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ABSTRACT

Development of off-flavors is a common phenomenon in different food materials and water resources. Off-flavors not only render the foods and water unfit for human consumption but also badly impact the commercial markets concerning these commodities. In the previous decade, a tremendous amount of research has been carried out on the issues related to development and effects of off-flavors. Different aspects of this research have been encompassed by this review work. Developments of off-flavors, their effects on humans and possible solutions have been discussed in detail with reference to the research work done in past ten years.

Mechanism of off-flavor development has been the main focus of research. Similarly, identification and isolation of the chemical compounds responsible for these off-flavors has been done with the help of modern and sophisticated research tools such as analysis techniques including chromatography and mass-spectroscopy.

Food and water off-flavors are still a great challenge to the researchers. Future research must be directed towards the development of techniques which ensure maximum yield of foods with optimum quality and minimum off-flavors.

ZUSAMMANFASSUNG

Die Entwicklung des Fehlgeschmackes ist ein häufiges Phänomen in verschiedenen Lebensmitteln, Nahrungsmitteln und Trinkwasser. Ein Fehlgeschmack macht nicht nur Lebensmittel und das Wasser für den menschlichen genuss untauglich, sondern hat auch auf den Handel mit diesen Produkten einen negative Einfluss. Im letzten Jahrzent, wurde in vielen Untersuchungen Fragen im Zusammenhang mit der Entwicklung und Auswirkung eines Fehlgeschmackes studiert. Verschiedene Aspekte dieser Forschungen und deren Ergebnisse wurden in dieser Literaturübersicht zusammengefasst und mögliche Lösungen auch im Detail hinsichtlich der in den letzten Jahrzehnt durchgeführten Forschungen diskutiert.

Der Mechanismus der Entwicklung verschiednen arten des Fehlgeschmacks sowie die Identifizierung und Isolierung von chemiche Substanzen, die für einen Fehlgeschmack verantwortlich sind, sind Schwerpunkte der modernen Forschung. Zukünftige Forschungen müssen auf Techniken gerichtet werden, die maximalen Ertrag an Nahrungsmitteln mit optimaler Qualität und minimalen Fehlgeschmack gewährleisten.

Chapter 1

MILK AND MILK PRODUCTS

MILK OFF-FLAVORS

Milk is a perfect diet for human beings regardless of age. It is a natural emulsion containing many important components with vital nutritional values and physiological functions. Most important constituents of milk are milk proteins (e.g. albumin and globulin), milk fats (e.g. cholesterol), vitamins (e.g. riboflavin), milk sugars (e.g. lactose) and other trace substances. Fresh milk has a typical flavor and aroma which may sometimes be altered to a variable extent in response to various factors. Most important factors affecting the flavor of fresh and stored milk are light, oxygen, internal enzymes, temperature treatments and microorganisms especially bacteria.[1]

In the previous decade, enormous research work has been done to identify and characterize these detrimental factors. Meanwhile certain approaches and treatment techniques have been developed to avoid or counteract the effects of these factors.

PHOTO OXIDATION

Of all the deteriorating factors, light performs probably the most important role. Light initiates a cascade of oxidative reactions which lead ultimately to formation of certain chemical species which impart unpleasant and unwanted off-flavor to milk. In retail outlets milk is displayed often in transparent containers under fluorescent conditions for sale.[1] Due to such exposure to light, milk is more prone to photo oxidative changes resulting in flavor defects.

Milk contains many substances which are light sensitive. Vitamins like riboflavin (vitamin B2) are rapidly degraded under influence of light or radiant energy. Recent studies have revealed that many naturally occurring tetrapyrrols in milk, e.g. porphyrins and chlorophyllic substances are also prone to photo degradation.[1], [2] All of these substances are in fact “Photosensitizers”. Tetrapyrrols and especially chlorophyllic substances are probably more active photosensitizers. The important naturally occurring tetrapyrrols in milk detected by Fluorescence Spectroscopy were protoporphyrin, hematoporphyrin, chlorophyll *a* and chlorophyll *b*.[3]

The photosensitizers readily absorb radiant energy throughout visible and UV light range as shown by a recent study in which milk was exposed to different excitation wavelengths of light.[2] It was found that the chlorophyllic substances are responsible for a major part of light-induced oxidation of milk. The photosensitizers absorb the radiant energy from light source and transfer it to highly reactive forms of oxygen such as singlet oxygen.[4] These reactive species then start an oxidative cascade involving other substances in the milk such as amino acids, vitamins (A, C, D, E) and fatty acids. Result is the destruction and loss of important and valuable nutritional components of milk. Riboflavin significantly affects the depletion of headspace oxygen in bottled milk stored under fluorescent conditions.[5] After absorbing visible light between 400-500 nm[2], it forms highly reactive triplet-excited state.[6] The triplet riboflavin is capable of further oxidizing the proteins leading to the formation of low-molecular weight sulfur compounds e.g. dimethyl disulfide imparting "burnt-feather" off-flavor to milk.[6]

An important compound formed by riboflavin photosensitization in milk is n-hexanal which imparts a "beany" flavor to soymilk.[5] Other important off-flavor imparting chemical entities formed due to photo oxidation of milk besides dimethyl disulfide (burnt-feather flavor) are pentanal, 1-octane-3-one, acetaldehyde and 1-hexen-3-one.[7]

Light-oxidized flavor of milk has a very low sensory threshold. A general consumer can detect the light-induced taste defect in milk which has been exposed to fluorescent light for approximately 1 to 2 hours.[8] As in retail setups milk is often exposed to fluorescent light for several hours, therefore most of the bulk marketed milk is prone to develop a consumer detectable light-oxidized flavor defect.

Two important light-induced off-flavors are "burnt sunlight flavor" and "cardboard flavor". The "burnt sunlight flavor" develops and predominates for 2-3 days. This flavor is attributed to the degradation of sulfur containing amino acids, mainly methionine. The "cardboard or metallic" flavor develops after two days and does not dissipate. It is thought to be produced due to lipid oxidation which is a continuity of photo oxidation.[7]

Where light is characterized as the most detrimental factor affecting milk flavor, several approaches and techniques have been tested and developed over the past decade to avoid and counteract photo oxidation of milk, especially in retail setups. As discussed above, riboflavin is the most active photosensitizer in milk. Blockade of the riboflavin excitation wavelengths

of light is the primary approach to deal with the problem. As riboflavin absorbs most of radiant energy between 400-500nm, blocking this particular wavelength was found to significantly reduce the formation of n-hexanal, the main degradation product of riboflavin and an important off-flavor compound.[9] Effects of photo oxidation can be more efficiently reduced if all visible and UV excitation wavelengths are blocked.

In this regard most important role is played by the packaging material. Transparent glass containers can not block any single wavelength of light. Traditional paper-coated cartons can not provide barrier to oxygen. Oxygen also amplifies the effect of light entering the container. Therefore, glass is considered to be most unsuitable packaging material for milk and milk products. Glass bottles can be overwrapped with iridescent films, significantly blocking entry of light [9] but this is not the permanent solution to the problem.

Different types of packaging materials have been studied. Most important of them is polyethylene terephthalate (PET). PET possesses some excellent characteristics which make it an outstanding packaging material. Most important character of PET is its chemical inertness. It is light weight, not easily breakable and is fully recyclable for both food and non-food applications.[10] The contents of PET bottles can be easily poured and the open bottle can be resealed, thus minimizing product recontamination and wastage.[11] PET is a good barrier to oxygen and reduces the adverse effects of light on milk quality in the form of pigmented bottled.[12] Another important packaging material with emerging demand is High Density Polyethylene (HDPE).

These packaging materials have been used in studies as such and with different modification. PET bottles were used in different colors in a study. Amber colored bottles were found to show greater resistance to entry of light into the containers.[7]

In another study, both PET and HDPE were used with different modifications.[12] All of the packaging materials provide sufficient protection against bacterial recontamination but show variable efficiency in blocking oxygen and light entry. Multilayer pigmented HDPE (white TiO_2/HDPE + Carbon black/HDPE + white TiO_2/HDPE), monolayer pigmented HDPE (HDPE + TiO_2) and white pigmented PET (PET + TiO_2) are the best alternative packaging materials for traditional coated paperboard cartons. [12]

Certain polyphenolic substances from plant sources have also been shown to resist photo oxidation in milk and milk-based beverages. These polyphenols like catechin from chocolate,

rutin from fruits and epigallocatechin gallate (EGCG) from tea are active quenchers of triplet excited state of riboflavin. During reduction of triplet-state riboflavin to doublet-state of riboflavin they also react with the reduced state showing dual role as triplet quenchers and as radical scavengers.[6]

Another important approach to overcome the defects of photo oxidation in milk is the addition of antioxidant compounds. Milk naturally contains very low concentrations of antioxidants such as α-tocopherol (13-30 μg/g of milk fat) and ascorbic acid (less than 20 mg/L) but processing treatments result in the depletion of these naturally occurring antioxidants.[13] Various studies have been carried out over time to seek various solutions. Pre-harvest approaches such as introduction of α-tocopherol into the muscles of dairy cows through injection or adding it into the cow feeds have been extensively evaluated. Similarly different processing or post harvest approaches have also been tried such as addition of vitamin C (ascorbic acid) to milk. α-tocopherol terminates the free radical chain by donating hydrogen or electrons to free radicals and converting them into more stable products. Ascorbic acid acts as an oxygen quencher, reacting with singlet oxygen and making it unavailable to take part in oxidation reactions. Both of these are very effective antioxidants.[13]

Both α-tocopherol and ascorbic acid have been used in different studies. When α-tocopherol is used alone as antioxidant, it significantly reduces the effects of photo oxidation but an overall effect is the increased lipid oxidation due to increased fatty acid contents in cattle feed. Dimethyl disulfide is considered to be the primary off-flavor compound of photo oxidation of milk. Addition of ascorbic acid alone as antioxidant significantly reduces the formation of dimethyl disulfide but itself negatively affects the milk flavor. In a recent study both of the antioxidants were used in different concentration alone and in combinations. A trained panel of judges analyzed the samples sensorially while GC-O was used to chemically assess the formation of off-flavor compounds. Moreover the thiobarbituric acid reactive substances (TBARS) assay was used to determine and verify the extent of oxidation. It was concluded that α-tocopherol/ascorbic acid combination is the only treatment that exerts no negative effect on milk flavor. It is suggested that the addition of a combination of 0.25/ α-tocopherol (1.25% α-tocopherol/g of fat) and 0.025 % ascorbic acid to reduced fat milk may protect its from light-induced oxidation.[13]

Another study suggests that addition of β-carotene protects against photo oxidation.[2] As chlorophyllic substances are also responsible for the major part of photo oxidation-induced flavor defects in milk, β-carotene absorbs a major portion of a particular light wavelength (500 nm) that sensitizes the naturally occurring chlorophyllic substances, thus by reducing the photo oxidative degradation of these chlorophyllic substances and development of off-flavor in milk.

Light not only affects the taste or flavor of milk but also produces detectable alteration to the aroma profile of milk. The aroma of fresh milk is mild with a slight cooked note from the thermal process of pasteurization. Using GC-O analysis, 9 odor active compounds have been detected in pasteurized milk. These include heptanal, indole, nonanal, 1-octen-3-ol, dimethyl sulfone, hexanal, 2-nonanone, benzothiazole and D-decalactone. Dimethyl sulfone produces the most intense odor followed by hexanal.[14]

At present, ultra high temperature (UHT) processing technique is being widely used to obtain milk with extended shelf-life making transportation and distribution easy, but prolonged storage increases the chances of photo oxidation. Many odor active compounds have been detected in milk with extended shelf-life, e.g. 2 heptanone (fruity, spicy, cinnamon odor) exerts highest aroma intensity, and was not reported in pasteurized milk while dimethyl sulfone (sulfurous odor) shows much lower odor intensity. 2-undecanone (floral and rose-like odor) is also present only in UHT treated milk.[14]

Many aroma-active compounds have been detected in light-activated milk. Heptanal (green/fish oil odor), pentanal (sour grass), heptanol (sweet), 1-octen-3-ol (mushroom odor), hexanal (cut grass), dimethyl disulfide (boiled potato & burnt-feather odor), 2,3-butanedione (cheesy) and other compounds with pungent or sulfurous odor are typical odor active compounds detected in photo oxidized milk.[14]

Aroma defects in milk can be overcome by addition of natural or synthetic antioxidants. Important synthetic antioxidants are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). As discussed above, naturally occurring antioxidants (α-tocopherol & ascorbic acid) are the best choice when used in combination. Timed-addition of the antioxidants can be achieved by controlled release of antioxidant substances from polymeric packaging material into the product.[14]

Recent studies suggest different techniques to detect and measure photo oxidation. Solid-phase fluorescence analysis is a non-destructive technique to measure extent of degradation of riboflavin in dairy products whereas fluorescence imaging is to used to visualize the intensity and propagation of this process.[1] The efficiency, simplicity and rapidity of this technique are helpful to detect early effects of photo oxidation in dairy products and packaging materials.

