated with intrinsic food properties (e.g., endogenous enzymes, substrates, sensitivity for light, oxygen) and (cross)contamination during harvesting, slaughter and processing in combination with temperature abuse. For fresh foods the primary quality changes may be catagorized as (i) bacterial growth and metabolism resulting in possible pH-changes and formation of toxic compounds, off-odours, gas and slime-formation, (ii) oxidation of lipids and pigments in fat-containing foods resulting in undesirable flavours, formation of compounds with adverse biological effects or discoloration. Although interrelated with the microbial spoilage, the last category is 'purely' chemical in nature and will, all other things being equal, increase in importance with decreasing temperature. Little is known about the relationship between microbial activity and (bio)chemical spoilage parameters under different packaging and storage conditions. Although there is much progress in the characterisation of the total microflora and metabolites developing during spoilage, not much is known about the identification of specific microorganisms in relation to food composition. Despite the fact that food spoilage is a huge economical problem world wide, it is obvious that the mechanisms and interaction leading to food spoilage are very poorly understood.

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1. Introduction

Food spoilage can be considered as any change which renders a product unacceptable for human consumption (Hayes, 1985). Food spoilage can be obvious e.g., physical damage, visible growth of microorganisms, slime formation or insect damage. However, when spoilage is due to changes in texture or the development of off-flavours caused by (bio)chemical or microbial reactions, the underlying mechanisms may be difficult to identify. Therefore, evaluation of spoilage will always, directly or indirectly be related to a sensory assessment. In contrast, biochemical or to a lesser extent, microbial analyses are less expensive, more objective and thus convenient. Consequently, for a limited number of foods various chemical and biochemical indices for spoilage have been proposed and used as measures of the quality or degree of spoilage. Microbial or biochemical spoilage indices will be discussed in this special issue.

The exact figures of the total economical losses due to food spoilage are unknown but the scarce figures available indicate that it constitutes an enormous financial loss. It is estimated that one-fourth of the world's food supply is lost through microbial activity alone (Anonymous, 1985). In the developed countries spoilage is mainly caused by psychrotrophic microorganisms, yeasts and moulds. In less developed countries food spoilage due to rodents and other animals is of major concern. Thus, food spoilage is an economical problem that is not yet under adequate control despite modern food technology and the range of preservation techniques available.

Food spoilage is a complex event, in which a combination of microbial and (bio)chemical activities may interact. The microbiology of food spoilage has over the years received considerable attention, and the characterisation of the typical microflora which develop on different types of foods during storage has been well documented (Mossel et al., 1995). Therefore, the major problems are to find the relation between microbial composition and presence of microbial metabolites, related to the evaluation and possible prediction of microbial spoilage (Borch and Agerhem, 1992; Drosinos and Board, 1994).

Classical microbial evaluation of especially perishable foods is of limited value for predictive prognoses since these foods are sold or eaten before the results of microbiological tests are available. New highly sensitive and possible specific microbial methods based upon immunological and molecular techniques have already been developed for the detection of pathogenic microorganisms (Huis in't Veld et al., 1994; Fung, 1994; Van der Vossen and Hofstra, 1996). These techniques could also be applied for the early detection of specific spoilage organisms (SSO). However, before such techniques can be used for the detection of SSO, those microorganisms must be identified for each type of product and their effect on

spoilage characteristics must be determined. As yet SSO are only known for a few products and selective or indicative methods for enumeration are not generally available. Table 1 summarizes the methods for the evaluation of SSO and some techniques which can be used to determine the spoilage domain of SSO are proposed.

Although the detection levels for the metabolites formed during (bio)chemical spoilage are generally low and thus more accessible, a major disadvantage is that the (bio)chemical processes related to food spoilage appear to be poorly understood. Even less is known about interactions between microbial and chemical spoilage reactions (Pittard et al., 1982; Dainty and Mackey, 1992; Dainty, 1996). Therefore we are still far away from assuring the quality of a food by predicting shelflife on the basis of specific spoilage indicators.

A unifying description of the interaction between the microflora developing in the product and the chemical changes in the same product presents a special challenge. Such an integrated understanding of each of the different types of products would indeed be beneficial in relation to the increasing interest in natural preservation systems such as microbially derived antimicrobial agents and antioxidants derived from plants.

A schematical representation of the complex mechanisms of food is presented in Fig. 1.

