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BT611T

Food Biotechnology (Theory)

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Lecture 1

INTRODUCTION TO FOOD, DEFINITIONS AND BASIC CONCEPTS**Module 1: Introduction to Food, Definition and basic concepts**

Food Science The study of food science involves understanding the nature, composition and behavior of food materials under varying conditions of growth, harvest, transportation, processing, storage, handling and use.

Food Technology: is the application of food science & engineering principles to the selection, preservation, processing, packaging, distribution, and use of safe, nutritious, and wholesome food.

Food Biotechnology:

Application of technology to modify genes of animals, plants, and microorganisms to create new species which have desired production, marketing, or nutrition related properties. Called genetically engineered (GE) or genetically modified (GM) foods.

Food Security: Food security is achieved when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life. (800 million people suffer from hunger).

Nutrition:

“The science of food, the nutrients and substances therein, their action, interaction, and balance in relation to health and dis-ease, and the process by which the organisms ingest, digest, absorb, transport, utilize, and excrete food substances” (The Council on Food and Nutrition of the American Medical Association).

Food is required for four main reasons:

- as a source of energy;
- as a source of raw materials for growth and development;
- to supply minute chemicals that serve to regulate vital metabolic processes; 
- to supply food components (phytochemicals) that retard the development and progression of degenerative diseases.

Module 2: Food composition

Carbohydrates

Carbohydrate is the collective name for polyhydroxyaldehydes and polyhydroxyketones, and these compounds form a major class of biomolecules that perform several functions in vivo, including the storage and transport of energy. Indeed, carbohydrates are the major source of energy in our diet. The name carbohydrate derives from their general empirical formula, which is $(CH_2O)_n$; however, the carbohydrate group contains several derivatives and closely related compounds that do not fit this general empirical formula but are still considered to be carbohydrates. There are three distinct classes of carbohydrates: *monosaccharides* (1 structural unit), *oligosaccharides* (2–10 structural units) and *polysaccharides* (more than 10 structural units).

Module 3: Water Contents in foods

It exists in three forms in foods

1. **Free Water:** This is form of water that retains its physical properties and hence acts as dispersing agent for colloids and solvents of choice.
2. **Chemically adsorbed water:** This water is held tightly and it is component of cell walls or protoplast and is held tightly with proteins.
3. **Chemically bound water:** This is form of water that is bound chemically, i.e. lactose monohydrate or also in form of some salts $Na_2SO_4 \cdot 10H_2O$ etc.

Module 4: Carbohydrates Contents of food

Monosaccharides

The monosaccharides are also termed *simple sugars*, are given the suffix *-ose* and classified as *aldoses* or *ketoses* depending on whether they contain an aldehyde or ketone group. The most common monosaccharides are either *pentoses* (containing a chain of five carbon atoms) or *hexoses* (containing a chain of six carbon atoms). Each carbon atom carries a hydroxyl group, with the exception of the atom that forms the carbonyl group, which is also known as the *reducing group*.

Monosaccharides are assigned optical configurations with respect to comparison of their highest numbered asymmetric carbon atom to the configuration of D-glyceraldehyde or L-glyceraldehyde. By convention, the carbon atoms in the monosaccharide molecule are numbered such that the reducing group carries the lowest possible number; therefore, in aldoses the reducing group carbon is always numbered 1 and in ketoses the numbering is started from the end of the carbon chain closest to the reducing group. Most naturally occurring monosaccharides belong to the D-series, i.e. their highest numbered carbon has a similar optical configuration to D-glyceraldehyde.

Oligosaccharides

Oligosaccharides contain 2–10 sugar units and are water soluble. The most significant types of oligosaccharide occurring in foods are disaccharides, which are formed by the condensation (i.e. water is eliminated) of two monosaccharide units to form a *glycosidic bond*. A glycosidic bond is that between the hemiacetal group of a saccharide and the hydroxyl group of another compound, which may or may not be itself a saccharide. Disaccharides can be homogeneous or heterogeneous and fall into two types:

Non-reducing sugars in which the monosaccharide units are joined by a glycosidic bond formed between their reducing groups (e.g. sucrose and trehalose). This inhibits further bonding to other saccharide units

Reducing sugars in which the glycosidic bond links the reducing group of one monosaccharide unit to the non-reducing alcoholic hydroxyl of the second monosaccharide unit (e.g. lactose and maltose). A reducing sugar is any sugar that, in basic solution, forms an aldehyde or ketone allowing it to act as a reducing agent, and therefore includes all monosaccharides.

Of the disaccharides, sucrose, trehalose and lactose are found free in nature, whereas others are found as *glycosides* (in which a sugar group is bonded through its anomeric carbon to another group, e.g. a phenolic group, via an O-glycosidic bond) or as building blocks for polysaccharides (such as maltose in starch), which can be released by hydrolysis. Probably the three most significant disaccharides in food are sucrose, lactose and maltose.