LIPOLYSIS & PROTEOLYSIS

Second important factor responsible for the production of off-flavor in milk is lipolysis and proteolysis. Lipolysis or lipid breakdown results in the release of free fatty acids producing a consumer detectable "rancid" flavor. Different types of lipids are naturally present in milk. In highest concentrations are the triglycerides (95-97%) followed by phospholipids (0.5-1%), cholesterol (0.3-0.5%), monoglycerides (0.03-0.01%) and other free fatty acid and cholesteryl esters.[15]

A native lipoprotein lipase (LPL) of milk is responsible for release of fatty acids from triglycerides.[16] Other lipases which cause the breakdown of milk lipids are lipase from somatic cell origin, bacterial lipases and other miscellaneous esterases. Lipolysis in milk may be light-induced (i.e. continuity of photo oxidation) or self-induced (auto oxidation). In fresh milk, milk lipids are naturally protected from action of LPL due to the presence of micellar membrane around milk fat globules (MFG), but mechanical handling and thermal processing results in the destruction of protective micellar membrane rendering milk fats more vulnerable to the actions of lipases, thus resulting in the production of rancid off-flavor in milk.

Fats are not only a source of energy for humans but also promote good health. It is well-established that n-3 fatty acids e.g. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) prevent humans from cardiovascular diseases, hypertension and certain autoimmune and inflammatory diseases.[17] Similarly conjugated linoleic acid (CLA) is identified to have many beneficial health effects. cis-9, trans-11-CLA isomer is found to be a potent anti carcinogen.[18] It also prevents development and causes regression atherosclerotic lesions in animal modals for coronary heart disease.

cis-9, trans-11-CLA is partially formed by bio dehydrogenation of linoleic acid but major portions are formed endogenously using vaccinic acid as a substrate.[19] Using this

information, efforts are being made to increase vaccinic acid fat contents in the diet of dairy cows. This dietary manipulation results in an increase in CLA concentration in milk. Fish oil is recognized as best supplemental source for omega-3 fatty acids but direct fortification of milk with fish oil poses certain problems, the most important of which is the development of a "fishy" off-flavor. Moreover fish oil has a greater susceptibility for oxidation producing more off-flavors, so addition of fish oil & linoleic acid to the animal feed seems to be the best alternative.[20] Addition of calcium salt of palm and fish oil alone or in combination with full fat extruded soybeans and soybean oil increased CLA, vaccinic acid, n-3 & and unsaturated fatty acid contents of milk without altering feed intake, milk yield and milk composition of dairy cows.[21]

Threshold for the detectable lipid oxidation off-flavor of milk caused by native lipoprotein lipase lies in the range of 0.32-0.35 mEq of FFA(free fatty acids)/Kg of milk.[16] A high correlation is found between short-chain free fatty acids determined quantitatively by solid-phase micro extraction/Gas chromatography(SPME-GC) and sensory scores by using a multiple regression analysis.

Proteolysis of milk results in accumulation of fractional peptides which impart "bitter" or "astringent" off-flavor to milk.[16] The principal proteinase in milk is plasmin which is a thermolabile, alkaline serine protease and exists in milk as a part of a complex system including zymogen, plasminogen, plasminogen activators and inhibitors.[22] It specifically targets caseins. Its activity increases with increasing milk somatic cell count (e.g. in mastitis). Second important protease in milk is an aspartyl protease cathepsin D which is derived from somatic cells.[22]

Approximately 80% of milk protein in bovine milk consists of a mixture of four phospho-proteins, αs1-, αs2-, β- and κ-caseins. These proteins exist as micelles in association with micellar or colloidal calcium phosphate.[23] Plasmin cleaves polypeptide chains after a cysteine or arginine residue producing λ-caseins, γ-caseins and proteose-peptones resulting in a bitter milk off-flavor. Ion-exchange chromatography or reversed-phase HPLC of either of whole caseins or fractions of milk proteins can be used to separate the proteolytic fragments.[22]

Alteration of conditions, e.g. freezing, temperature treatments and fractionation techniques like microfiltration affect the activity of milk proteases.[22] Plasmin activity greatly influences

the active ripening of cheese which is a positive effect regarding proteolysis but in milk excessive protease activity always results in the production of off-flavors. Plasmin activity not only affects the quality of fresh raw milk but also significantly influences the rate and extent of hydrolysis of β-caseins during cold storage of milk, e.g. at 5°C or 20°C.[23]

Microorganisms also play a vital role in auto-oxidation specially proteolysis of milk. When milk is stored for long time at the farms after milking, the risk of spoilage due to mesophillic bacteria can be avoided but it furnishes the problem of spoilage by psychotrophic bacteria which grow rapidly in unpasteurized raw fresh milk stored at low temperatures.[24] Most of the organisms are thermolabile and are destroyed during pasteurization but proteolytic enzymes released by these bacteria cause breakdown of milk proteins thus producing off-flavors.

Different protease-producing species of microorganisms include *Pseudomonas, Bacillus, Clostridium, Proteus, Escherichia, Micrococcus, Mycobacterium, Flavobacterium* and *Chryseobacterium*.

Proteolytic enzymes produced by bacilli manifest themselves by increase in non-protein nitrogen concentration and by para-κ-casein formation accompanied by casein micelles destabilization and milk coagulation.[25] *Pseudomonas* spp. produce only one type of protease which especially affect κ-casein, casein micelles and colloidal calcium phosphate. *Pseudomonas* are the most common post-pasteurization contamination. However, *Bacillus cereus* seems to be the most important microorganism limiting pasteurized milk shelf-life. Similarly *Escherichia coli* significantly increases plasmin and gelatinase activity.[26] During capillary electrophoresis some para-κ-casein related peaks were observed in raw and pasteurized milk stored at 6°C for 5 or more days, and in UHT treated milk at 20°C for 30, 60 or 90 days. Presence of these peaks is attributed to protease activity from psychotrophic bacteria. Thus presence of para-κ-casein can also be used as indicator of bacterial milk proteolysis.[27]

Proteolysis in milk can be studied either as quantification of proteolytic changes or determination of proteolytic enzyme activity. Methods for detection of milk protein degradation products like capillary electrophoresis[27] and the Kjeldahl method are reliable but time consuming and inconvenient for manufacture use. Spectrophotometric methods are simple and quick, but their sensitivity is relatively low.[28] Although microbiological analyses

are unable to detect enzymes indigenous to milk, they seem to be very useful and important tool for dairy-plant laboratories. Usage of GTY-M agar enables simultaneous estimation of total mesophillic contamination and percentage of proteolytic microorganisms in a single step.[28]

TEMPERATURE

Another important factor affecting the overall sensory characteristics of milk is temperature. Heating treatments are crucial to clear the milk from bacteria and to enhance the shelf-life period. Most commonly two types of temperature treatments are used in the dairy industry:

Pasteurization during which milk is heated to a minimum temperature of 66°C continuously for 30 minutes with agitation or at 72°C for 15 minutes.

Ultra High Temperature (UHT) during which milk is normally heated at a temperature exceeding 135°C for 2-5 seconds. Where normal pasteurization temperature is effective in destroying pathogenic bacteria, it is insufficient to inactivate thermo resistant spore formers e.g. *Mycobacterium paratuberculosis*.[29] The basis of UHT is the sterilization of food before packaging and then filling into pre-sterilized container in sterile environment. It offers advantages like high quality and extended shelf-life.

Fresh bovine milk has a particular delicate flavor which can be masked by off-flavor compounds. These compounds are directly responsible for rejection of the product by the consumer. The processes involving extensive heating impart a strong ‘cooked’ flavor to milk.[29] This cooked flavor is easily detectable due to milk´s naturally mild, slightly sweet flavor.

Ultra pasteurization can extend the shelf-life up to 45 days [29] but can promote thermally derived off-flavor compounds such as aldehydes, methyl ketones and various other compounds.[30] These temperature treatments not only affect the milk flavor but the aroma profile as well. 41 impact odorants have been identified and isolated using Aroma Extract Dilution Analysis(AEDA). Most important of these are 2-methyl-3-furyl disulfide and bis-(2-methyl-3-furyl) disulfide. [31]

Solvent-assisted Flavor Evaporation (SAFE), Aroma extract dilution and sensory evaluation revealed the epicatechin containing milk has low cooked flavor intensity. Moreover it did not increase the bitterness intensity significantly.[32]

Different techniques are being introduced to detect and quantify thermally generated off-flavors in milk. Atmospheric Pressure Chemical Ionization Mass Spectroscopy (APCIMS) is a useful real-time monitoring tool for thermal flavor generation. This technique can be used to follow the effects of heating temperature and moisture contents on the formation of selected compounds in skim milk powder.[33]

Headspace SPME coupled with gas chromatography (HS-SPME/GC) is found to be very sensitive and can be used to quantify thermally derived volatile compounds in milk.[30] Sensitivity of this technique allows quantification of very low concentrations of off-flavor compounds in milk samples. Due to its accurate determination of compounds of interest, the simple steps, and short time required for the extraction & analysis, HS-SPME/GC shows a high potential to achieve rapid quantitative analysis of volatiles in milk when large number of samples are needed to be analyzed.[30]

Summarizing, it can be stated that a variety of factors affect the development of off-flavors in milk. Milk is sensitive to light, temperature and lipid oxidation etc. Light, being oxidation-inducing factor plays most important role initiating cascade of oxidation reactions. Development of packaging materials with high light resistance is the demand of time.

Milk contains high concentration of fats (triglycerides, phospholipids, cholesterol and other free fatty acids). Degradation of these fats by milk native lipase or lipases from other sources results in increased free fatty acid concentration rendering “rancid” off-flavor to milk. Fish oil and other sources of beneficial omega-3 fatty acids are being added to cattle diet to enhance the nutritional value of milk but on the other hand these substances are making milk more prone to fatty acid oxidation. Caseins are the natural milk proteins. Their degradation by internal proteases or proteases from external sources like bacteria results in the accumulation of fractional peptides resulting in “bitter” or “astringent” off-flavor in milk.

High-temperature treatments are inevitable to remove micro organisms during milk processing but these high temperatures may negatively affect the milk proteins resulting in development of off-flavors.

More research should be focused on the development of techniques that would readily detect off-flavors in milk even in low concentrations. Milking systems in cattle farms and transportation chain should be optimized to avoid the development of off-flavors in milk during its delivery from the cattle farms to the end consumer.

SOYMILK OFF-FLAVORS

Soymilk, a milk variant, is a stable emulsion of oil, water and proteins. It is produced by soaking dry soybeans and grinding them with water. It contains almost same proportions of protein as bovine milk. It is considered as the best alternative of milk for the people who cannot tolerate milk. Some of numerous health benefits of soymilk are anti-carcinogenicity, reduced risk of coronary heart disease and hormone deficiencies.[34]

Development of different off-flavors is also a common phenomenon in soymilk. Important volatile substances of soymilk extracted by SPME and quantified by GC are hexanal, hexanal, 2-nonanol, 1-octen-3-ol, 2,4-decadienal and dimethyl trisulfide. Dimethyl trisulfide has not been detected in freshly prepared soymilk, but was present in rehydrated soymilk prepared from commercial powder products.[35] Grinding soybean at low temperature during soymilk manufacturing is an economical method to produce soymilk having less off-flavor and high protein content.[36]

Isolation of volatile compounds from soybean varieties growing in different locations was done using dynamic headspace analyzer and capillary gas chromatography. Soybean varieties and growing locations have significant effects on volatile components of soymilk. The higher the soybean protein content, the greater are the volatile compounds of soymilk in concentration.[37]

Development of certain ‘Beany’ or ‘grassy’ flavor on storage limits the utilization and popularity of soymilk among the consumers.[38] This beany off-flavor is attributed to 2-pentylfuran which is formed from linoleic acid by singlet oxygen.[37] When soaked soybeans are blanched and ground with hot water at a temperature between 80°C and 100°C, lipoygenase (LOX) activity gradually decreases and thereby decrease the volatile compounds responsible for beany and non-beany flavor.[39]

CHEESE

Cheese is a fermented milk-based food product. Over 500 cheese varieties are officially listed by International Dairy Federation. Many local cheese varieties also exist. The flavor profiles of cheeses are complex and variety- and type- specific.[40] According to the 'Component Balance Theory', proposed in 1950s, typical required cheese flavor is the result of correct balance and concentration of a wide variety of volatile flavor compounds.

The flavor compounds of cheese are formed due to degradation of milk components e.g. lactose, milk fats and milk proteins[40] during ripening period. A disturbance of balance between concentrations of cheese volatiles results in the generation of cheese off-flavors.