Table 1
Methods for the characterisation of specific spoilage organisms[a]

Method	Comparison of results from product and model substrate experiments
Spoilage potential (qualitative)	Microorganisms are isolated from products at sensory rejection and the ability of isolates to produce off-odours is determined by inoculation of substrates
Spoilage activity (quantitative)	The concentration of groups of bacteria is determined at the time of sensory rejection and the concentration of these bacteria at the time of off-odour detection is determined in model substrates
Yield factor determination (quantitative)	Numbers of groups of bacteria and the concentration of selected metabolites are determined in products and the increase in concentration of these bacteria and of selected metabolites are then determined in model substrates
Chemical spoilage profiles (qualitative or quantitative)	Chemical spoilage profiles of naturally spoiled products compared to chemical spoilage profiles of isolated microorganisms grown in model substrates

[a] Adapted from Dalgaard, 1993.

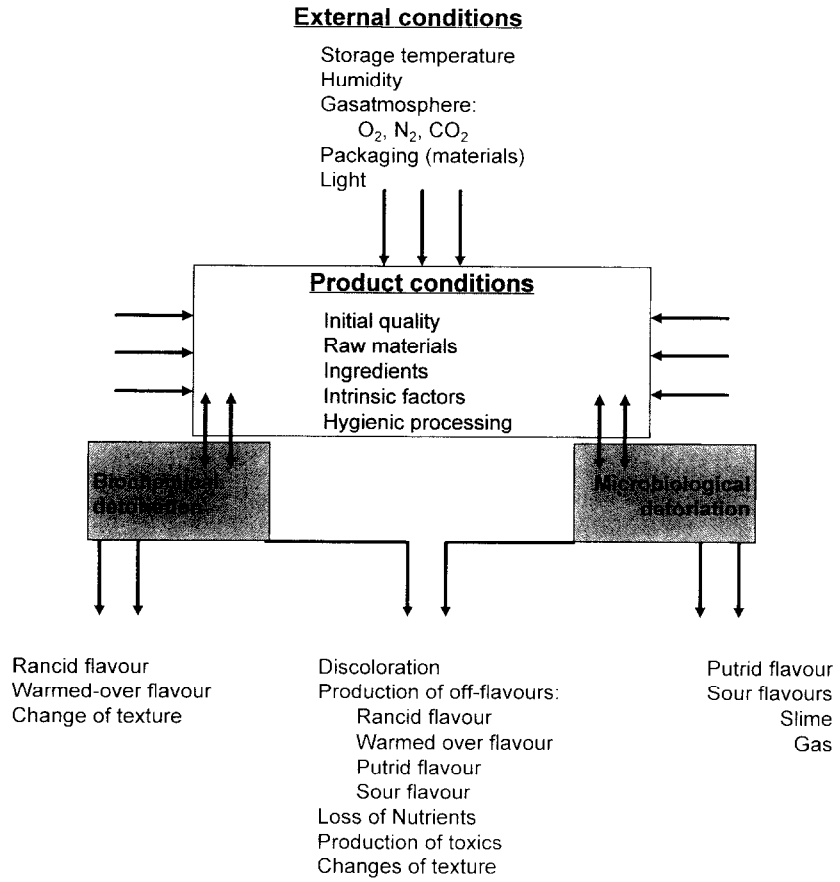

Fig. 1. Quality detoriation during storage of foods.

2. Factors determining microbial spoilage of foods

Spoilage of food and beverages is the result of microbial activity of a variety of microorganisms. The microbial flora that colonizes a particular food or beverage depends highly on the characteristics of the product and the way it is processed and stored. The parameters affecting proliferation of microorganisms in foods can be categorized into four groups: (i) intrinsic parameters; (ii) extrinsic parameters; (iii) modes of processing and preservation; and (iv) implicit parameters (Mossel et al., 1995).

It must be realized that any of the above mentioned parameters will influence the effect of others. Therefore the overall effect of a combination of parameters is generally much higher than the perceived effect of each individual parameter.

2.1. Intrinsic parameters

Intrinsic parameters are the physical, chemical and structural properties inherent in the food itself. The most important intrinsic factors are water activity, acidity, redox potential, available nutrients and natural antimicrobial substances.

2.2. Extrinsic parameters

Extrinsic parameters are factors in the environment in which a food is stored, notably temperature, humidity and atmosphere composition.

2.3. Modes of processing and preservation

Physical or chemical treatments often result in changes in the characteristics of a food product, determining the microflora associated with the product.