Sucrose is the substance known commonly in households as sugar and is found in many plant fruits and saps. It is isolated commercially from sugar cane or the roots of sugar beet. Sucrose is composed of a-D-glucose residue linked to a b-D-fructose residue and is a non-

reducing sugar. Its systematic name is α -D-glucopyranosyl-(1 $\leftrightarrow$ 2)- β -D-fructofuranoside (having the suffix *-oside*, because it is a non-reducing sugar). It is the sweetest tasting of the disaccharides and is an important source of energy.

Lactose is found in mammalian milk and its systematic name is β -D-galactopyranosyl-(1 $\leftrightarrow$ 4)- β -D-glucopyranose. To aid the digestion of lactose, the intestinal villi of infant mammals secrete an enzyme called lactase (β -D-galactosidase), which cleaves the molecule into its two subunits β -D-glucose and β -D-galactose. In most mammals the production of lactase gradually reduces with maturity into adulthood, leading to the inability to digest lactose and so-called *lactose intolerance*. However, in cultures where cattle, goats and sheep are milked for food there has evolved a gene for lifelong lactase production.

Maltose is formed by the enzymatic hydrolysis of starch and is an important component of the barley malt used to brew beer. It is a homogeneous disaccharide consisting of two units of glucose joined with an α (1 $\rightarrow$ 4) linkage, and is systematically named 4-O- α -D-glucopyranosyl-D-glucose. Maltose is a reducing sugar and the addition of further glucose unit yields a series of oligosaccharides known as maltodextrins or simply dextrins.

Polysaccharides

Polysaccharides are built of repeat units of monosaccharides and are systematically named with the suffix *-an*. The generic name for polysaccharides is *glycan* and these can be *homoglycans* consisting of the one type of monosaccharide or *heteroglycans* consisting of two or more types of monosaccharide.

Polysaccharides have three main functions in both animals and plants: as sources of energy, as structural components of cells, and as water-binders. Plant and animal cells store energy in the form of *glucans*, which are polymers of glucose such as starch (in plants) and glycogen (in animals). The most abundant structural polysaccharide is cellulose, which is also a glucan and is found in plants. Water-binding substances in plants include agar, pectin and alginate.

Polysaccharides occur as several structural types: *linear* (e.g. amylose, cellulose), *branched* (e.g. amylopectin, glycogen), *interrupted* (e.g. pectin), *block* (e.g. alginate) or *alternate repeat* (e.g. agar, carrageenan). According to the geometry of the glycosidic linkages, polysaccharide chains can form various conformations, such as *disordered random coil*, *extended ribbons*, *buckled ribbons* or *helices*. One of the most important properties of a

great number of polysaccharides in foods is that they are able to form aqueous gels and thereby contribute to food structure and textural properties (e.g. mouth-feel).

Starch

Starch occurs in the form of semi-crystalline granules ranging in size from 2 to 100 μm , and consists of two types of glucan: *amylose* and *amylopectin*. Amylose is a linear polymer of $\alpha(1\rightarrow4)$ linked α -D-glucopyranose and constitutes 20–25% of most starches. Amylopectin is a randomly branched polymer of α -D-glucopyranose consisting of linear chains with $\alpha(1\rightarrow4)$ linkages with 4–5% of glucose units also being involved in $\alpha(1\rightarrow6)$ linked branches. On average the length of linear chains in amylopectin is about 20–25 units.

Module 5: Proteins contents of Food

Proteins are polymers of amino acids linked together by peptide bonds. They can also be referred to as *polypeptides*. Proteins are key constituents of food, contributing towards organoleptic properties (particularly texture) and nutritive value. Proteins participate in tissue building and are therefore abundant in muscle and plant tissues.

Amino acid structure

The general structure of an *amino acid* consists of an amino group (NH_2), a carboxyl group (COOH), a hydrogen atom and a distinctive R group all bonded to a single carbon atom, called the α -carbon. The R group is called the *side chain* and determines the identity of the amino acid.

Amino acids in solution at neutral pH are predominantly *zwitterions*. The ionization state varies with pH: at acidic pH, the carboxyl group is un-ionized and the amino group is ionized; at alkaline pH, the carboxyl group is ionized and the amino group is un-ionized.

There are 20 different amino acids that are commonly found in proteins. The R group is different in each case and can be classified according to several criteria into four main types: *basic*, *non-polar* (hydrophobic), *polar* (uncharged) and *acidic*. The four different functional groups of amino acids are arranged in a tetrahedral array around the α -carbon atom; therefore, all amino acids are optically active apart from glycine. Of the possible L- or D-isomers, proteins contain only L-isomers of amino acids.