PHOTO OXIDATION

Now-a-days consumers demand transparent packaging for the food products. Moreover environmental concerns have let down the use of metallic packaging and aluminum foils.[3] Concept of transparent packaging has satisfied the aesthetic demand of consumer but has made milk products more prone to the damage caused by light. Light depletes different components of cheese like vitamins, e.g. riboflavin which then initiate a cascade of oxidation reactions ultimately resulting in the formation of such compounds which directly or indirectly impart off-flavors to cheese. Riboflavin is considered to be the primary photosensitizer in dairy products which absorbs visible light in spectral region between 400-500nm. It is also a good absorber of UV light. So called 'warm' light (spectral region of much yellow, orange and red light) is considered more harmful than 'cold' light (spectral region of much blue and violet light). In *Havarti* cheese exposed to single wavelength of 366, 405, 436nm, white fluorescent light is found to be more harmful,[41] inducing formation of off-flavors after a few hours. Pink light retards the oxidation processes while green light gives best protection against the formation of off-flavors. Natural porphyrins and chlorophyll residues also act as photosensitizers and impart off-flavors to various cheese varieties.[3] Front-face fluorescence spectroscopy is a non-destructive and effective method for both quantitative and qualitative evaluation of photo oxidation as it measures the actual initialization of oxidation process and degradation of several photosensitizers in intact cheese. [3]

LIPOLYSIS

n-6 fatty acids such as linoleic and γ-linoleic acid are the essential fatty acids that need to be incorporated into the diet as they cannot be synthesized by the body. The adequate intake recommended by USDA for n-3 and n-6 fatty acids is estimated at 1.0-1.6g/d and 12-17g/d respectively.[42] DHA and EPA are incorporated in various food products. Cheese can be fortified with DHA and EPA by two approaches. Using DHA/EPA fortified milk for cheese making or by directly incorporating DHA/EPA during cheese making.[43]

DHA and EPA are polyunsaturated fatty acids. These highly unsaturated fatty acids tend to oxidize producing certain off-flavor compounds so during incorporation of these unsaturated fatty acids, it is important to achieve the desired concentration without causing the formation of off-flavor.

Fortification of cheddar cheese with low amounts (18mg/serving size DHA & 32mg/serving size EPA) does not produce any off-flavor in the cheese.[43] Fortification of cheese with higher concentrations of polyunsaturated fatty acids results in rancid or fishy off-flavor which dissipates with aging. Development of fishy off-flavor is catalyzed by presence of oxygen, light and metals. Formation of certain compounds, e.g. short-chain aldehydes, alcohols and esters impart an off-flavor due to their volatile nature. Disappearance of fishy off-flavor may be due to decrease in the rate of oxidation, bacterial metabolism or masking effects of flavors developed during cheese aging.[43] Unlike fluid milk, addition of vitamin E (α-tocopherol) has no positive effect on the degradation of unsaturated fatty acids. Use of anti-oxidants increases the intensity of some odor compounds, e.g. butanoic acid, but the indicators of unsaturated fatty acid degradation such as heptanal are much higher thus indicating the uselessness of addition of anti-oxidants to cheeses.[44]

BACTERIA-INDUCED PROTEOLYSIS

Bacteria are used as important adjuncts to the cheese flavor. During cheddar cheese maturation, bacteria catabolize the aromatic amino acids produced by degradation of caseins. The result of these catabolic reactions is the formation of certain flavor compounds which enhance the cheese flavor positively. Due to these characteristics, bacteria are used widely as flavor adjuncts during cheese ripening. Most commonly used bacterial species as starter culture are *Enterococcus, Leuconostoc mesenteroides* (sub spp. *cremoris* and *lecon*), *Streptococcus thermophillus, Lactobacilli* (*Lb. delbruckii* sub spp. *Bulgaricus, Lb.*

acidophilus, Lb. casei and *Lb. helveticus*) and *Lactococcus lactis* (sub spp. *cremoris*). Sulfur compounds produced by these starter cultures speed up the cheese ripening process and impart desirable flavors to the cheese. On the other hand, catabolism of aromatic amino acids by these bacteria promote the production of off-flavor compounds. Catabolism products of aromatic amino acids manifest themselves as distinct flavors e.g. phenylalanine catabolites phenyl acetaldehyde and 2-phenethyl alcohol impart floral, rose-like off-flavors whereas tyrosine catabolite p-cresol imparts barny, medicinal or utensil-like off-flavors.[45] Similarly important off-flavor catabolites of tryptophan are indole and skatole.[46]

Mechanism of amino acid catabolism in cheese involves different pathways. Catabolism by *Lactococci* and *Lactobacilli* is initiated by aminotransferase (ATase) enzyme that converts aromatic amino acids to their corresponding α-keto acids.[45] These α-ketoacids may further be converted to benzaldehyde, phenyl acetaldehyde, 2-phenethyl alcohol and other aroma compounds. Different enzymes involved in tryptophan catabolism are tryptophanase, tri-2-monooxigenase, indole acetamide hydrolase and indole acetaldehyde dehydrogenase.[46]

Enhanced expression of certain enzymes like D-hydroxyisocaproic acid dehydrogenase suppresses the spontaneous degradation of α-keto acids and decreases the amino acid catabolism-induced off-flavor generation but this effect also retards cheese flavor development.[45]

A variety of off-flavors is found in cheeses. The most important and often encountered problem is the bitterness of cheese. Microflora used as starter culture for cheese production degrades the milk caseins to polypeptides consisting of 2-23 amino acids residues. Many of these polypeptides are hydrophobic and positively contribute to required cheese flavor but when their concentration crosses threshold levels, bitter taste is detectable in cheese. Bitterness is a particular problem in low-fat cheeses. The reason behind this phenomenon is that the bitter peptide sequences being hydrophobic, partition into the fat phase and their bitter taste is no more perceivable.[40] Bitterness is a major defect in Gouda, Camembert, Cheddar and Gorgonzola cheeses.[47] Increasing sodium chloride concentration in cheese inhibits many bitter peptides and thus can effectively suppress bitter off-flavor.[40] A potato-like off-flavor has also been detected in Gruyère cheese attributed to the presence of 2,3-diethyl-5-methylpyrazine, methanethiol and organic acids.[48]

There is a great difference in the type of milk used for cheese manufacturing. Raw unpasteurized milk contains higher microbial counts which rapidly degrade milk proteins and stimulate the production of aromatic acids which further degrade to certain off-flavor compounds. Moreover these amino acids may be converted to biogenic amines during cheese ripening. Biogenic amines like histamine, tyramine and phenyl ethylamine are potentially toxic. Therefore it is suggested that pasteurized milk should be used for cheese production.[49]

WHEY PROTEINS

During the cheese production process, addition of rennet or acids naturally coagulates the primary milk protein casein forming a solid component (curd or cheese) and a pale greenish yellow liquid that is slightly opalescent (whey). Whey contains great amount of proteins. There are different types of whey proteins. Whey protein concentrate (WPC) contains 25-89% protein and small amounts of lactose, fats and minerals. Whey protein isolate (WPI) is the purest form of whey protein & contains 90% protein with minimal lactose and virtually no fat. Whey proteins are readily bioavailable and accumulate into the muscles helping in protein synthesis. Due to these cheese characteristics, whey proteins are used as nutritional components in various beverages or used wholly as powdered food supplement for reconstitution. Primary proteins in whey proteins are α-lactalbumin and β-lactalbumin.[50]

Whey proteins are highly susceptible to off-flavor generation. Riboflavin of milk reacts with fluorescent light to produce super oxide anions and its interaction with milk proteins oxidizes methionine to methional which yields a dirty potato flavor. Similarly storage is also an important factor producing off-flavors. Major storage-related off-flavor associated with dried whey and whey products is a ‘stale aged’ flavor.[51] Other predominant storage related off flavors are cardboard, raisin, fatty and cucumber.[50] Stale off-flavor mostly limits the use of whey proteins in food products. Dimethyl disulfide, pentanal, hexanal, heptanal, 2-heptanol, 2-octanone, octanol and 2-nonanol are found to be the substances responsible for storage-related off-flavors in whey protein concentrate.[52]

Temperature abuse also badly affects the whey protein flavor initiating the browning of sugar components (Maillard reaction or Maillard browning). Extent of Maillard browning can be significantly reduced by reducing sugar contents e.g. lactose.[50] Therefore, sweet whey powder is more prone to Maillard reaction than WPC and WPI. Aliphatic aldehydes, methyl ketones, 1-pentanol, 1-octen-3-ol are most important products of lipid oxidation imparting

off-flavors to whey proteins. Different compounds isolated from whey proteins are associated with typical off-flavor attributes. Dimethyl trisulfide is associated with 'cabbage' off-flavor in whey protein isolate.[51] Pentanal has cardboard flavor[52] while 2,4-decadienal produces astringent flavor.[53]

Coming to the conclusion, we can say that light, lipolysis, proteolysis and temperature variations are the main factors which affect the flavor and aroma profile and milk and milk products.

Light induces photo oxidation resulting in accumulation of off-flavor compounds. It also initiates lipolysis. Lipolysis is the breakdown of milk fats resulting in milk rancidity. Proteolysis, on the other hand is breakdown of milk proteins (caseins), rendering off-flavors to milk. Temperature treatments also significantly alter flavor and aroma profile of milk.

Different techniques such as SPME, GC, SAFE and capillary electrophoresis can be used to determine the extent of deterioration produced by these factors. Selection of suitable packaging materials and addition of appropriate amounts of anti-oxidants may limit the oxidative damage.
Besides milk, milk products such as cheese, whey proteins and soymilk are also affected by these deteriorating factors in terms of flavor and aroma.

Chapter 2

MEAT

MEAT OFF-FLAVORS

Meat is the most important source of proteins in human diet. Meat palatability directly correlates with meat flavor. Other properties such as juiciness and tenderness of meat are very important but flavor probably plays most important role in consumer preference and acceptation of meat products.

Meat flavor is a complicated phenomenon. Hundreds of compounds such as hydrocarbons, aldehydes, ketones, alcohols, furans, triphenes, pyrrols, pyridines, pyrazines, oxazoles, thiazoles and sulfurous compounds contribute to the typical flavor and aroma of meat and ultimately influence the flavor perception of meat by the consumer.

Meat is obtained from different sources, important of which are cattle, pigs, lamb and poultry. Each variety of meat possesses a particular flavor and aroma. Different factors such as lipid contents, oxidation, feed & diet, myoglobin and pH directly affect the inherent flavor of meat.[55]

High lipid contents including phospholipids and polyunsaturated fatty acids render meat more prone to lipid oxidation. Concentration of metal ions, oxygen, salts and other pro-oxidants also play an important role in this regard. Different compounds formed this reaction cascade result in development of rancid off-flavor in meat.

Diet differences also affect the flavor of meat. Grain-fed animals contain more lipid contents as compared to grass-fed animals. Hay diets have also been introduced and are found to produce meat with less desirable flavor. Type of feed actually affects changes in lipid deposition and fatty acid composition.[55]

Change in pH also affects flavor development in meat. Concentration of nitrogenous compounds increases with increase in pH. Fresh meat has a pH of around 5.5-6.0 with a good buffering ability. [55] Another important factor is the type of muscle used, as different muscles contain variable fatty acid contents thus showing difference in flavor profile.

Raw meat has little aroma and only a blood like flavor.[56] Perceivable meat flavor is thermally derived during cooking. Sweet flavor of meat is attributed to sugars and amino acids. Sour flavor arises from amino acids coupled with organic acids, where as inorganic salts and sodium salts of glutamate and aspartate generate saltiness and savory taste in meat. Bitterness in meat is likely due to the presence of hypoxanthine, anserine and carnosine.[56]

Phospholipids in the meat react with cysteine and ribose to produce mild, slightly beefy compounds. Oxidation of polyunsaturated fatty acids results in the formation of aldehydes which impart an overall meaty, tallow odor to meat.[57]

Carbohydrates give rise to furans which react with the sulfur containing amino acids cysteine to yield roasted meat aromas. Carbohydrates also produce lactones which impart dairy, sweet or waxy flavor notes.[58] Another important meat flavor is 'umami' which is described as savory or brothy and is attributed to monosodium glutamate, inosine monophosphate and guanosine monophosphate (GMP).[56]

BEEF OFF-FLAVORS

Beef is the most widely used meat variety throughout the world, right from restaurants to fast-food chains to household cooking. Appearance, texture and flavor of beef are three important sensory properties which affect the consumer preference. Muscles from beef shoulder (Chuck), hips (Round) and back (Loin) are most commonly used.[55]

Different important off-flavors in beef are rancid, oxidized, metallic, sour, fatty, burnt, salty, bloody, grassy, livery, painty and gummy.[59] Certain flavors are formed upon cooking. Maillard reaction or non-enzymatic browning is very important in this regard.[60] During this reaction amino compounds condense with carbonyl group of a reducing sugar in the presence of light. Glycosylamine produced as a result of this reaction after rearrangements and dehydration forms furfural, furanone derivatives, hydroxyketones and dicarbonyl compounds.[55] As the reaction proceeds through different pathways and rearrangements, melanoids are ultimately formed which are brown, high molecular weight polymers[60] and impart certain off-flavors to meat.