2.4. Implicit parameters

Implicit parameters are mutual influences, synergistic or antagonistic, among the primary selection of organisms resulting from the influence of the above mentioned parameters. Thus, implicit parameters are the result of the development of a microorganism which may have a synergistic or antagonistic effect on the microbial activity of other microorganisms present in the food product (Mossel et al., 1995). Synergistic effects include production or availability of essential nutrients due to the growth of a certain group of microorganisms, allowing development of other organisms which otherwise were unable to grow. Likewise, changes in pH value, redox potential and water activity may enable the development of microorganisms less tolerant to these inhibitory factors, yielding secondary spoilage. Antagonistic processes include competition for essential nutrients, changes in pH value or redox potential or the formation of antimicrobial substances e.g., bacteriocins which may negatively affect the survival or growth of other microorganisms (Stiles and Hastings, 1991; Kim, 1993; Huis in't Veld et al., 1996; Abee et al., 1996).

Another important phenomenom, which deserves attention in food preservation is the homeostasis of microorganisms (Gould, 1988). If the homeostasis of a microorganism i.e., their internal equilibrium, is disturbed by preservative factors in foods, they will not multiply, i.e. they remain in the lag-phase or even die, before their homeostasis is re-established. For instance, in an acid food they will actively expel protons against the pressure of a passive proton influx. Another important homeostatic mechanism regulates the internal osmotic pressure (osmohomeostasis). Cells have to maintain a positive turgor by keeping the osmolarity of the cytoplasm higher than the environment and they generally achieve this using so-called osmoprotective compounds such as proline and betaine (Gould, 1988; Leistner and Gorris, 1995).

We still do not have enough knowledge about how microorganisms behave under stress situations which regularly occur in foods. Most of our knowledge of how microorganisms respond to changed environments comes from extrapolation from pure cultures in laboratory experiments. However, it is becoming clear that microorganisms possess a series of mechanisms whereby they can adapt rapidly to particular environments, enabling them to colonize and grow on diverse substrates and to adapt to a wide range of hostile conditions.

One of the most fruitful research themes of recent years has been the discovery that microorganisms are not limited to specific ranges of temperature, pH and water activity, but can adapt to survive at values outside those found in these laboratory experiments.

The above mentioned accumulation of osmoprotectants is a major adaptive response to an osmotic stress in microorganisms (Gutierrez et al., 1995). Microorganisms possess also the ability to adapt to and tolerate low pH environments (Hill et al., 1995) or higher temperatures by the induction of heat-shock proteins or chaperones (Sanchez and Lindquist, 1990; Sanchez et al., 1992; Mager and Ferreira, 1993).

Another mechanism is that external stimuli stimulate a transmembrane sensor protein (Gross, 1993). The extracytoplasmic domain senses the environment and transfers a signal to a regulatory protein in the cytoplasm which through phosphorylation leads to specific binding to the genome, DNA supercoiling, alterations RNA polymerase specificty etc. which result in major changes in the gene transcription or translation and hence gene expression. These mechanisms have to be understood before we can control and predict the growth of spoilage microorganisms in foods.

We also need to understand how microorganisms react to external stresses, such that we can find ways of interfering with diverse mechanisms whereby they develop resistance to physical and chemical treatments.

In this overview first the microorganisms associated with food spoilage are discussed. Subsequently the production of off-flavours by both endogenous enzymes as well as enzymes of microbial origin will be presented. Finally mechanisms involved in chemical spoilage are discussed.

3. Bacteria in food spoilage

Spoilage is most rapid and evident in proteinaceous foods such as meat, poultry, fish, shellfish, milk and some dairy products. These foods are highly nutritious, possess a neutral or slightly acid pH and a high moisture content and therefore permit growth of a wide range of microorganisms. The pattern of microbial spoilage has been found to be similar for these type of proteinaceous foods (Fig. 2). Initially, SSO are present in low quantities and constitute only a minor part of the natural microflora. During storage, SSO generally grow faster than the remaining microflora and produce the metabolites responsible for off-odours, off-flavours or slime and finally cause sensory rejection. The cell concentration of SSO at rejection

may be called the minimal spoilage level and the concentration of the metabolite that corresponds to spoilage can be used as an objective chemical spoilage index (CSI) (Dalgaard, 1993).