Peptide bonds

The *peptide bond* is the covalent bond between amino acids that links them to form peptides and polypeptides. A peptide bond is formed between the α -carboxyl group and the α -amino group of two amino acids by a condensation (or dehydration synthesis) reaction with the loss of water. Peptides are compounds formed by linking small numbers of amino acids (up to 50). A polypeptide is a chain of 50–100 amino acid residues. A protein is a polypeptide chain of 100+ amino acid residues and has a positively charged nitrogen-containing amino group at one end (*N-terminus*) and a negatively charged carboxyl group at its other end (*C-terminus*).

Molecular structure of proteins

Primary structure

The *primary structure* of a protein is simply the sequence of amino acids listed from the N-terminal amino acid. There are more than a billion possible sequences of the 20 amino acids and every protein will have a unique primary structure which determines how the protein folds into a three-dimensional conformation.

Secondary structure

The secondary structure of a protein describes the arrangement of the protein backbone (polypeptide chain) due to *hydrogen bonding* between its amino acid residues. Hydrogen bonding can occur between an amide hydrogen atom and a lone pair of electrons on a carbonyl oxygen atom.

Module 6: Fats

Lipids are a group of molecules that contribute to the structure of living cells and are also used in the body for the purpose of energy storage. Dietary lipids have important roles for provision of energy and as carriers of fat-soluble vitamins. Generally speaking, all lipids are soluble in non-polar organic solvents and have low solubility in water. Dietary lipids are commonly referred to as *oils* and *fats*. Edible oils are liquid at room temperature, whereas fats are solid or semi-solid at room temperature. The lipids found in oils and fats are chemically very diverse, but are predominantly long-chain *fatty acid esters*. Other lipid types encountered in foods are also either fatty acids or derivatives of fatty acids, and include *triglycerides*, *phospholipids*, *sterols* and *tocopherols*.

Lipids can be broadly classified into three main groups:

Simple lipids yield two classes of product when hydrolyzed, e.g. glycerides (acylglycerols) which are hydrolyzed to give glycerol and a fatty acid.

Complex lipids yield three or more classes of product when hydrolyzed, e.g. phospholipids, which are hydrolyzed to give alcohols, fatty acids and phosphoric acid.

Derived lipids are non-hydrolyzable and do not fit into either of the above classes, e.g. sterol, tocopherol and vitamin A.

Lipid structure and nomenclature

Fatty acids

A *fatty acid* is a carboxylic acid having a long unbranched aliphatic tail or chain, and can be described chemically as an *aliphatic monocarboxylic acid*. The aliphatic chain can be either *saturated* (no double bonds between carbons) or *unsaturated* (one or more double bonds between carbons). Saturated

Fatty acids have the general chemical structure $\text{CH}_3(\text{CH}_2)_{n-2}\text{CO}_2\text{H}$, and commonly contain an even number of carbon atoms from $n = 4$ to $n = 20$.

Triglycerides:

While fatty acids are the most common structural component of lipids, oils and fats are largely composed of mixtures of *triglycerides*. Triglycerides are also known as *triacylglycerols* and are esters of three fatty acids with glycerol.

The three fatty acid residues may or may not be the same, i.e. they can be simple or mixed triglycerides. Natural oils and fats will therefore contain a characteristic profile of different fatty acids dependent on their source. For example, fish oils are rich in long-chain polyunsaturated fatty acids (PUFAs) with up to six double bonds, while many vegetable oils are rich in oleic and linoleic acids. Vegetable oils are important in the diet since the body is unable to synthesize linoleic acid, which is an important precursor of *prostaglandins*, a class of hormones that are involved in inflammation and smooth muscle contraction.

Module 7: Minerals

When testing for minerals in the food laboratory, the first step is to remove all water and then all organic matter from the product being tested. After these steps, most of the food (often more than 97%) is gone. What remains is the inorganic material or ash. The human body consists of about 3% minerals, most of which is in the skeletal system. Although the minerals exist in minute amounts, their functions are necessary for normal growth and reproduction. Minerals are generally categorized as "major" and "trace" based on the amounts in the body. Here, a very brief overview of the importance of each mineral is discussed. Further reading on the subject is recommended for students of nutrition. All minerals are important but only two are included on the nutritional labels; thus these are mentioned first.

Iron

Iron is considered a trace mineral because it is needed in such small amounts. Of all the required nutrients, iron may be consumed in insufficient amounts in the industrialized world more commonly than any other. One reason may be the poor absorption of iron sources such as the iron phosphates and iron phytates found in plants. Animal sources and those from soluble salts used in food fortification are generally absorbed more efficiently. Iron is an essential part of both the blood pigment hemoglobin and the muscle pigment myoglobin. It is included in some enzymes. Deficiencies of iron cause anemia. The amount of iron needed is related to growth rate and blood loss. Women who are of menstruating age should take special caution to ensure sufficient intake. Iron toxicities are rare but care should be taken when storing iron supplement tablets. As few as 6 to 12 tablets could prove fatal if taken by a small child. Good sources of iron are liver, meats, eggs, oatmeal, and wheat flour. As mentioned previously, both vitamin E and vitamin C have been shown to aid in iron absorption.