Different compounds are directly correlated with certain off-flavors. Heptanal is related to oily, fatty and rancid flavor notes. Hexanal produces fatty, fishy off-flavor while benzaldehyde is correlated with burning aromatic taste. Nonanal and 2-nonanone are responsible for floral and fruity flavor where as pentanal has pungent, acrid taste and pentane has very slight warmed-over flavor and oxidized taste.[55] Different factors such as age of animal, animal breed, lipid content, diet, oxidation, myoglobin and pH affect the inherent flavor of beef.[61]

BEEF BREED & FLAVOR

Different breeds of beef vary in composition of nitrogen & sulfur compounds, free amino acids, alcohols, aldehydes, and ketones.[62] Friesian cattle has a different volatile profile than

Pirenaica cattle.[63] Similarly, lean meat from Wager cattle has better sensory quality than that from dairy cattle.[56]

CATTLE DIET & BEEF FLAVOR

Difference in cattle diet produces meat with variable taste and flavor both in ruminants and non-ruminants. More than 40% beef flavor variation is due to difference in cattle diet.[64] Any change in animal diet pre-slaughter significantly alters the physical properties of meat.[61] Meat flavor characteristics of grain-fed cattle are better than forage- or grass-fed cattle.[56] Grass-fed beef is less prone to lipid oxidation due to presence of antioxidants vitamin E, carotenoids and flavonoids.[61] Moreover, grain feeding increases the polyunsaturated fatty acid content. These polyunsaturated fatty acids are susceptible to lipid oxidation. High phospholipid contents are related to off-flavors in ground beef.[56] When cattle are fed on grain diet, 'stale' off-flavor decreases and roasted beef flavor is increased presumably due to increased phospholipids, phosphatidylcholine and phosphatidylethanolamine. Lactone also correlates with roasted-beef flavor. On the other hand, diterpenes correlate with increased 'stale' off-flavor.[56]

Diets which constitute of fish products, soybeans or certain oils such as canola or palm oil are rich in EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid).[65] Both EPA and DHA are polyunsaturated fatty acids which improve overall nutritional value of beef but at the same time diets high in polyunsaturated fatty acids may contribute to the appearance of off-flavors in beef including 'fishy'[66] and 'rancid' off-flavors.[61]

AGING & BEEF OFF-FLAVORS

Post-harvest aging definitely affects the overall flavor profile of beef muscles. Aging conditions such as oxygen, time of aging, temperature and humidity also affect the beef flavor.[56] On one hand, aging enhances some positive flavor notes such as 'beefy', 'brothy' and 'brown-caramel' but on the other hand, some off-flavors such as 'cardboard', 'bitter' and sour are also produced.[63], [64] Concentration of certain chemical substances such as nonanal, butanoic acid and 1-octen-3-ol increases with aging.[67] These substances are correlated with off-flavors.

After the slaughter, loss of circulatory competency results in the accumulation of metabolic by-products such as lactic acid in the muscles. pH in the muscles decreases post-mortem. Many thiol proteases are activated at this low pH of about 3-4. These enzymes correlate with 'rancid', 'sour' and 'salty' off-flavors. Aging enhances lipid oxidation resulting in increased carbonyl compounds which impart noticeable off-flavors.[56] Similarly, aging in high oxygen atmosphere results in increased 'burnt', 'toasted' off-flavor.

ENHANCEMENT & BEEF FLAVOR

Enhancement treatments are commonly used to improve the sensory qualities of beef.[68] Enhancement improves not only the tenderness but beef flavor is also affected. Traditionally, enhancement is done with solution of water, salt and phosphates.[69] Salt enhances the flavor but it is also a pro-oxidant. Phosphates on the other hand retain the fluids in the meat and chelate the free ionic iron thus making it unavailable to catalyze lipid oxidation. Enhancement solutions may also contain flavor enhancers or organic acids such as sodium diacetate which extend the beef shelf-life by suppressing the growth of micro organisms.[56]

Hexametaphosphate, sodium tripolyphosphate, tetrasodium pyrophosphate, sodium lactate and calcium lactate are commonly used as components of enhancement solutions.[56] A new procedure consists of injecting a solution comprised of water, salt, ammonium chloride and carbon monoxide. This treatment is highly effective in both high-value cut loin muscles and low-value beef chuck and round muscles.[69]

Enhancement generally ranges from 6%-12% of initial weight. Higher concentrations may creat off-flavors.[70] Enhanced beef is much saltier, more intensely beef flavored and has less off-flavor and 'beany/grassy' aroma than non-enhanced beef.[61]

HEAT & BEEF FLAVOR

Typical meaty flavor of beef develops during heating (cooking). Cooking temperature and cooking medium both affect the flavor and tenderness of beef.[61] Flavor of cooked beef depends on the water soluble components of food. At least seventeen compounds contribute to specific cooked beef aroma.[56] Rate of heat penetration, cooking time, total moisture and juiciness varies with type of muscle and type of heat treatment (deep fat frying, oven roasting, oven braising and pressure braising).[61] Increasing or decreasing cooking temperature also affects the formation of flavor compounds in beef. In general, the higher the degree of heating, the higher the concentration of aliphatic aldehydes, benzenoids, polysulfides, heterocyclic compounds and lipid-derived volatiles.[56]

Heating flavor also develops via browning or Maillard reaction in which amino acids and sugars condense to form aldehydes and amino ketones.[56] Similarly, urea is generated during beef heating, which due to its reducing nature produces important nitrogen containing volatiles such as pyrazines and thiazoles.[58]

MUSCLE TYPE & BEEF FLAVOR

As different muscles differ in tenderness and juiciness, there is difference in the occurance of off-flavors among various muscles. In general, beef round muscles have less off-flavors than loin and chuck beef muscles.[72] Hexanal, an indicator of lipid oxidation, is present in almost

double amount in chuck muscles than that in round muscles, where as sirloin muscles contain least amount of hexanal.[56]

LIVER-LIKE OFF-FLAVOR IN BEEF

Liver off-flavor in beef is a sporadic but very important off-flavor which renders the meat unacceptable for the consumer.[61] Potential causes of the liver-like off-flavor in beef are lipid oxidation, heme iron content and elevated degrees of doneness.[73] Sulfur containing compounds such as thiols, sulfides, thiazoles and sulfur-substituted furans interact with carbonyl compounds to produce livery flavor attributes.[56]

Lipid oxidation may be induced by prolonged aging, heating or light penetration. Lipid oxidation is a chain process. Once started, it continues on and on to produce deteriorating effects on meat. It is accomplished in three stages: initiation, propagation and termination. Initially, due to the triggering factor (e.g. light), free radicals are produced. Each of these free radicals then reacts with other chemical entities to generate more and more free radicals. The higher the concentration of phospholipids, phosphatidylcholine and phosphatidylethanolamine, the greater will be the occurrence of livery off flavor.[56] Similarly, 16-, 17-, 18- and 20-carbon chain fatty acids (2-decanal, 2-undecanal, propanoic acid, vaccenic acid, cis-11,14 eicosadienoic acid, 5,8,11,14,17-eicosapentaenoic acid) also correlate with liver-like off-flavor in beef.[72] On the other hand, oxidation of arachidonic acid is also associated with metallic and liver flavor.[61]

Animal diet plays an important role in this regard, as carcasses from the grain-fed cattle are richer in polyunsaturated fatty acids such as conjugated linoleic acid (CLA) than those of pasture-fed animals. The higher the concentration of free polyunsaturated fatty acids, the greater will be the degree of lipid oxidation.[56]

Free ionic iron also plays a vital role in propagation of lipid oxidation. In raw beef muscles, iron exists as a component of heme group of myoglobin and hemoglobin, but heat during cooking causes hemoglobin degradation resulting in the increased concentration of free ionic iron.[61] This free ionic iron is a known pro-oxidant and greatly accelerates lipid oxidation and thereby development of liver off-flavor.[74] Therefore intensity of liver off-flavor in beef will be increased with increasing internal cook temperature (greater degree of doneness) as more ionic iron will be liberated.[61]

Age of the animal may also affect the development of liver-like off-flavor, as it was found that beef from bulls has higher livery odor and flavor than that from heifers. This fact is related to higher 2-propanone and ethanol concentration in bulls than heifers.[63] Moreover, carcass maturity can also affect iron content and may increase off-flavor notes.[56]

As it is hypothesized that residual blood hemoglobin results in development of liver off-flavor, complete bleeding post-mortem is the best way to avoid liver off-flavor. Post-mortem electrical stimulation (ES) is one of the important techniques used mainly in USA. ES increases blood loss from carcasses.[61] It is most effective procedure in the young animal carcasses with greater tenderness.

Lipid oxidation (another main cause of liver off-flavor in beef) can be prevented by removal of oxygen and addition of proper antioxidants.[61] Rancidity can be reduced by packaging beef (both steaks and ground beef) in low oxygen, modified atmosphere with 0.4% carbon monoxide.[75] High oxygen concentration (usually 80%) is widely used during packaging of fresh meat to maintain red color but it makes the beef more prone to development of rancid or liver-like off-flavors.[76] Therefore, use of antioxidants is inevitable especially for packaging of fresh meat. α-tocopherol (vitamin E), sodium tripolyphosphate (STP), phytic acid and milk mineral (MM) are important antioxidants used during meat packaging. α-tocopherol slows down the propagation step of lipid oxidation,[61] whereas polyphosphates chelate the free ionic iron, making it unavailable to catalyze lipid oxidation.[77] MM is found to be an effective antioxidant in ground beef.[77]

WARMED-OVER FLAVOR (WOF)

Warmed-over flavor is a primary cause of deterioration in cooked, refrigerated and pre-cooked, frozen meat products. The term 'Meat Flavor Deterioration' is now used to include both, the development of WOF and concurrent loss of desirable meat flavor. WOF includes a variety of odors and flavors such as 'stale', 'cardboard' , 'painty' and 'rancid'.[78]

WOF is mainly caused by lipid oxidation of beef. Polyunsaturated fatty acids (PUFA) located in the plasma membranes as phospholipids are more prone to oxidation.[78] Oxidation of these PUFA results in the formation of smaller molecules such as pentanal, hexanal and 2,4-decadienal having typical WOF.[78] lipid oxidation leads to the formation of free radicals.[79] Heat, light and various oxidases trigger oxidation of PUFA. Heat causes proteins to coagulate, disrupting the plasma membrane structure and releasing PUFA. Moreover, free ionic iron is released from hemoglobin and myoglobin which further catalyzes the oxidation reaction.[78]

Ions other than iron (such as copper from water), processing equipments, spices, oxygen (key component of oxidation) and light (photo activating wavelengths) are all contributing factors to the development of WOF.[78]

Lipid oxidation can be inhibited or at least minimized by using antioxidants. Antioxidants used may be: natural antioxidants (e.g. α-tocopherol, ascorbic acid and erythorbic acid,

chelating antioxidants (EDTA, citric acid, phosphate) and free radical terminators (e.g. butylated hydroxytoluene and tetrabutyl hydroquinone).[79] Herbs and spices such as rosemary, marjoram, sage, thyme, mace, allspice and clove also contribute some antioxidants.[78]

PORK OFF-FLAVORS

Pork is a meat variety widely used throughout the world. Pork quality can be defined on indexing parameters (e.g. lean carcass yield, carcass fat content), sensory parameters (aroma, flavor, appearance and taste) and nutritional parameters (feed type, feed patterns).[80]

Development of off-flavors is common to pork. Probably most important cause of pork off-flavor like other meat varieties is lipid oxidation. Pork meat contains high levels of unsaturated fatty acids as compared to meat from ruminants.[81] Therefore, it is more susceptible to lipid oxidation. Factors such as increased levels of unsaturated fats, polyunsaturated fatty acids, oxygen, heat, UV light, metal ions, meat/heme pigments and oxidative enzymes accelerate lipid oxidation in pork.[82] Pre-cooked pork patties are highly sensitive to lipid oxidation with significant development of off-flavor and loss of desired meat flavor upon reheating following chilled storage.[83] Diet of finishing pigs has a great impact on post-mortem lipid oxidation.[81] Feeds containing greater amounts of polyunsaturated fatty acids (PUFA) definitely improve the nutritional properties of pork meat but at the same time higher concentration of these PUFA makes the meat more prone to off-flavor development.[84], [85]

Saturated fatty acids pose more health problems than their unsaturated counterparts. Polyunsaturated fatty acids are a vital component of food. Manipulation of the pig diet improves PUFA profile of pork meat.[80] Addition of flax seeds to pig diet is one of the intervention in this regard. Flax seed oil contains approximately 72% polyunsaturates and is the richest oil seed source of linolenic acid (58%). Therefore, feeding flax (which contains 40%-45% oil) to finishing pigs increases the concentration of beneficial omega-3 (n-3) fatty acids in carcass fat and produces pork with value added potential.[86] Other oils such as canola oil, rapeseed oil and fish oil can also be used instead of flaxseed oil[80] to achieve good concentration of omega-3 fatty acids in pork muscles. But increased concentration of PUFA due to addition of these oils in pig diet makes the pork meat more vulnerable to lipid oxidation and consequently developing off-flavors. On the other hand, addition of dietary distiller dried grains with solubles (DDGS) to pig diet decreases saturated fatty acid content

and increases the polyunsaturated fatty acid content without significantly altering the flavor profile of pork meat.[87]