Changes in the extrinsic conditions (e.g., refrigeration, MAP) is the only way to delay spoilage. However, storage at adequate low temperatures will not prevent spoilage but will limit spoilage to psychrotrophic microorganisms. In general they comprise, in addition to some Gram-positive rods (lactic acid bacteria) and spore-forming bacteria (Clostridia), largely the Gram-negative, rod-shaped, non-spore-forming bacteria (Pseudomonaceae). Although several yeasts and moulds are psychrotrophic, they do not generally compete well with bacteria at low temperatures but they may become important in situations where bacteria have difficulty in growing, i.e. in acid foods, or foods with high sugar or salt concentration.

For convenience, the spoilage microorganisms will be divided into broad categories: Gram-negative rod shaped bacteria, Gram-positive spore forming bacteria, lactic acid bacteria, other Gram-positive bacteria (e.g., *Brochotrix thermosphacta*), yeasts and moulds.

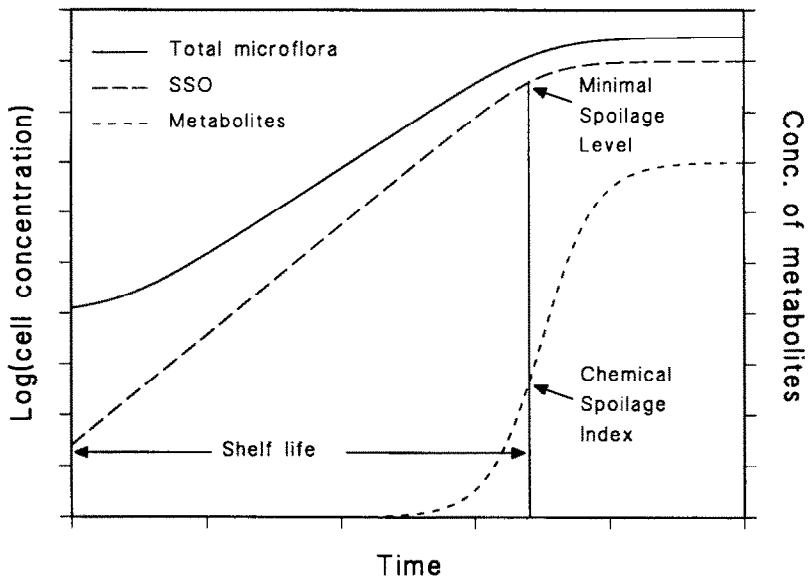

Fig. 2. General pattern of microbial spoilage. SSO, specific spoilage organisms; MSL, minimal spoilage level; CSI, chemical spoilage index (adapted from Dalgaard, 1993).

3.1.1. Gram-negative rod shaped bacteria

Pseudomonas spp. are the most common spoilage organisms, particularly in aerobically stored foods with a high water content and natural pH, e.g., red meat (Dainty and Mackey, 1992; Dainty, 1996; Borch et al., 1996), fish (Gram and Huss, 1996), poultry (Lahellec and Colin, 1979; Gallo et al., 1988; Regez et al., 1988), milk and dairy products (Walker and Stringer, 1990; Craven and Macauly, 1992a; Craven and Macauly, 1992b; Craven and Macauly, 1992c). *Pseudomonas* spp., like most of the other Gram-negative rod shaped bacteria, usually comprise only a small proportion of the initial microflora of fresh foods. They are, however, widely distributed in the environment and may contaminate foods from many sources and are able to utilise a wide range of materials as substrates for growth.

Food spoilage due to pseudomonads may occur in a number of ways. In foods of animal origin the non-protein nitrogen fraction (NPN) will be first metabolized. Subsequently the production of lipases or proteases will liberate fatty and amino acids which after metabolism can also result in off-odours, off-flavours and rancidity. At later stages the production of extracellular slime and the development of often pigmented growth becomes visible (Dainty and Mackey, 1992; Dainty, 1996).

A number of other Gram-negative rod shaped bacteria may also grow rapidly at chill temperatures and spoil foods, such as *Aeromonas*, *Photobacterium*, *Shewanella* and *Vibrio*. Some or all of the above bacteria have been shown to contribute to the spoilage of chilled red meat, cured meats, poultry, fish, shellfish and milk and dairy products. *Vibrio* spp. are unusual as most are halophilic (salt-loving) and so may cause spoilage of sea-fish and cured meats. Like the pseudomonads, other Gram-negative bacteria also cause food spoilage due to NPN metabolism. Subsequently foods will spoil by the production of enzymes (resulting in odour and flavour defects), slime production and the formation of visible, often pigmented colonies (Walker and Stringer, 1990).