Calcium

Calcium is one of the minerals that humans require in the greatest amounts (phosphorus is the other). It is required for bone and tooth structure and is necessary for the function of nerves and muscles. Calcium is also needed in the blood clotting mechanism. Deficiencies can lead to osteoporosis, especially in older women. It is suggested that increased calcium intake, especially when young, may help prevent this in later years.

Vitamin D is essential for calcium absorption and lactose has been shown to aid in this also.

This makes vitamin D fortified milk an excellent, if not the best source of calcium. Calcium

deficiencies may be widespread in our society, but unlike most other deficiencies, symptoms are not apparent until later in life. Care should be taken to ensure sufficient calcium intake.

Sodium

In humans, sodium is required in the extracellular fluids to maintain osmotic equilibrium and body-fluid volume. As salt is a major food ingredient there is little evidence of deficiencies except in diseases involving prolonged vomiting or diarrhea. The consumption of salt and other sodium sources should be constrained. Sodium has been shown to aggravate hypertension (high blood pressure) and it is recommended that daily consumption levels be kept between 1100 and 3300 mg. The average in the typical American diet is closer to 6000 mg.

Chlorine

Chlorine is also involved in extracellular fluids as the major negatively charged ion. It has a role in controlling blood pH and is necessary in the production of hydrochloric acid of gastric juice. It is a component of table salt and is never really lacking in the diet. If great losses of body fluids occur, as in vigorous exercise, the amount of chlorine may have to be replenished.

Potassium

Potassium is present in the body cells as the chief intracellular cation and is associated with the function of muscles and nerves and with the metabolism of carbohydrates. It is important in maintaining the fluid volume inside cells, and the acid-base balance. Good sources of potassium are meats, eggs, oranges, bananas, and fresh milk. Cell membranes are quite permeable to potassium, but as it leaks out, a highly active membrane pump returns it to the cell in exchange for sodium. This is critical because if as little as 6% of the potassium contained in the cells were to escape into the blood, the heart would stop.

Phosphorus

About 85% of the phosphorus in the body is found combined with calcium. It is also part of the body's major buffers (phosphoric acid and its salts) and it is part of both DNA and RNA, the genetic code material present in every cell. Some lipids contain phosphorus in their structure and these phospholipids help transport other lipids in the blood. Phosphorus also

plays a key role in energy transfer as it is part of the energy carrier of the cells, ATP. Some sources of phosphorus are meats, fish, eggs, and nuts.

Magnesium

Magnesium is a minor component of bones and is present in soft tissue cells, where it is involved in protein synthesis. Deficiencies of magnesium are unusual and good food sources are most vegetables, cereals and cereal flours, beans, and nuts.

Sulfur

Sulfur is present in virtually all proteins and plays a most important role in forming the cysteine bridges that are essential for protein conformation. Deficiencies of sulfur are associated with protein deficiency and if foods containing sufficient protein are eaten then sulfur amounts will be adequate.

Trace Minerals

Iodine

Iodine is part of the hormone thyroxine which regulates metabolic levels. Deficiency of iodine leads to low-level metabolism, lethargy, and goiter, which results in an enlarged thyroid gland. There is rarely a deficiency of iodine when saltwater fish are available and eaten. Today, iodized salt prevents the deficiency but there is some concern about overconsumption of the mineral. Fast food operations use iodized salt liberally, and iodates are used by some bakeries as dough conditioners. Symptoms of toxic levels are similar to those resulting from a deficiency, that of an enlarged thyroid gland. With the identification of the problem, food industries are reducing use of iodine-containing products but care must still be taken.

Fluorine

The fluorine ion is present in body tissue in trace amounts and helps to prevent tooth decay. Drinking water is the chief source of fluorine and fish is also a good source. If fluorine is taken in excessively high doses for long periods of time, it is toxic. Too much fluorine in the form of supplements can cause fluorosis, a mottling of the tooth enamel.

Copper

Copper aids in the utilization of iron in hemoglobin synthesis and is required by some body enzyme systems. It can be toxic in high concentrations and these can be reached if copper utensils are used for storage or distribution of acid foods (copper tubing in machines that dispense lemonade or other acid beverages). Deficiencies are virtually unknown and some food sources are fruits, beans, peas, eggs, liver, fish, and oysters.

Cobalt

Cobalt is a component of vitamin B12, the only component present in the body known to contain this element. Sufficient amounts of cobalt are present in most foods and some may be absorbed into food from cooking utensils. Even though it is part of vitamin B12, it does not replace the need for the vitamin.