Salt (sodium chloride) is used as enhancement solution to improve the texture and overall sensory properties of meat post-mortem. It binds the proteins and retains the fluid in the muscles thus increasing tenderness and maintaining juiciness of meat. In addition to texture improvement, salt is also used to enhance flavor and to extend the shelf-life of meat by limiting the growth of pathogenic bacterial species.[88]

Salt acts as a pro-oxidant at different concentration. It is believed to displace bound iron from hemoglobin, resulting in increased concentration of free ionic iron which accelerates lipid oxidation and hence off-flavor development.[88]

Synthetic antioxidants such as butylated hydroxyltoluene (BHT) and propyl gallate (PG) retard lipid oxidation and extend shelf-life of meat,[89], [90] but the trend is being shifted from synthetic antioxidants towards plant-derived food ingredients, naturally acting as antioxidants.[82] Rosemary extract is shown to effectively maintain low thiobarbituric acid-reactive substances (TBARS) values in raw frozen sausage.[91]

Plant extract or animal products, such as aloe vera, fenugreek, ginseng, mustard, rosemary, sage, soy protein, tea catechins and whey protein concentrates are very effective antioxidants when incorporated into cooked pork patties.[92] Similarly, paprika and garlic also effectively inhibit lipid oxidation.[89] Food with high oxygen radical absorbance capacity (ORAC) values such as dried plums (containing phytophenolic compounds) also effectively inhibit oxidation of low density lipids (LDL).[82]

Like other meat varieties, pork meat also readily develops liver-like off-flavor. The key compounds responsible for the development of liver-like off-flavor (a combination of ‘metallic’, ‘cardboard’ and ‘fishy’ off-flavors) in pork meat, as characterized by simultaneous steam distillation-solvent extraction (SDE), gas chromatography (GC) and gas chromatography-olfactometry (GC-O) are aldehydes (1-89%), ketones (0.6%), alcohols (0.6%), furans (0.1%), thiazoles (0.2%), phenols (0.3%) and acids (80.1%).[93]

Marbling or intramuscular fat (IMF) contents play an important role in determining the tenderness and positive flavor of meat.[94] In particular, degree of marbling influences sensory traits of texture, tenderness, flavor and juiciness.[95] Generally, higher level of marbling is positively correlated with good pork flavor,[96] whereas reduced IMF is correlated with strong off-flavors.[94]

A typical off-odor is observed in the meat from intact male pigs which limits their use despite of high production efficiency.[80] This off-odor is mainly due to male sex hormone derivative

androstenone and skatole which is an indole derived from microbial breakdown of tryptophan in hind gut. Skatole in high concentration produces a 'boar taint'. As it is derived from microorganisms, use of antibiotics or probiotics in food regimens may reduce its concentration ultimately reducing 'boar taint' in pork meat.[80]

LAMB OFF-FLAVORS

According to the surveys, the consumers rank lamb meat last among other meat varieties (beef, chicken, fish, pork, turkey and veal).[97] There is an overall negative perception from consumers regarding lamb flavor which must be assessed and resolved in order to increase the consumption of lamb meat in human diet.

The basic meaty flavor resides in the water-soluble portion of meat, where as species-specific flavor of meat resides in lipid-soluble fraction. Branched chain fatty acids, carbonyl compounds, sulfur containing compounds, lipid oxidation products, phenols and many other compounds are responsible for specific lamb flavor.[97] Higher concentration of alkyl phenols is also correlated with specific lamb-flavor.

Lamb and goat fat contains branched chain fatty acids (BCF), ranging from 8-10 carbons, which play an important role in flavor development.[97] These fatty acids such as 4-methyloctanoic acid (MOA), 4-ethyloctanoic acid (EOA) and 4-methylnonanoic acid (MNA) are also the main determinants of mutton odor and their concentrations are influenced by factors such as age and nutrition.[98] Concentration of these branched chain fatty acids is greater when lambs are fed on barley or diets containing propionate.

Lipid or fat concentration also significantly affects the flavor profile of lamb meat but this lipid concentration in turn depends on the nutritional regimen. About 244 compounds have been identified with specific differences for nutritional regimen.[97]

Type of pasture consumed pre-slaughter can alter the lamb meat flavor.[99] The animals fed on concentrates possess high ammonia, bitter, metallic and rancid off-flavors and low mutton flavor.[100] Similarly, animals finished on rye grass or clover pasture have high sheep meat flavor and possess low barnyard or rancid off-flavors than the lambs finished on either dried Lucerne or maize concentrate.[101]

Manipulation of lamb diet alters the fatty acid profile of lamb meat. Generally, grain feeding (especially corn) increases the unsaturated fatty acid content and concentration of saturated fatty acids is decreased. Feeding higher levels of high metabolizable energy (diets consisting

of alfalfa, corn and soybean) increases 18:1 acid content in lamb meat, thereby decreasing saturated fatty acid concentration.[97]

There is difference in overall lipid composition, fatty acid deposition and composition of certain volatile compounds among different lamb species. Mutton flavor intensity is almost similar among Romney, Hampshire, Columbia, Rambouillet and Merino species but unsaturated fatty acid content is higher in finer-wool breeds.[97]

Concluding the discussion, meat (whether red or white) is undoubtedly the best natural source of proteins and an important component of human diet. Beef, pork, chicken, fish, veal, turkey and lamb are the prime meat varieties consumed worldwide. Meat flavor is greatly influenced by various factors such as lipid concentration, age of animal, breed of animal, type of diet consumed by the animal, type of muscle used and specific treatments post-mortem (e.g. enhancement injections). Raw meat has little aroma and only a blood-like flavor. The desired meaty flavor develops during heating (cooking). Lipid oxidation is the major cause of off-flavor development in all meat varieties. Lipid oxidation is a chain reaction which ultimately leads to formation of certain chemical entities which possess specific off-flavors and off-aromas.

Lipid oxidation in meat can be inhibited or delayed using anti oxidants such as α-tocopherol, ascorbic acid etc. Spices used during packaging and cooking also possess antioxidant properties. Similarly, manipulation of animal diet regimen can positively alter the fatty acid composition of meat and hence can improve overall flavor and aroma profile.

Most challenging area in meat research is the development of new and advanced techniques for processing, analyzing and storage of meat. Moreover, special attention must be paid to animal diet regimens in future research.

Chapter 3

AQUACULTURE OFF-FLAVORS

OFF-FLAVORS IN DRINKING WATER

Water is the most basic necessity without which no life is possible. Fresh water bodies and water reservoirs have been for centuries used to furnish the human needs. With the invention of sophisticated observatory devices such as microscope, it came into knowledge that water contains a variety of living organisms both from animal and vegetable origin. Water is used for various purposes such as domestic supplies, aquaculture, food industries and water-dependent industries.[102]

Taste and odor in water has always been a problem both from the water authorities and consumer's point of view. With the increased trend of usage of surface water, the need to maintain water quality in the finished product of a water treatment plant is being emphasized.[103] In the last decade many algal metabolites were isolated from water which were associated with bad odor and off-flavors in water.[104]

Majority of all biological taste and odor outbreaks in drinking water characterized world wide are caused by microbial production of geosmin and 2-methylisoborneol (2-MIB)[105] These two saturated bicyclic terpenoids are chemically named as trans1,10-dimethyl-trans-9-decalol (geosmin) and 1,2,7,7-tetramethyl-exo-bicyclo[2.2.1]-heptan-2-ol (2-methylisoborneol). Geosmin is typically described as having an 'earthy' odor while odor due to 2-MIB is referred to as 'musty'.[104] Other important taste and odor compounds (TOC) in water reservoirs are methyl tertiary butyl ether (MTBE) having 'terpentine' or 'oily' taste; 2, 4-heptadienal; decadienal; octanal ('fishy' or 'rancid' odor); chlorine ('chlorinous' odor); chlorophenols and iodoform ('medicinal' odor); volatile organic compounds ('paint-like' odor); metals ('metallic' odor) and green algae ('grassy' odor).[106] General TOC present in water do not contribute harmful risks to human health but may be indicative of the toxicity potential of organisms producing them.[107] In a recent study, it was shown that geosmin and 2-MIB do not possess cytotoxic potential towards cultured human-derived cells even at a concentration of 700ng/L.[108] TOC have very low sensory thresholds (even at ng/L levels). Under typical bathroom and shower stall settings, geosmin and nonadienal are detectable at aqueous concentration exceeding 10ng/L (at 42 °C) whereas 2-MIB is only detectable above 20ng/L (at 42 °C).[109]

Production and concentration of taste and odor compounds (TOC) is influenced by various environmental factors such as temperature, light and nutritional levels.[110] Temperature affects the production of geosmin by algal cells. Geosmin production is generally increased at low temperature, reaching at its maximum value at the optimum temperature for the given species.[102] Concentration of intracellular geosmin increases with cellular growth at optimum temperature, whereas extracellular geosmin concentration decreases with decrease in temperature. Higher temperature may also favour the release of geosmin into the medium thus increasing the concentration of extracellular geosmin.[102] On the other hand, at low temperatures, synthesis of intracellular geosmin is increased.[111]

Similarly, at optimum light intensity (depending upon the algal species), synthesis of geosmin is maximum. As a general trend, at low light intensity, geosmin concentration increases. It is probably because the geosmin precursors (geranyl pyrophosphate and farnsyl pyrophosphate) in thylakoid membranes of algal cells attempt to synthesize geosmin rather than chlorophyll when photosynthetic activity is decreased. Similarly, at optimum light intensity, the concentration of extracellular geosmin increases with increase in cellular growth.[102] Another important point is that although concentration of extracellular geosmin increases as more geosmin is extracellularly released along with the growth stage, great concentration of geosmin is still retained intracellularly by algal cells with increase in growth.[102] Therefore, it has been suggested that filtration media should be used as first step for eradication of off-flavors in order to avoid cell disruption which would cause enhanced release of geosmin into the medium.[112]

Nutritional factors promoting biomass also promote the production of geosmin. Both ammonium-N and nitrate-N affect the geosmin production. Increase in ammonium-N concentration causes an increase in geosmin synthesis, whereas higher concentrate of nitrate-N suppresses the synthesis of geosmin. Geosmin/mg biomass is also positively correlated with ammonium-N concentration. Greater phosphorous-phosphate concentration also increases geosmin synthesis.[111]

Quality of water also correlates with the occurrence of odor and flavor compounds. This correlation can be determined using physicochemical and biological parameters of water. These parameters can be studied through principal component analysis. Eutrophication of water affects the geosmin production positively whereas anthropic contributions increase the 2-MIB concentration.[113]

Geosmin and 2-MIB are tertiary alcohols which exist as their both enantiomer forms. Naturally occurring (-)-enantiomers are ten times more potent than (+)-enantiomers in

generation of malodors.[105] These odor and taste compounds are biologically originated from a number of sources. Important of these sources are benthic and pelagic aquatic microorganisms found in source water such as lakes, reservoirs and running waters; industrial wastes[105]; human-spread pollutants and increased eutrophication. Both heterotrophic and photoautotrophic organisms are involved in production of odor and taste compounds.

Actinomycetes are the important heterotrophic producers of geosmin and 2-MIB. *Streptomyces*, which are aerobic filamentous *Actinomycetes* are most important in this regard.[105] Non-streptomycete *Actinomycetes* such as *Nocardia* are also important sources of production of geosmin and 2-MIB.[114] In a study, high concentrations of geosmin were detected in sediments of a Chinese reservoir and eight *Streptomycetic* strains isolated from these sediments were verified as producers of geosmin and 2-MIB by Headspace Solid-Phase Micro-Extraction/Gas Chromatography/Mass Spectroscopy (HSPME-GC-MS).[115]

Actinomycetes are Gram positive, filamentous, spore-forming bacteria, which form colonies with a leathery surface and greyish color on agar media.[116] These are the most common microorganisms in most aquatic environment.[117] TOC production by *Actinomycetes* is studied using culture-dependent techniques such as growth on selective or general agar media.[118] Culture-independent techniques such as fluorescence *in situ* hybridization (FISH), targeting rRNA are potent techniques to quantify and determine the identity of active microorganisms in natural systems.[119]

Cyanobacteria (previously called 'Blue-Green algae') are major photoautotrophic producers of geosmin and 2-MIB.[105] Cyanobacteria may be planktonic, benthic or epiphytic. Visible surface blooms of cyanobacteria are usually considered to be primary sources of source water odor.[105] Due to increase in cyanobacterial blooms, the occurrence of a number of toxic metabolites (i.e. algal toxins) in water supplies has also increased. These algal toxins pose direct threat to human health. *Oscillatoria splendida, Oscillatoria geminate, Lyngbya subtilis* and *Oscillatoria limnetica* are the primary cyanobacterial species which produce geosmin and 2-MIB.[120] Traditional techniques such as plate-count method and growth on selective media are not sufficient tools to enumerate the microbial species producing TOC. Production of geosmin and 2-MIB is a complex process with considerable variations among microbial taxa, therefore, biochemical and molecular data should also be integrated with modern analysis techniques such as FISH and FISH CARD (Catalyzed Reporter Deposition).[105]

2-MIB is a monoterpene and geosmin is an irregular sesquiterpene.[105] Three different pathways have been proposed for biosynthesis of these two TOC which are namely MEP (2-methyl erythritol-4-phosphate) pathway, MVA (Mevalonate) pathway and Leucine pathway.