At a temperature above 5–10°C enterobacteriaceae generally dominate over *Pseudomonas* spp. and become responsible for spoilage. Vibrionaceae spoil fish at higher temperatures. Spoilage is characterised by the production of gas, acid, slime, rope, bitter flavours and off-odours. The presence of enterobacteriaceae is often used as an indication for possible faecal contamination, inadequate processing or post-process contamination.

3.1.2. Gram-positive, spore forming bacteria

Many foods undergo a heating or pasteurisation process. Thus microorganisms capable of surviving this process are significant (e.g., *Bacillus* and *Clostridium* spp.), particularly if they are able to grow at chill temperatures. Growth of the spore forming bacteria tends to be much slower than that of the Gram-negative bacteria, but most of these latter bacteria are eliminated by heat processing.

The *Bacillus* spp. are largely aerobic in nature. Perhaps, best recognised is *B. cereus* which may grow at low temperatures (5°C or less) and produce enzymes

which result in 'sweet curdling' and 'bitty cream' in milk (Coghill and Juff, 1979; Cousin, 1982; Walker et al., 1989). Other *Bacillus* spp. may grow at temperatures of 0–2°C (Cousin, 1982).

Most spoilage *Clostridium* spp. are unable to grow at refrigerator temperatures (i.e. 5°C or less), but at slightly higher temperatures may produce gas resulting in 'late blowing' of hard cheeses during maturation (Cousin, 1982; Walker, 1988).

Recently some psychotrophic *Clostridium* spp. have been observed to spoil vacuum-packed meat and fish with extended shelf life such as vacuum-packed beef and ham (Dainty, 1996) and sous-vide cooked beef (Lawson et al., 1994; Hansen et al., 1995).

3.1.3. Lactic acid bacteria

Lactic acid bacteria spoil foods by the fermentation of sugars to form lactic acid, slime and CO_2 leading to a drop in pH and off-flavours. These bacteria tend to grow slowly at refrigeration temperatures and are under aerobic conditions generally out-competed by pseudomonads. Generally, they are present in the initial microflora in low numbers and are therefore rarely responsible for the spoilage of fresh proteinaceous foods.

Lactic acid bacteria, however, have been identified as the major spoiling microorganisms of vacuum-packed meat and poultry (Dainty et al., 1983; Mead, 1983; Borch et al., 1996) and are also suggested as possible spoilers of lightly preserved fish products. Cured and fermented meat products may also be spoiled by lactic acid bacteria, as the pH or other preservation methods in the food again prevent the growth of the normal spoilage microflora. Typical lactic acid bacteria are identified as *Lactobacillus*, *Streptococcus*, *Leuconostoc* and *Pediococcus* spp.

3.1.4. Other Gram-positive bacteria

Brocothrix thermosphacta is a Gram-positive rod which may be occasionally present on fresh meats (Gardner, 1981). The increased use of modified atmosphere packaging and vacuum packaging will often allow *Br. thermosphacta* to dominate the microflora.

Micrococcus spp. are able to grow in the presence of salt and may be responsible for the spoilage of cured meat products such as bacon producing slime, souring or pigmented growth. These microorganisms also often predominate in freshly collected milk (Thomas, 1974). Many strains are thermoduric and may survive milk pasteurisation causing subsequent spoilage, particularly if the other more rapidly growing spoilage organisms have been eliminated by the heat treatment.

3.2. Milk and dairy products

During extended refrigerated storage of milk heat-stable enzymes of microbial origin may be formed. These can biochemically alter the products, eventually causing spoilage. Two types of enzymes are particularly important in the formation of off-flavours: (i) lipases and (ii) proteinases.

3.2.1. Lipases

Lipase activity has been reported for most psychotrophs isolated from milk and milk products. *Pseudomonas*, *Flavobacteria* and *Alcaligenes* species are the most lipolytic bacteria (Muir et al., 1979). Microbial lipases are heat stable. Lipase activity in milk leads to the preferential release of medium- and short-chain fatty acid from triglycerides, hydrolysis of as little as 1–2% triglycerides leading to rancid off-flavour. Milk naturally also contains high levels of indigenous lipase (Olivecrona and Bengtsson-Olivecrona, 1991). It is therefore extremely likely that indigenous as well as microbial lipases are important in the development of lipolytic rancidity in milk.