Zinc

Zinc is a cofactor in more than 70 enzymes that perform specific tasks in the eyes, liver, kidneys, muscles, skin, bones, and male reproductive organs. These include carbohydrate and protein metabolism and nucleic acid synthesis. Deficiencies are rare but dwarfism, gonadal atrophy, and possible damage to the immune system have been attributed to a deficiency of the mineral. Good sources are protein-rich foods such as shellfish, meat, and liver.

Manganese

Manganese is needed for normal bone structure, fat production, reproduction, and functioning of the central nervous system. Deficiencies result in bone disorders, sexual sterility, and abnormal lipid metabolism. Meats are a source of the mineral but adequate supplies are found in most human diets.

Selenium

Selenium acts as an antioxidant in conjunction with vitamin E. Its major food sources are meat, seafood, and grains. A deficiency results in anemia, muscle pain, and sometimes heart failure.

Module 8: Vitamins

There are a number of vitamins required in small amounts by the human body for sustaining life and good health. Some are fat-soluble; others are water-soluble

Fat-Soluble Vitamins

Vitamin A

Vitamin A is found only in animals, although a number of plants contain carotene, from which vitamin A can be produced in the body once the plants containing carotene are eaten. Vitamin A may be formed in the body from the yellow pigments (containing carotene) of many fruits and vegetables, especially carrots. Vitamin A, which is required for vision and resistance to infection, is also found in fats and oils, especially in the liver oils of many saltwater fish. Epithelial cells (those cells present in the lining of body cavities and in the skin and glands) also require vitamin A. Deficiency in vitamin A may cause impairment in bone formation, impairment of night vision, malfunction of epithelial tissues, and defects in the enamel of the teeth.

Vitamin A previously was expressed in international units (IU). Because of the different biological activities of vitamin A sources such as beta-carotene, other carotenes, and preformed vitamin A (retinol), vitamin A is now expressed in terms of retinol equivalents (RE). To convert, 1 IU = 0.3 RE. The definition of RE is given by the Food and Nutrition Board as:

1 RE = microgram *all-transretinol* = 6 micrograms of beta-carotene = 12 micrograms of other provitamin A carotenoids.

The Reference Daily Intakes (RDIs), on which the % daily values on the nutritional labels are based, for vitamin A are 5000 IU based on the 1968 RDAs. This corresponds to 1500 REs. Vitamin A is routinely added to milk. The 1989 revised RDA for vitamin A is 1000 REs for adult males and 800 for females.

Vitamin D

Vitamin D (calciferol or activated ergosterol) is necessary for normal tooth and bone formation. Deficiencies in vitamin D result in rickets (deformities of bone, such as bow-legs and curvature of the spine) and tooth defects. Fish oils, and especially fish liver oils, are excellent sources of vitamin D. The human body is also able to make vitamin D by converting sterols such as cholesterol with ultraviolet light from the sun or an artificial source. The RDA for vitamin D was in the past expressed in IUs and 400 was the RDS. It is

now expressed in micrograms (Ilg) as cholecalciferol and $10 \text{ Ilg} = 400 \text{ IU}$. Vitamin D, as vitamin A is also routinely added to milk.

Vitamin E

Vitamin E has four different forms (the tocopherols) that have the same name except with the prefixes alpha-, beta-, gamma-, and delta-. The four compounds are closely related, with slight differences in structure, but alpha-tocopherol is the most common and serves as an antioxidant that serves to prevent the oxidation of body components, such as unsaturated fatty acids. Vitamin E has also been shown to enhance the absorption of iron. Diets excessive in polyunsaturated fats can lead to the formation of peroxidized fatty acids that could reach dangerous levels. Vitamin E may prevent this. Good sources of Vitamin E are corn oil, cottonseed oil, and peanut oil.

While the symptoms of vitamin E deficiency in humans are not clearly established, experiments with various animals have shown that vitamin E deficiency has an adverse effect on reproduction, with apparent irreversible injury to the germinal epithelium. Other symptoms noted in animal studies include injury to the central nervous system, growth retardation, muscular dystrophy, and interference with normal heart action.

The RDA for vitamin E is now given in milligrams of alpha-tocopherol equivalents (a-TE). The old measurement was in IUs and 30 was the RDA ($10 \text{ a-TE} = 30 \text{ IU}$).

Vitamin K

Vitamin K is essential for the synthesis of prothrombin, a compound involved in the clotting of blood. Cabbage, spinach, cauliflower, and liver are especially good sources and it is also synthesized by bacteria in the human intestine. Antibiotic therapy that destroys intestinal organisms can produce deficiencies of vitamin K.