Most important of these is MEP.[121] In a study, two compounds were introduced to a medium containing *Streptomyces*: deuterated [5,4-$^{2}H_2$]1-deoxy-D-xylulose and [4,4,6,6,6-$^{2}H_5$]-mevalolactone. Labeled geosmin was produced with former whereas no geosmin production was observed with mevalolactone. This confirms that MEP isoprenoid pathway is most predominant pathway for geosmin production.[122] MVA pathway is used primarily for the synthesis of geosmin and other isoprenoids in myxobacteria[123] and during stationary growth phase in *Streptomycetes*.[105] Myxobacteria also use the minor pathway starting with leucine.[123] During genetic studies, certain protein domain has been identified which is required for biosynthesis of geosmin.

A supposed flow diagram indicating important steps in different biosynthesis pathways for biosynthesis of geosmin and 2-MIB is given in Fig.1:

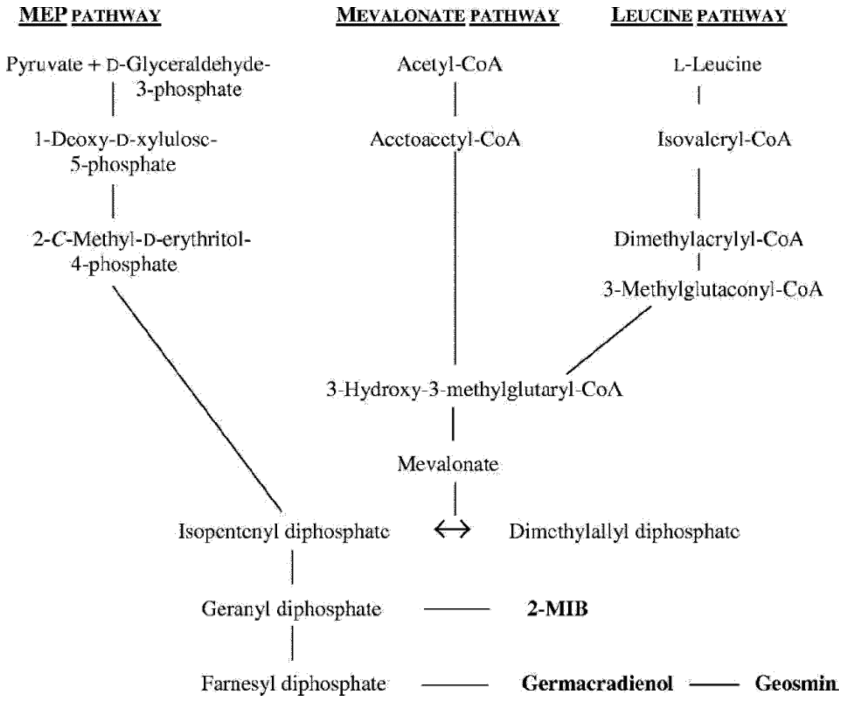

Fig.1: Proposed Biosynthetic pathways for geosmin and 2-MIB[105]

Chronic causatives of aquaculture malodors and off-flavors geosmin and 2-MIB are detectable by humans at parts per trillion (ppt) levels. A variety of techniques have been used to determine and analyze both TOC qualitatively and quantitatively. Certain extraction techniques such as closed-loop stripping analysis (CLSA), liquid-liquid extraction (LLE), solid phase extraction[125], solid-phase microextraction [126], [127], purge and trap, stirbar sorptive extraction (SBSE)[127] and headspace single dynamic microextraction (HS-SDME) have been widely used for extraction and determination of geosmin and 2-MIB.[128] Solid-

phase microextraction technology (SPME) augments both headspace and purge & trap techniques.[129] Relatively low cost and ease of use make SPME widely used analysis technique.[130] Using SPME, geosmin and 2-MIB can be readily detected in water at concentrations lower than ppt range.[131] On the other hand, SPME is a time-taking process. Membrane-assisted solvent-extraction (MASE) is an alternative to SPME, which is a liquid-liquid extraction technique.[131] A recent extraction technique is dispersive liquid-liquid microextraction (DLLME).[132] Recently a modification has been made to DLLME, by implication of ultrasound energy to assist the dispersion and is a more environment-friendly technique.[133] Dynamic headspace coupled to $^{1}D/^{2}DGC$-MC is also a useful technique in this regard.[134] Similarly, extraction of samples directly in the sampling bottle with dichloromethane is a practical method for extraction of multiple water samples for 2-MIB and geosmin on a research vessel during a survey cruise.[135]

Geosmin and 2-MIB, the two most commonly occurring taste and odor compounds in fresh and reservoir waters, are relatively resistant to degradation due to their peculiar physico-chemical properties.[105] These compounds are also stable to chemical treatments.[136] A variety of processes and techniques have been implied to remove geosmin and 2-MIB from water. These include addition of powdered or granulated activated carbon, biological sand filtration, ozonation etc.

Conventional water treatment procedures include general steps of coagulation, flocculation, sedimentation and filtration. The conventional treatment techniques may remove 2–MIB and geosmin when these TOC are contained within algal cells[137], but these processes are totally ineffective in the treatment of dissolved geosmin and 2-MIB[138] because of their uncharged organic nature, aqueous solubility, hydrophobicity and volatility.[139] Conventional procedures may involve slow or rapid sand filtration. Slow sand filtration is the oldest technique which is cost-effective whereas rapid sand filtration is a relatively modern adaptation. Rapid sand filtration involves the passage of water through the filter under gravity or applied pressure.[137]

Activated carbon is widely used for removal of pollutants from water both in powdered and granulated form. It has been shown that powdered activated carbon (PAC) is not quite efficient in removal of geosmin and 2-MIB from water mainly due to the presence of natural organic material (NOM) in water sources. Natural organic material is present in all natural water sources. NOM is an aggregation formed as a result of animal and plant material breakdown.[140] Humic substances are the main constituent of NOM. These may be divided into fulvic acid, humic acid (HA) humin. Humic substances badly impact the quality of water

by imparting colors and acting as precursors for chlorinated substances.[140] NOM is present in water in greater concentration than geosmin and 2-MIB (in mg/L concentrations) and offers hindrance in removal of these substances by competing with geosmin and 2-MIB for adsorption sites on activated carbon.[141] Therefore, NOM compromises the adsorption efficiency of activated carbon significantly.[142] Moreover, activated carbon looses its absorptiveness when saturated with moisture thus allowing the pollutants to leach out into the water.[140] Dosing quantity of PAC also poses a problem, as under-dosing results in insufficient removal of TOC from water and over-dosing results in exorbitant costs.[137] Granular activated carbon (GAC) is preferably used when odor episodes are more frequent.[137] Geosmin and 2-MIB face a competition for adsorption sites on GAC.

Introduction of the polymeric molecules in the treatment of taste and odor episodes in water is an advanced intervention. Polymer use for abatement of geosmin and 2-MIB has replaced many previously used processes which were not significantly efficient for the purpose. Variety of polymeric substances is now-a-days being used to clear up the water from TOC.

Cyclodextrins (CD) are cyclic glucose oligomers made from enzymatic degradation of starch through the action of *Bacillus macerans*.[140] They are also known as cycloamyloses, cyclomaltoses and Schardinger dextrins.[143] The three most commonly known CD substances, α, β and γ consist of cyclic 6, 7 and 8-membered glucose units respectively. There is a central cavity in cyclodextrin molecules which provides an excellent site for hydrophobic molecules such as organic compounds.[144] Cyclodextrins remove organic pollutants from water through formation of inclusion complexes. Cyclodextrin molecule (acting as 'host') encapsulates the organic molecule (the 'guest') thus forming a complex.[140] The ability of CD molecule to form inclusion complexes depends on the relative size of both 'host' and 'guest' and on the energy that pulls the organic molecule into the CD cavity.[145] These polymers should be introduced into the treatment system at later stage after ozonation, UV treatment and activated carbon treatment.[140]

Dendrimers are highly branched macro-molecules which consist of a central core, repeating units and terminal functional groups.[146] Two commonly used dendrimers are polypropylene imine (PPI) and polyamido amin (PAMAM).[147] Due to their greater surface area and high solubility, molecular uniformity and internal cavities, they are being implied for removal of organic pollutants such as geosmin and 2-MIB from water.[148] PPI dendrimers blended with polyethylimine and polyglycerol polymer with β-CD have been synthesized[149] and are found to efficiently remove organic pollutants from water. Ceramic porous filters also show greater efficiency when impregnated with dendrimers.[150]

With the advancements in technology, new approaches are being adopted to resolve environmental problems. Nanotechnology (nanocatalysts) is used in water treatment due to nano size, structure, density and reactive surface sites on these molecules.[147] ZnO immobilized thin films[151], nano-scale iron particles[152], titanium oxide + ferric oxide (TiO_2/Fe_2O_3)[153] and aluminum oxide particles[154] have been successfully used for removal of pollutants from water. Enzyme nanoparticles such as manganese peroxidase (MnP), lignin peroxidase (LiP) and lactase.[155]

Chlorination and Ozonation are two important techniques implied for removal of TOC from water. Addition of chlorine is one of the most widely used water treatment process for taste and odor control. Chlorine is an excellent disinfectant for water. Most disinfected drinking water contains 0.2-1mg/L of chlorine.[156] It is highly effective in removal of microbial activity from drinking water but unfortunately does not completely remove geosmin and 2-MIB from water. Rather it can increase the release of geosmin and 2-MIB by lysing the cells of algae and *Actinomycetes*.[157]

In a study, using Flavor Profile Analysis (FPA), it was showed that chlorine possesses masking effect on both geosmin and 2-MIB[158] but latter on further studies using Friedman analysis of the paired comparison test proved that chlorine only had a little masking effect on organic TOC (geosmin & 2-MIB) and hence chlorination alone is not an effective strategy for reducing or masking other odors in drinking water. Moreover, chlorine itself has an offending odor, therefore, adding excess of chlorine will not enhance the organoleptic quality of drinking water.[157]

Chlorine not only has an inadequate masking effect on geosmin and 2-MIB in water sources but it also interferes with the analysis of these compounds. Solid-phase micro extraction is a protocol technique for the analysis of TOC in water. Presence of chlorine in the sample water may strongly affect the adsorption of organic compounds (TOC) onto the SPME-adsorbents. Therefore, the adsorbents in the SPME fiber coating such as Carboxen (CAR) and divinylbenzene (DVB) may be influenced by the presence of residual chlorine.[159] The residual chlorine significantly reduces the observed concentrations of geosmin and 2-MIB. Depending on the analyte and chlorine concentrations, a reduction of 10-50% for 2-MIB and 10-74% for geosmin was found. The impact increases with decrease in the concentration of these TOC in water or with increase in the concentration of free chlorine. Dechlorination of water samples through addition of sodium thiosulfate while applying SPME for the extraction of geosmin and 2-MIB from chlorinated drinking water is mandatory.[159] Another problem associated with chlorination is the formation of disinfection byproducts (DBP) such as

trichloromethane. These byproducts may pose a health threat such as asthmatic episodes and increased concentration of high-density lipoproteins and cholesterol.