3.2.2. Proteinases

The major cause of bitterness in milk and milk products is the formation of bitter peptides due to the action of proteinases. Proteinase activity has been detected in many bacterial species, in particular *Pseudomonas*, *Aeromonas*, *Serratia* and *Bacillus* species. Heat stability of proteinases from several bacterial species was investigated by Griffiths et al. (1981). Strict quality control is therefore critical in UHT milk products to ensure that heat-stable proteinases do not cause bitter off-flavours. The most investigated source of bitter peptides is the casein.

3.3. Meat and fish

Off-flavours which develop due to surface microbial contamination are major causes of spoilage in meat. Dainty et al. (1983) showed the first signs of spoilage to be caused by the formation of fruity, sweet-smelling esters, followed by the formation of putrid sulphur compounds. These workers identified *Pseudomonas* species as the main bacterial contamination. Many putrid odours arise due to decomposition of proteins and amino acids by anaerobic bacteria. Volatiles produced include indole, methanethiol, dimethyl disulphide and ammonia (Dainty, 1996). Rancidity problems which occur are usually due to oxidation of unsaturated lipids and are not associated with microbial growth (Kramlich et al., 1975).

In the case of fish, Huss (1995) and Gram and Huss (1996) describe a four-phase pattern for the changes in flavour quality after harvest. Initial microbial contamination and growth is by aerobes, which act on carbohydrates giving carbon dioxide and water. As the surface becomes covered and slime builds up, conditions become more favourable to the growth of anaerobes. Reduction of trimethylamine oxide to the unpleasant, fish-smelling trimethylamine, catalysed by trimethylamine-*N*-oxide reductase, is carried out by many bacteria. Many off-flavours are also associated with the breakdown of sulphur-containing amino acids. Typical volatile products are hydrogen sulphide, methyl mercaptan and dimethyl sulphide.

4. Yeasts and moulds in food spoilage

Yeasts and moulds can be found in a wide variety of environments, such as in plants, animal products, soil, water and insects. This broad occurrence can be explained by the fact that yeasts and moulds can utilize a variety of substrates such as pectines and other carbohydrates, organic acids, proteins and lipids. Moreover, yeasts and moulds are relatively tolerant to low pH, low water activity, low temperature and the presence of preservatives. It is also notable that yeasts can utilize food ingredients, such as organic acids like lactic, citric and acetic acids, that are generally considered to have an inhibitory effect on the growth of many microorganisms. Even common preservatives such as benzoate, propionate and sorbate can be utilized by some yeast species (Miller, 1979).

Contamination of foods and beverages by yeasts and moulds has been extensively reported (Baleiras Couto, 1995). Contemporary work has also reported the occurrence of yeasts in fresh seafood, packaged meats, delicatessen salads (Fowler and Clark, 1975; Koburger, 1971; Koburger, 1972), and in fresh vegetables (Winter et al., 1971). For an exhaustive list of specific habitats and possible spoilage yeast species, the overviews by Fleet (1990); Fleet (1992); Deák (1991); Samson et al. (1984); Filtenborg et al. (1996) are recommended.

Changes induced by spoilage of yeasts and moulds can be of a sensory nature, recognizable in the product's appearance by the production of slime, quite often pigmented growth on the surface, fermentation of sugars to produce acid, gas or alcohol or the development of off-odours and off-flavours.

In addition to visible spoilage, moulds can also spoil foods through the formation of mycotoxins. It has now been established that more than 200 different types of moulds do form substances that are orally toxic to man, when growing in certain foods. Although most research has been carried out on the metabolites of *Aspergillus flavus*, it is quite obvious that in addition to the so-called aflatoxins, many other mycotoxins may be of great significance.

5. Off-flavours produced in different foods due to endogenous enzymatic activities

Off-flavours which arise in foods fall into the three following categories (Springett, 1993): (i) off-flavours preformed in the food; (ii) off-flavours formed as a result of cellular disruption; and (iii) off-flavours as a consequence of endogenous or microbial enzymes.

Off-flavours may be preformed in the food due to normal biochemical metabolism or as stress metabolites. These type of off-flavours are dependent to a large extent on agronomic factors such as varietal differences, feeding or fertilizer regimes, level of water used, spacing, etc. This will not be further discussed in this paper.