The significant symptom of vitamin K deficiency in humans and in animals is the loss of the ability of the blood to clot which is, of course, a dangerous condition that can result in death whenever bleeding occurs. It is believed that humans ordinarily

receive adequate amounts of vitamin K in the diet. The RDA ranges from 5 ~g in infants to 80 ~g in males over 25 years of age.

Water-Soluble Vitamins

Vitamin B1 (Thiamin)

Thiamin is involved in all bodily oxidations that lead to the formation of carbon dioxide. It is also necessary for nerve function, appetite, and normal digestion as well as growth, fertility, and lactation. The symptoms of thiamin deficiency are retardation of growth, palpitation and enlargement of the heart, hypertension, and beri-beri. The various effects of a disturbance of the nerve centers such as forgetfulness or difficulty in thinking are other manifestations of vitamin B1 deficiency.

Important to the food scientist is the sensitivity of vitamin B1 to sulfur dioxide (SO₂) and to sulfite salts. SO₂ destroys vitamin B1 and should not be used as a preservative in foods that are a major source of the vitamin. FDA and meat inspection laws prohibit such use. Thiamin is stable to heat in acid foods but is less so in neutral and alkaline foods. This should be taken into account when processing foods.

Good sources of the vitamin are fresh pork, wheat germ, and cereals containing bran and some fair sources are beef and lamb.

Vitamin B2 (Riboflavin)

Vitamin B2 makes up a part of enzyme systems involved in the oxidation and reduction of different materials in the body. Deficiency of riboflavin generally results in growth retardation and may result in vision impairment, scaling of the skin, and lesions on mucous tissues. Neuritis is another effect of deficiency. Milk, liver, and eggs are good sources while meats and leafy green vegetables are moderate sources. Riboflavin is sensitive to light, so packaging such as cardboard and other light-resistant containers for milk are used.

Niacin (Nicotinic Acid)

Niacin is part of enzyme systems regulating reduction reactions in the body. It is also a compound that dilates blood vessels. It is part of the coenzyme nicotinamide adenine dinucleotide (NAD) which is involved in glucose breakdown. Deficiency of niacin causes pellagra (a disease that causes diarrhea, dermatitis, nervous disorders, and sometimes death). Good sources are yeast, meat, fish, poultry, peanuts, legumes, and whole grain.

Vitamin B6 (Pyridoxine)

Other substances closely related to this vitamin are pyridoxal and pyridoxamine. Vitamin B₆ is part of the enzyme system that removes CO₂ from the acid group (COOH) of certain amino acids and transfers amine groups (NH₂) from one compound to another in the body. Although the vitamin is needed for processes such as those mentioned, it seems a deficiency does not cause a well-recognized disease. Bananas, barley, grain cereals with bran, muscle meat, liver, and green vegetables are all among sources of this vitamin. Vitamin B₆ has been used by women taking steroid contraceptive pills, and it has been used in treatment of such ailments as premenstrual syndrome (PMS) but this is not recommended without valid blood or urine tests to show low B₆ levels.

Biotin

Biotin is reported to be a coenzyme in the synthesis of aspartic acid, which plays a part in the aminase system and in other processes involving the fixation of carbon dioxide. Deficiency of this compound is unusual, although it can be tied up by a substance in raw egg whites called avidin and the deficiency has been demonstrated in feeding studies with mice. Because of the production of biotin by microbial flora of the intestines, the dietary requirement for this compound is unknown. Liver is an excellent source of biotin and peanuts, peas, beans, and whole cooked eggs are good sources.

Pantothenic Acid

Pantothenic acid, a vitamin required for normal growth, nerve development, and normal skin, is a component of coenzyme A and others involved in metabolism. Pantothenic acid is widespread in foods, so obvious symptoms of a deficiency are rare in humans. Organs of animals (liver, heart, kidney), eggs, whole wheat products, and peanuts are excellent sources.

Choline

Choline is generally listed with the B vitamins and is consumed in adequate amounts in a normal diet as well as being produced by intestinal microbial flora (other growth factors such as biotin, para-aminobenzoic acid, and inositol are also produced in this manner). It is a component of cell membranes and brain tissue and it functions as part of the substance acetylcholine, which is one of the brain's principal neurotransmitters.

Vitamin B₁₂ (Cyanocobalamin)

This vitamin is a very complex chemical compound and is the largest vitamin molecule. It is required for the normal development of red blood cells, and a deficiency in vitamin B12 causes acute pernicious anemia and a variety of other disorders. Cobalt is part of Vitamin B12's structure, thus giving rise to the requirement for this mineral in nutrition. Some vitamin B12 is synthesized by bacteria in the intestine but intake of 2 g per day for adults is recommended.