Ozonation is also widely used to remove organic pollutants (TOC) from water. It is the triatomic form of oxygen and is a highly effective water treatment for the removal of 2-MIB, geosmin and other organic compounds.[150] Ozone decomposition is a series of chain reactions initiated by hydroxide ion and the concentrations of both ozone and hydroxide are crucial for the removal of TOC from source water.[161] However, requirement of high doses[150], rapid depletion of ozone due to the presence of natural organic material (NOM)[161] and formation of toxic by-products such as bromates as a result of reaction between ozone and organic pollutants are major disadvantages ozonation.[150]

Peroxene, an advanced oxidation technique involving the use of ozone in combination with hydrogen peroxide, is more effective than ozonation alone for the removal of geosmin, 2-MIB and other volatile organic compounds. High cost and disinfection byproducts (DBP) such as cytotoxic bromates are major disadvantages of this technique.[150]

Both geosmin and 2-MIB are susceptible to biological degradation. A variety of microorganisms is responsible for biodegradation of these TOC. The most important of these bacteria are *Pseudomonas aeruginosa, Bacillus subtilis, Flavobacterium multivorum, Bacillus cereus, Arthrobacter atrocyaneus, Bacillus fusiformis, Enterobacter spp, Rhodococcus moris, Alphaproteo bacterium* and *Sphingomonas*.[162]

The susceptibility of both geosmin and 2-MIB is attributed to their structures which are similar to biodegradable alicyclic alcohols and ketones, though no definite metabolic pathways for these substances have yet been elucidated.[162] Biodegradation pathway of geosmin is thought to be similar to that of cyclohexanol. The assumption is based on the structure similarities between both the compounds. In this pathway, cyclohexanol is converted into an alicyclic ketone which is further converted into its corresponding lactone by monooygenases and subsequently broken down by ring cleavage to an acidic alcohol that can be oxidized to a diacid.[137]

On the other hand 2-MIB has a similar structure to that of the bicyclic monoterpene camphor, so its biodegradation may follow the same pathway involving formation of unstable lactone followed by sequential ring cleavage.[137] Biodegradation of both 2-MIB and geosmin is determined to be a pseudo-first order reaction, as the degradation rates are affected by the initial concentration of the biofilm but not the initial concentration of TOC.[162]

The biodegradation phenomenon can be applied to set water free of TOC. The task can be accomplished by using biological filtration. It is a technique which utilizes a biologically

active film (biofilm) containing diverse colonies of heterotrophic bacteria which oxidize the organic matter, utilizing it as an energy supply source.[137] The product water from biological filtration contains great number of bacteria. These bacteria may be later inactivated by disinfection processes but the microorganisms still may contribute to higher particle counts and turbidity of product water.[163] However, biological filtration produces water with particle count consistently below 0.1 NTU (Nephelometric Turbidity Unit), so the quality of product water is not compromised.[164]

The most crucial role is played by the selection of filter media. Most commonly used filter medium is granular activated carbon (GAC) due to its adsorptive and attached biological properties. Sand is also used being an inert medium with attached properties. A combination of GAC/sand is the best filter medium because it is shown to establish biofilms more rapidly and to be more resistant to oxidant residuals.[165] Moreover, GAC due to its adsorptive characteristics can entrap the additional compounds which may not be biologically removed. Presence of active degrading organisms in the influent to filter, optimum filter operating conditions for bacterial attachment and sufficient contact between target compounds and degrading organisms are the factors that govern the effective biodegradation of TOCs.[137]

The biological degradation also possesses some disadvantages. Firstly, both 2-MIB and geosmin are mostly seasonal compounds, so the bacteria are expected to survive even in the absence of these two compounds. Secondly, this technique is also temperature sensitive as microbial growth is significantly decreased in winter season due to decrease of temperature below optimum bacterial growth temperature.[150]

OFF-FLAVORS IN FISH-CULTURE

Fish are the most important part of aquaculture. Different species of fish are exposed to metabolic substances and TOCs through the ingestion of producers like blue-green algae; consumption of contaminated food items such as detritus or absorption of dissolved compounds from water such as leakage due to cell lysis.[166] Ingestion of surface scum by Catfish while feeding on floating food pellets[167] and ingestion of cyanobacteria by planktivorous fish, such as carp and tilapia[166] are examples of such exposure. Dissolved TOC are transported across membranous structures. Exposures to TOC also occurs through aquatic food web, involving the accumulation of there metabolites in consumer tissues thus facilitating transfer through the food chain.[166] Trophic transfer also plays a role.[168] Aquatic plants and macroalgae are also important vectors for these TOC.[169]

Fish is major source of animal protein. Cultured fish constitute over 13% of the animal protein intake for the human population according to WHO 2007. Occurrence of easily detectable and objectionable odor is a great hindrance to the growth of fish culture industry. Production of TOC is a common phenomenon in aquaculture. Algae produce over 200 of such TOC.[170] Filamentous cyanobacterial species produce more than 25% off-flavor compounds[166], the most important of which are geosmin and 2-MIB. Recently, two important off-flavor compounds have also been identified from cyanobacteria.[171] These are hydroxyl ketones formed via fermentation pathways and nor-carotenoids such as β-cyclocitral resulting from the degradation of carotenoids.

TOC affect many commercially important fish species including Nile tilapia (*Oreochromis niloticus*), Shrimp, Atlantic Salmon (*Salmo salar*), Rainbow trout (*Salmo gairdneri*), Catfish species, Cultured large mouth bass (*Micropterus salmoides*) and White Sturgeon (*Acipenser transmontanus*).[166]

There are over 1,250 species of Catfish. Eight out of these are commonly known. These include Channel Catfish, Blue Catfish, White Catfish, Flathead Catfish, Speckled Bullhead Catfish, Brown Bullhead Catfish and Yellow Bullhead Catfish.[172] Channel Catfish (*Ictalurus punctatus*) is the leading cultured catfish. They are a healthy source of protein with exceptional flavor and efficiently convert feed to flesh.[172]

Development of off-flavor is the most serious problem faced by catfish farmers. Off-flavor phenomenon has a high occurring rate and significantly damaging effects economically. Most important and frequent off-flavor compounds are geosmin and 2-MIB. Catfish off-flavors occur as seasonal episodes with peak production during the warmer months.[173] 'Earthy' malaroma is caused by geosmin while 2-MIB is responsible for generation of 'musty' aroma. Other objectionable odors may include 'woody', 'grassy', 'rotten' and 'diesel' etc.[174] after absorption through the gills, the TOC are transferred into the flesh and are deposited there.[172] Due to hydrophobic nature and high lipid solubility, these molecules are easily deposited in the flesh.[175] Different environmental factors and weather conditions affect the production of these TOC and their accumulation in the fish flesh. Both of the TOC, geosmin and 2-MIB are positively correlated with air and soil temperatures and negatively correlated with wind velocity. Moreover, a correlation is also found between 2-MIB concentration and maximum humidity. Rainfall has a definite effect on geosmin, being a risk factor for its production. On the other hand, rainfall is negatively correlated with 2-MIB concentration.[175]

Development of off-flavors in catfish is difficult to determine through mechanical instrumentation as it is time consuming and therefore cannot be used as routine quality

control protocol.[172] Currently, determination of off-flavors in catfish is carried out by sensory analysis. Professional panelists judge the quality of fish on the basis of presence or absence of off-flavors. Pre-harvest sensory evaluation is done at various points before marketing the fish.[174] As a newly developed technique, solid phase micro extraction, coupled with gas chromatography/mass spectroscopy (SPME-GC/MS) can be successfully used to readily detect geosmin and 2-MIB in water at concentrations less than 10ng/Kg.[176]

Once off-flavors develop, it is usually very difficult to eliminate TOC completely. Decreasing the stocking densities may control the production of geosmin and 2-MIB by minimizing the amount of nutrients and thus discouraging the blue-green algae and *actinomycetes*. As it is hypothesized that both of these TOC are produced seasonally in warmer months of the year, it may be a good approach to harvest catfish before the summer season when warmer temperatures encourage the TOC production in water.[172] Different methods and techniques are at present used to abolish the off-flavors and off-aromas from cultured fish. The most commonly used is purging out the TOC from fish by holding the fish in smaller ponds and continuously flushing it with well-water until the TOC purge out, but it is a time-taking process and also results in fish with low body weight due to minimum addition of food into the ponds.[177] Another technique for elimination of geosmin and 2-MIB from catfish is the acidic dehydration. Both, geosmin and 2-MIB, being tertiary alcohols are easily susceptible to dehydration. 2-MIB dehydrates to 2-methylenebornane and 1-methyl camphene whereas geosmin dehydrate to form argosmin.[178] In this process, citric acid is used for dehydration. Vacuum tumbling allows the citric acid solution to be massaged into the catfish tissue rapidly without deterioration of color and form. The 2% citric acid solution in combination with vacuum tumbling significantly reduces 2-MIB concentration in catfish fillets as a result of either indirect physico-chemical effect or chemical degradation.[178] A modification of this technique involves the extraction of myofibrillar proteins from collagen and fat using an acid solubilization technique that separates these fleshy components based on their solubility differences. This technique results in increase in the tissue surface area thus facilitating the acid to come in contact with the tissues and providing efficient dehydration of TOC as compared to tumbling technique.[179]

Copper sulfate pentahydrate (CSP)[180] and other copper-based algaecides[173] have also been used to control the TOC producing algae in catfish ponds. Applications of CSP have been effective in controlling snails in channel catfish production ponds. Snails serve as intermediate hosts for the digenetic trematoda *Bolbophorus damnificus* which badly affects catfish and fingerling catfish.[181] Using Diuron ($C_9H_{10}Cl_2N_2O$) is an alternative approach to

the use of copper containing herbicides. Diuron is a broad spectrum herbicide with algaecidal properties at low concentrations. It also possesses a wide margin of safety and long-term persistence in the pond environment because the chemical is decomposed by natural microbiological activity.[173] However, when diuron is applied to eutrophic aquaculture ponds, it will suppress plant growth, resulting in reduced rate of oxygen production and ammonia assimilation, both of which may lead to water quality deterioration that can stress fish.[173]

Ozonation also helps to get rid of catfish off-flavors. Ozone is a strong oxidant which is also implied in the removal of TOC during water treatment. Ozone is believed to directly attack the bacterial cell wall. The free radicals of ozone breakdown the double bonds in the cell wall and destroys the permeability of the structure resulting in breakdown and eventual lysis of the cell.[172]

Development of off-flavors is not only a problem with culturing of catfish but the other species of fish are also badly affected due to the occurrence of off-flavors. Flatfish are a widely used variety of fish. Important of these are rock fish (*Sebastes spp.*), petrale sole (*Eopsetta jordani*), dover sole (*Solea sole*a) and English sole (*Parophyris vetulus*). Petrale sole and dover sole are in high demand but demand for English sole is continuously decreasing due to its small size (small fillets) and presence of metallic or iodine-like off-flavor. This off-flavor is attributed to the presence of bromophenols which are also positive flavor substances at low concentrations.[182] Tilapia is second largest farmed fish in the world with most production coming from Asia and Latin America.[183] Most of the tilapia is cultured in ponds supplemented with organic or inorganic fertilizers. Tilapia pond culture is dependent on green water establishment with fertilizer application. Geosmin and 2-MIB readily contaminate tilapia species. Other fish species contaminated by geosmin and 2-MIB are Rainbow trout (*Onchorhynchus mykiss*)[185], white shrimp (*Penaeus vannamei*)[186] and Mackerels.[187]

Summarizing the whole discussion, aquaculture industry encounters frequent episodes of off-odors and off-flavors. Domestic water supply authorities continuously face the challenge to abate water off-flavors. Most of the odor and flavor compounds are produced by micro organisms, mainly cyanobacteria and *actinomycetes*. Geosmin and 2-MIB are the two most important and frequently occurring tertiary alcohols that cause malodors and off-flavors. Different techniques are presently implied to counteract their effects. These TOC can be removed using different approaches such as ozonation and biological degradation. The problem of off-flavors is not only restricted to fresh and reservoir water but cultured fish are

also at risk for off-flavor development. It is very difficult to completely abolish the TOC which are taken in by the fish.

It is mandatory to continue research on the development of newer techniques to determine TOC concentrations. Moreover, new and safe ways to eliminate the TOC must be researched which may reduce off-flavor problems without compromising the final product quality.

Chapter 4

FRUIT AND VEGETABLE OFF-FLAVORS

Fruits and vegetables make a large component of human nutrition. Like milk, water and meat, fruits and vegetables are also prone to development of off-flavors. Various factors affect the development of these off-flavors.

The flavor of the fruits and vegetables depends upon the taste and aroma.[188] Taste is the balance between sweetness and sourness or acidity and low or no astringency whereas aroma denotes the concentrations of odor-active volatile compounds.[189] Sweetness is determined by the concentrations of the predominant sugars e.g. sucrose and fructose, whereas concentrations of predominant organic acids e.g. citric acid, tartaric acid contribute to the sourness as well as some amino acids such as aspartic and glutamic acid determine the sourness of fruits and vegetables. Similarly, minerals such as calcium, phosphorus and potassium combine with the organic acid and influence the buffering capacity and the perception of acidity.[188]

Variations in flavonol polymers (proanthrocyanidins or condensed tannins) such as polymer size, extent of galloylation and formation of derivatives affect the astringency.[190] Aroma-active volatiles are largely esters, alcohols, aldehydes and ketones.[188]

FACTORS AFFECTING FRUITS AND VEGETABLE FLAVOR

a) Genetic Make-Up

Different varieties of fruits and vegetables possess different flavor profiles mainly due to the differences in genetic make-up. A study of 40 apple cultivars by gas chromatography revealed that although some common volatiles were important in all cultivars, the overall apple aroma was not the result of same compounds in every cultivar.[191] Similarly, in tomatoes, great variations exist in levels of flavor-active volatiles in different varieties and genetic variation e.g. insertion of certain geans, alter the flavor profile of tomato varieties. Transgenic fruit with certain antisense enzymatic activity have reduced levels of important flavor compounds and as a result are more prone to the development of off-flavors.[192]

b) Pre-Harvest Factors

Pre-harvest factors such as climatic conditions (temperature, sunlight and water availability)[192], cultural practices (planting density, tree pruning and fruit thinning)[188] affect the natural flavor profile of the fruits and vegetables. Similarly, chemical applications and

fertilization may also affect internal flavor quality of fruits and vegetables.[192] These factors result in high yield but often result in less than optimal flavor quality.[188]

c) Harvest Maturity

Harvest maturity is the second most important factor that affects the development of off-flavor in fruits and vegetables, as the concentrations of the compounds which impart particular flavor to fruits and vegetables increases with ripening and maturation.[1] Harvesting at improper maturity stage results in decreasing flavor quality and increases physical damage as well.[191] Non-fruit vegetables are best tasting when harvested immature, while fruit vegetables (e.g. tomatoes) and fruits are best tasting when harvested fully ripe.[188]