After harvest or slaughter the structural integrity of foods begins to break down due to damage from handling and normal decay processes. This will result in the mixing of compartmentalized enzymes and substrates and the generation of possible

flavour compounds. Although in some cases the production of flavour compounds along this way is a typical characteristic of the product (e.g., onions, garlic) generally this leads to the formation of off-flavours and spoilage of the product.

As by-products of growth and metabolism microorganisms produce a range of chemicals which alter the quality attributes of foods such as off-flavours, (myco)toxins (Filtenborg et al., 1996), and biogenic amines (Ten Brink et al., 1990; Halász et al., 1994) ultimately rendering the product inedible or unsafe.

5.1. Fruit and vegetables

5.1.1. Citrus fruit

A flavour defect which constitutes a major problem world-wide to the citrus industry is bitterness due to formation of limonin. It was shown (Maier and Beverley, 1968; Maier and Margdeth, 1969) that intact fruits were not bitter and did not contain limonin itself but a non-bitter precursor, limonoate A-ring lactone. When juice is extracted this non-bitter precursor is slowly converted to limonin under acidic conditions and is accelerated by the presence of limonin D-ring lactonase.

5.1.2. Legumes

The enzyme lipoxygenase is believed to be ubiquitous amongst eukaryotic organisms (Whitaker, 1991). This enzyme poses a particular problem in legumes such as soy beans, winged beans, lentils, green beans, etc., giving rise to a range of off-flavours described variously as beany, grassy and rancid. Flavour problems with lipoxygenase have been summarized recently by O'Connor and O'Brien (1991).

There has been considerable interest in lipoxygenase in soy beans, related to the formation of volatile aroma compounds. Soy beans contain high levels of lipoxygenase, constituting 1–2% of the protein (Axelrod et al., 1981). The oil fraction contains 55% linoleic acid and 8% linolenic acid, which are substrates for lipoxygenase. Volatiles produced lead to a range of off-flavours described variously as grassy, beany, rancid, etc.

5.1.3. Brassicas

Perhaps the most important group of compounds to the flavour of Brassicas are the volatile degradation products of endogenous glucosinolates (Kjaer, 1960). Brassicas also contain thioglucosidase (myrosinase) enzymes, which are released when the cells of the plant are disrupted and which then come into contact with the glucosinolates. This leads to a range of potent flavour compounds dependent upon: (i) the specific glucosinolate involved; and (ii) the conditions of reaction. In many instances this gives rise to desirable flavour notes e.g., 2-propenylisothiocyanate, derived from the thioglucosidase-initiated breakdown of 2-propenyl glucosinolate (sinigrin), is an important flavour component of black pepper. However, the undesirable bitter note in *Brassica oleraceae* cultivars in particular Brussels sprouts has been attributed to the formation of goitrin (5-vinyloxazolidine-2-thione) from the glucosinolate progoitrin (2-hydroxy-3-butenyl glucosinolate) (Fenwick and Griffiths, 1981; Fenwick et al., 1983).

5.2. Wine and beer

Enzymes are essential for the conversion of grape juice into wine, and malt extract into beer. This includes the formation of many flavour compounds, together with the typical ethanol component of these drinks. Off-flavours which arise are invariably associated with fermentation problems or microbial contamination.

5.2.1. Wine

Grass-like tastes which may arise in wines are caused by the formation of hexanal, *cis*-hexen-3-al and *trans*-hexen-2-al. These compounds are formed during the juice extraction phase due to the breakdown of membrane equivalent alcohols. Although sensorically the alcohols are not as potent as the aldehydes, it is believed they can also contribute to the grass-like aroma (Villetaz and Dubourdieu, 1991).

A range of off-flavours, collectively known as 'cork taint', may arise in wines and spirits. The major cause of cork taint is believed to be 2,4,6-trichloroanisole (TCA) (Tanner et al., 1981).

5.2.2. Beer

Perhaps one of the most studied off-flavour problems in beer is the formation of the butter-like compound diacetyl. α-Acetolactate is an intermediate in the biosynthetic pathway from pyruvate to leucine and valine, within a normal metabolizing yeast. However, it is possible for α-acetolactate to pass into the bulk of the wort where chemical oxidative decarboxylation converts it into diacetyl. Given sufficient time, the yeast will absorb the diacetyl and convert it via acetoin to 2,3-butanediol. However, if the yeast is removed, diacetyl will accumulate leading to the off-flavour (Slaughter and Priest, 1991).