Folacin

Folacin is required for the formation of blood cells by the bone marrow and is involved in the formation of the blood pigment hemoglobin. It is also required for the synthesis of some amino acids. Deficiency results in some types of anemia including pernicious anemia. The RDA is increased from 180 µg to 400 µg for pregnant women, as it is believed the vitamin may act in prevention of some birth defects. It is part of the coenzyme system tetrahydrofolate (THF), which is required for the synthesis of new genetic material and therefore new cells. Liver, leafy vegetables, legumes, cereal grains, and nuts are all sources of the vitamin.

Module 9: Fiber

Dietary fiber includes the nondigestible carbohydrates. These may be either water soluble or water-insoluble. The water-insoluble group includes products such as wheat products and wheat bran etc. The water-soluble fibers, includes such as some cereal brans and pectin.

Module 10: Proximate Analysis of Food

Food Matrix is complex and consists of carbohydrates, proteins, fats, minerals, vitamins, water and fibre.

Broader classes of these closely related groups are termed as proximate constituents. Proximate analysis puts together all these closely related groups together.

Module 11: Estimation of moisture

Water contents are required to be determined for processor as well as consumer for variety of reasons. Although measuring water contents appears trivial but in fact it might be very challenging to accurately measure the amount of water in many cases due to unique

challenges it offers for accurate removal of moisture from foods. There are different methods for moisture analysis depending upon type of food and requirement of analysis.

Drying methods

Distillation methods

Drying Methods:

Air Oven Method:

Dry the flat bottom dish with tightly fitted lid at 100°C with heat first and weigh and tare the balance.

Add 2-10 g of food, loose the cover and heat for 2-3 hrs at 100°C.

Remove and cool in dessicator, repeat the process till 2 mg different persists.

Calculate moisture loss

$$\% \text{ Moisture (wt/wt)} = \frac{\text{wt H}_2\text{O in sample}}{\text{wt of wet sample}} \times 100$$

$$\% \text{ Moisture (wt/wt)} = \frac{\text{wt of wet sample} - \text{wt of dry sample}}{\text{wt of wet sample}} \times 100$$

Vacuum Oven:

This method is suitable for spices, meats or high sugar samples. It involves generally a

Pressure of 100 mg or less for 4-6 hrs heating and 60-70°C.

The procedure requires removal of dish and cooling in dessicator.

Again heat for 1 hour and cool in dessicator till 2-3mg difference exists.

Calculate moisture loss.

Infra red drying:

Infra red lamps with a filament temperature of 2000-2500 K can penetrate heat to food and shorten drying time to 20-25 minutes only.

Distillation Method:

Food sample is heated with a solvent with higher boiling point and lower specific gravity than water. Solvent include xylene, toluene and carbon tetrachloride etc.

Water is received in a graduate cylinder and is measure directly from the graduated cylinder.

Method:

1. Add 10-40 g sample into flask and 75-100 ml solvent to cover food product.
2. Distill @ 1-2 drop per minute unless all clear water is achieved.
3. Wash the condenser and continue condensing for 5 minutes.
4. Cool and read the volume of water.

Module 12: Estimation of ash in food

Dry ashing refers to use of muffle furnance capable of mainting temperature of 500-600C.

Water and organic matter are voporized and organic matter are burnt with the presence of oxygen to carbon dioxides and oxides of nitrogen. Most minerals are burnt to oxides of sulphates, phosphates, chlorides and silicates. Elements such as Hg, Se, Pb Fe may partially volatalised by this procedure, so other methods may be utilized if there is a need for further analysis of these metals.

Wet Ashing: is a procudere for oxidizing organic substances by using acids or oxidizing agents or combinations by using both. Minerals are solubilized without volatilization. Wet ashing is preferable to dry ashing as a preparation of specific mineral analysis.

Importance of Ash in Food Analysis:

Calculation of total ash may be needed in many cases for food analysis. Its is an important step for proximate analysis of food for nutritional purposes. Different foods have specific

amount of ash and we can determine rate of adulteration or contamination of these foods by measuring total ash contents.

Determination of Ash Content

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. Analytical techniques for providing information about the total mineral content are based on the fact that the minerals (the analyte) can be distinguished from all the other components (the matrix) within a food in some measurable way. The most widely used methods are based on the fact that minerals are not destroyed by heating, and that they have a low volatility compared to other food components. The three main types of analytical procedure used to determine the ash content of foods are based on this principle: *dry ashing*, *wet ashing* and *low temperature plasma dry ashing*. The method chosen for a particular analysis depends on the reason for carrying out the analysis, the type of food analyzed and the equipment available. Ashing may also be used as the first step in preparing samples for analysis of specific minerals, by atomic spectroscopy or the various traditional methods described below. Ash contents of fresh foods rarely exceed 5%, although some processed foods can have ash contents as high as 12%, *e.g.*, dried beef.