Flavor quality of climacteric fruit (which continue to ripe after harvesting, e.g. apple and banana) develops better if harvested after the start of ripening. On the other hand, flavor quality of non-climacteric fruit (which do not ripen after harvesting, e.g. citrus and strawberries) generally declines after harvest. On the other hand, fruit of both climacteric and non-climacteric type will be of inferior quality if harvested immature, even if held under optimal post-harvest conditions.[191] Apples harvested at premature stage and artificially exposed to optimal post-harvest conditions never come up with the good eating quality.[193]

d) Post-Harvest Factors

It is not enough to harvest fruit with good flavor but this flavor must be maintained or enhanced during storage and marketing.[188] A variety of procedures and techniques are utilized post-harvest to achieve the goals of shelf-life extension, post-harvest decay control and pest elimination.[191] Mainly used techniques involve temperature treatments (cold and hot), irradiation, chemical application and different storage atmospheres, e.g. controlled atmosphere (CA) and modified atmosphere (MA).[191]

Storage and marketing conditions result in a change in volatile contents of fruit thus impacting the flavor.[194] Optimal post-harvest handling conditions e.g. time, temperature, relative humidity and atmospheric composition etc. are important to identify.[188] Flavor loss is the direct result of losses in sugars, acids and aromatic volatiles. Moreover, due to fermentative metabolism and resulting accumulation of off-flavor compounds, unpleasant flavors are produced in fruits and vegetables. Similarly, interactions of fruits and vegetables with air, water or packaging materials may alter the natural flavor profile.[195] Processing methods especially thermal treatments sufficiently alter textural and flavor quality.[188] tomatoes stored at low temperatures show reduced level of important aroma volatiles as compared to the tomatoes stored above 20 °C.[196]

Controlled atmosphere (CA) storage reduces the emission of volatile substances as compared to air-stored fruits and vegetables. Significantly prolonged CA storage may result in valuable flavor and aroma loss.[191] Similarly, use of certain packaging materials and edible coatings such as waxes and shellac films create a modified atmosphere (MA). Due to application of gas resistant coating, the internal CO_2 build-up increases with reduced oxygen supply and this leads to accumulation of anaerobic metabolic products including ethanol and acetaldehyde which badly affects the natural flavor profile of fruits and vegetables.[191] Application of different chemical procedure such as ethylene treatment (to synchronize ripening on banana and tomato and for degreening of citrus) and pressure infiltration of apple also result in flavor degradation.[191]

OFF-FLAVORS IN CITRUS FRUITS

Citrus fruits, especially oranges, are one of the most popular fruits used worldwide. Different varieties of oranges such as Navel, Tangerines and Mandarins are used throughout the world. The typical sour-sweet taste of citrus fruits is determined by the relationship between the soluble solid content (SSC) or total soluble solids (TSS) and the titratable acidity (TA).[197] This relative ratio is termed as Fruit Maturation Index.[198] Sugars make up approximately 80-85% of TSS and therefore TSS can be easily measured using a refractometer, whereas TA is determined usually by titrating juice samples with sodium hydroxide (NaOH).[199] In unripe citrus fruits, organic acids predominate, whereas sugar are present in main proportion in ripe fruits.[197] Mandarin flavor is affected by different fruit components. Non-volatile components such as lipids, proteins, flavonoids, polysaccharides and pectin affect the mandarin flavor by forming covalent and hydrophobic interactions with volatile compounds either by absorbing them or affecting their partition coefficient and volatilization.[200] Non-volatile carotenoids such as β-cryptoxanthin and cis-violaxanthin are the precursors of potent aroma-active volatiles.[201] Approximately 42 volatile constituents are present in mandarins, important of which are myrcene (musty, wet-soil flavor), limonene, linalool (floral, citrus flavor) and gamma-terpinene (woody flavor).[198]

Temperature treatments affect these aroma-active volatile constituents. Curing of citrus fruits involves holding the fruit at high temperatures of 30-38 °C and 94-98% relative humidity for a period of three days.[202] Curing is done post-harvest to allow wound healing, to reduce decay development and to enhance chilling tolerance.[198] Ethylene degreening is used to promote external color development of green or poor-colored citrus fruit i.e. to accelerate degradation of the green chlorophyll pigments and accumulation of orange/yellow carotenoid

pigments.[203] Ethylene degreening may affect the sensory quality of the fruit by enhancing the accumulation of off-flavors.[198] Similarly, waxing of citrus fruits (to impart shine and reduce water loss and shrinkage). Application of waxes restricts the gaseous exchange through the peel surface thus modifying the internal atmosphere (more CO_2 and lesser O_2) and promoting anaerobic metabolism. This anaerobic metabolism leads to the accumulation of ethanol and acetaldehyde as off-flavor compounds.[198] Polyethylene based coatings are much more permeable to gases than shellac and wood-based coatings and hence are more suitable for the purpose.[204]

OFF-FLAVORS IN APPLE

Apple (*Malus sylvestris/domestica*) is one of the most important economical crops cultivated throughout the world. Various apple cultivars are grown in different parts of the globe. Most important apple cultivars include 'Red Delicious', 'Golden Delicious', 'Gala', 'Fuji', 'Granny Smith' and 'McIntosh'.[205]

Fruit aroma is a complex mixture of a large number of volatile compounds that contribute to the overall sensory quality of fruit specific to species and cultivars.[206] Most volatile compounds produced by apple fruit are esters, alcohols, aldehydes, acids, ketones and terpenes, of which esters play the most important role in aroma development.[207] Ethylene is continuously produced during ripening process and plays a significant role in flavor development.[205] Like citrus fruits, hypoxia deteriorates the intrinsic flavor of apple fruit. Hypoxic conditions result in accumulation of off-flavor producing substances in fruit matrix leading to inferior flavor quality.[206] Post-harvest temperature treatments affect concentrations of volatile substances in apple and the patterns of concentration change during storage show themselves in a cultivar specific manner. Exposure of apples to lower temperatures for more than 3 months decrease the concentrations of flavor-active volatile compounds by 30-60%.[206]

OFF-FLAVORS IN TOMATO

Tomato (*Lycopersicum esculetum*) is a very popular and important fruit but is mostly used as vegetable and salad. Tomato is rich in vitamins A and C and carotenoids with a specially appealing flavor. It was originated in tropical America, probably in Mexico or Peru. It is a member of family *Solanaceae* and is botanically a berry fruit.[208]

Composition of tomato varies with species, stage of maturity, climatic conditions and pre-and post-harvesting handling. Tomato is a mixture of many non-volatile and volatile compounds. Of non-volatile compounds, sugars form major (about 50%) portion. These include primarily

reducing sugars, glucose and fructose with minute quantities of saccharose, raffinose, arabinose, xylose, galactose and myoinositol. Other important polysaccharides include cellulose, pectin, arabinogalactans, xylans and arabinoxylans. Organic acids form second major portion (about 15%) of non-volatile contents. Important are citric and malic acids. Glutamic acid, gamma-aminobutyric acid, glutamine and aspartic acid are the important free amino acids in tomato. Important minerals present in tomato are potassium and phosphorus which affect the taste of tomatoes due to their buffering quality.[208] The most characteristic phytonutrient in tomato is lycopene, a carotenoid with high capacity to detoxify reactive oxygen species (ROS). Additionally, lycopene is responsible for the reddening of tomatoes, due to the differentiation of chloroplasts into chromoplasts.[209] About 400 volatile compounds have been identified in tomatoes using modern isolation and extraction techniques such as solid phase micro extraction (SPME), Gas chromatography/mass spectroscopy (GC-MS), Gas chromatography/Isotope ratio mass spectroscopy (GC-IR) and Nuclear Magnetic Resonance (NMR). Thirty (30) of these compounds are very important with regard to flavor activity. Important aroma-active compounds of tomato are hexanal, cis-3-hexenal, trans-2-hexenal, cis-3-hexenol, 2-isobutylthiazole, 6-methyl-5-hepten-2-one, β-ionone, geranylacetone, 1-penten-3-one, 3-methylbutanal, 3-methylbutanol, phenylethanol, 2-pentenal, acetone, ethanol and methanol. Combination of these volatile compounds in appropriate concentrations produces the aroma of fresh ripe tomatoes.[208]

As tomato fruit flavor is a delicate balance between the concentrations of sugars and acids, therefore, for better tomato fruit flavor, a high sugar concentration is necessary together with a relatively high acid content. A low sugar concentration with a high acid level causes tartness in tomatoes while high sugar and low acid contents produce a sweet-mild flavor. On the other hand, low concentrations of both sugars and acids result in an insipid flavor.[209]

Post-harvest physiological, chemical, biochemical and microbiological qualities of tomato partly depend upon pre-harvest factors such as genetic, climatic, biotic, edaphic, chemical and hormonal factors.[210] Environmental factors such as high solar radiation, temperature and vapour-pressure deficiency negatively affect the tomato flavor. These adverse environmental conditions can generate reactive oxygen species in cherry tomatoes[211] leading to oxidative stress.[212] This oxidative stress is one of the main physiological processes that can alter or reduce yield, nutritional and organoleptic quality and antioxidant activity of different crops.[209] Similarly, exposure to high temperatures inhibits lycopene synthesis and degrades β-carotene due to the presence of ROS.[213] High temperatures induce overheating of tomatoes and block lycopene accumulation, forming discolored zones known as ‘sunscald’.

Yellow/orangish rings or spots appear around the abscission zone of the fruits causing significant losses to both the grower and consumer.[214] Many vegetables are usually treated with chlorinated water after washing to reduce microbial load but this process may cause off-flavors.[210] An alternative to this technique, vegetables can be treated with ionized saline water (anolyte).[215] Packaging, storage temperatures and pre-packaging disinfecting negatively affect physiological weight loss[216], total soluble solids (TSS) and titratable acidity (TA).[210] Similarly, refrigeration induced changes in level of 3-methylbutanal, linalool, guialol, hexanol, trans-2-hexenal and trans-3-hexenol may alter the flavor perception of tomatoes.[217]

Summarizing the whole story, it can be said that fruits and vegetables are very important part of human diet and nutrition. They possess valuable components which are beneficial for human health and development. Natural flavor profile of the fruits and vegetables is affected by a variety of factors. These include genetic make-up, pre-harvest factors (climatic conditions, cultural practices) and post-harvest treatments and handling.

It is the need of time to address the issues of off-flavor development in fruits and vegetables in interest of both the producer and the consumer. Future research must be oriented towards the development of new techniques to identify and quantify the off-flavors quickly and accurately. Similarly, efforts should be targeted to the selection of such breeds which are more yielding and are less prone to off-flavors in order to fulfill the ever increasing demand of the consumer for tasteful and nutritionally rich fruits and vegetables.

CONCLUSION

Off-flavors are very common in foods and other natural resources. They are responsible for a great damage to the food products and water resources. The frequency of off-flavor development and their severity is dependent on a variety of factors. On one hand, natural factors such as climatic conditions, temperature und natural composition of the foods affect the flavor of these commodities negatively and on the other hand, factors related to processing of food products and drinking water has an equally significant impact on the flavor profile.

Milk and milk products (including cheese and whey proteins) are particularly sensitive to the deteriorative effects of light and temperature variation. Similarly, natural fat components as well as additional fats added as a reinforcement to improve the nutritional quality may produce off-flavors by induction of vicious oxidative cycle ultimately making the milk and milk products unsuitable for human use. Oxidation also negatively affects the flavor and aroma of meat rendering it rancid and distasteful. Flavor of milk and meat is also affected by the packaging materials and storage conditions.

Natural water resources are very suitable for the growth and propagation of certain organisms such as algae. These algal species produce complex organic compounds such as geosmin and 2-MIB which alter the taste of water. Most importantly, these off-flavor compounds are not easy to remove with traditional water purification techniques. These compounds also directly affect the sensory quality of fish cultured in these water resources. Like milk products, water and fish, fruits & vegetables are also prone to the development of off-flavors. A variety of pre- and post-harvest factors such as climatic conditions, cultural practices and post-harvest processing affect the overall flavor and aroma profile of fruits and vegetables.

A variety of techniques are being used to identify and quantify the development of off-flavors in foods and water. Most important of these are chromatography (with various modifications), microextraction, olfactometery and mass spectroscopy. Occasionally, these techniques are also used in combination.

Different solutions are being searched to cope with the off-flavors such as the use of nanotechnology to make the water free from geosmin and 2-MIB. New research should be directed towards the development of such analytical methods which are sensitive enough to detect the off-flavor producing substances in very minute quantities. Moreover, by utilizing latest advancements in the field of genetic engineering, certain foods can be designed which provide maximum nutrition with minimum off-flavor development.

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Literature and Poetry

Mysticism

Discussions on general issues

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