The interrelationship between microbial enzymes, endogenous enzymes and off-flavours is very complex. Although some aspects, such as bitterness in milk and diacetyl in beer, have been well studied, formation of off-flavours due to enzymes, both microbial and endogenous, is often poorly understood.

6. Chemical spoilage

Although chemical and physical spoilage processes cannot be totally separated, their main contributions to food spoilage are characterized by flavour and colour changes due to oxidation, irradiation, lipolysis (rancid) and heat. These changes may be induced by light, metal ions or excessive heat during processing or storage. Chemical processes also may bring about physical changes such as increased viscosity, gelation, sedimentation or colour change.

6.1. Lipid oxidation

Lipid oxidation is one of the most common causes of deterioration of food quality. Unsaturated fats are oxidized by free radical autoxidation, a chain reaction

process catalyzed by the products of the reaction. The susceptibility to and the rate of oxidation increase as the number of double bonds in the fatty acid increases. Oxidation of oils may also be initiated by lipoxygenase or photosensitizers (King et al., 1993).

Many foods or food ingredients (plants, fruits, roots and meats) contain components which possess so called antioxidant properties (King et al., 1993). Examples of well known natural antioxidants are ascorbic acid, vitamin E (tocopherols), caretenoids and flavonoids. Antioxidants inhibit lipid oxidation by acting as hydrogen or electron donors, and interfere with the radical chain reaction by forming nonradical compounds that will not propagate further radical reaction.

6.1.1. Enzymatic oxidation

Lipoxygenase occurs in many plants and catalyzes the oxidation of unsaturated fatty acids containing a *cis*, *cis* 1,4-pentadiene system to their corresponding monohydroperoxides. These peroxides have the same structure as those obtained by autoxidation. Lipoxygenase is a metal bound protein with a Fe-atom in its active centre. Plant lipoxygenases produce *cis*, *trans*-conjugated monohydroperoxides as primary products. Naturally occurring substrates include linoleic, linolenic and arachidonic acids (King et al., 1993).

6.1.2. Lipolysis

Fat containing foods, e.g. milk, can undergo a number of subtle chemical and physical changes caused by lipolysis. Lipolysis can be defined as the enzymatic hydrolysis of fats by lipases. The accumulation of the reaction products, especially free fatty acids is responsible for the common off-flavour, frequently referred to as rancidity. The lipolytic enzymes could either be endogenous of the food product, e.g. milk, but could also be derived from psychrotrophic microorganisms. In contrast to bacterial lipases, the endogenous milk enzymes have shown to be sensitive to heat (Muir et al., 1979).

6.1.3. Discoloration

The red or brown colour of meat depends on the different forms of myoglobin due to different oxygen partial pressure at the meat surface. The depth of the oxymyoglobin layer responsible for the red colour depends on the oxygen penetration into the meat (Belitz and Grosch, 1987).

Also during spoilage fish flesh may turn yellow due to oxidation of caretenoid pigments and lipids in tissues (Colby et al., 1993).

The most important colour changes in stored fruits and fruit products are caused by chemical reactions known as browning reactions. Browning of fruits may be enzymatic or non-enzymatic. Polyphenoloxidases (PPO) and peroxidases found in fruit tissues can catalyze oxidation of certain endogenous phenolic compounds to quinones that polymerize to form intense brown pigments (Lewis and Shibamoto, 1986).

7. Conclusions

Although food spoilage is a major economical loss, the underlying integrated mechanisms are still poorly understood. It is obvious that the presence of high numbers of spoilage microorganisms will eventually lead to deterioration of foods but the time between the moment of reaching these high levels of microorganisms and the actual spoilage may vary considerably depending on the type of food, the actual intrinsic, extrinsic and implicit factors and the activity of specific spoilage microorganisms (SSO). In order to minimise food spoilage and be able to predict the quality or shelflife of a particular food, a better understanding of the mechanisms underlying food spoilage is essential. As a consequence, there is a need for the identification and control of growth of SSO present on different food commodities. As yet not many SSO have been identified. Therefore, the estimation of the quality of a food product still relies on the quantification of total numbers of microorganisms, which in some cases is a very poor reflection of the actual quality. In addition to the identification of SSO, a better understanding of the complex interaction between SSO and other microorganisms or their metabolites (synergism/antagonism) is needed. Finally the interaction between microbial spoilage and (bio)chemical spoilage has to be elucidated.

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