Sample Preparation

As with all food analysis procedures it is crucial to carefully select a sample whose composition represents that of the food being analyzed and to ensure that its composition does not change significantly prior to analysis. Typically, samples of 1-10g are used in the analysis of ash content. Solid foods are finely ground and then carefully mixed to facilitate the choice of a representative sample. Before carrying out an ash analysis, samples that are high in moisture are often dried to prevent spattering during ashing. High fat samples are usually defatted by solvent extraction, as this facilitates the release of the moisture and prevents spattering. Other possible problems include contamination of samples by minerals in grinders, glassware or crucibles which come into contact with the sample during the analysis. For the same reason, it is recommended to use deionized water when preparing samples.

Dry Ashing

Dry ashing procedures use a high temperature muffle furnace capable of maintaining temperatures of between 500 and 600 °C. Water and other volatile materials are vaporized and organic substances are burned in the presence of the oxygen in air to CO₂, H₂O and N₂. Most minerals are converted to oxides, sulfates, phosphates, chlorides or silicates. Although most minerals have fairly low volatility at these high temperatures, some are volatile and may be partially lost, *e.g.*, iron, lead and mercury. If an analysis is being carried out to determine the concentration of one of these substances then it is advisable to use an alternative ashing method that uses lower temperatures.

There are a number of different types of crucible available for ashing food samples, including quartz, Pyrex, porcelain, steel and platinum. Selection of an appropriate crucible depends on the sample being analyzed and the furnace temperature used. The most widely used crucibles are made from porcelain because it is relatively inexpensive to purchase, can be used up to high temperatures (< 1200°C) and are easy to clean. Porcelain crucibles are resistant to acids but can be corroded by alkaline samples, and therefore different types of crucible should be used to analyze this type of sample. In addition, porcelain crucibles are prone to cracking if they experience rapid temperature changes. A number of dry ashing methods have been officially recognized for the determination of the ash content of various foods (AOAC Official Methods of Analysis). Typically, a sample is held at 500-600 °C for 24 hours.

Advantages: Safe, few reagents are required, many samples can be analyzed simultaneously, not labor intensive, and ash can be analyzed for specific mineral content.

- *Disadvantages:* Long time required (12-24 hours), muffle furnaces are quite costly to run due to electrical costs, loss of volatile minerals at high temperatures, *e.g.*, Cu, Fe, Pb, Hg, Ni, Zn.

Recently, analytical instruments have been developed to dry ash samples based on microwave heating. These devices can be programmed to initially remove most of the moisture (using a relatively low heat) and then convert the sample to ash (using a relatively high heat). Microwave instruments greatly reduce the time required to carry out an ash analysis, with the analysis time often being less than an hour. The major disadvantage is that it is not possible to simultaneously analyze as many samples as in a muffle furnace.

Wet Ashing

Wet ashing is primarily used in the preparation of samples for subsequent analysis of specific minerals. It breaks down and removes the organic matrix surrounding the minerals so that they are left in an aqueous solution. A dried ground food sample is usually weighed into a flask containing strong acids and oxidizing agents (*e.g.*, nitric, perchloric and/or sulfuric acids) and then heated. Heating is continued until the organic matter is completely digested, leaving only the mineral oxides in solution. The temperature and time used depends on the type of acids and oxidizing agents used. Typically, a digestion takes from 10 minutes to a few hours at temperatures of about 350°C. The resulting solution can then be analyzed for specific minerals.

- **Advantages:** Little loss of volatile minerals occurs because of the lower temperatures used, more rapid than dry ashing.
- **Disadvantages** Labor intensive, requires a special fume-cupboard if perchloric acid is used because of its hazardous nature, low sample throughput.

Determination of Water Soluble and Insoluble Ash

As well as the total ash content, it is sometimes useful to determine the ratio of water soluble to water-insoluble ash as this gives a useful indication of the quality of certain foods, *e.g.*, the fruit content of preserves and jellies. Ash is diluted with distilled water then heated to nearly boiling, and the resulting solution is filtered. The amount of soluble ash is determined by drying the filtrate, and the insoluble ash is determined by rinsing, drying and ashing the filter paper.

Module 13: Estimation of carbohydrates in food

A large number of analytical techniques have been developed to measure the total concentration and type of carbohydrates present in foods (see *Food Analysis* by Nielssen or *Food Analysis* by Pomeranz and Meloan for more details). The carbohydrate content of a food can be determined by calculating the percent remaining after all the other components have been measured: $\% \text{carbohydrates} = 100 - \% \text{moisture} - \% \text{protein} - \% \text{lipid} - \% \text{mineral}$. Nevertheless, this method can lead to erroneous results due to experimental errors in any of the other methods, and so it is usually better to directly measure the carbohydrate content for accurate measurements.

Monosaccharides and Oligosaccharides

Sample Preparation

The amount of preparation needed to prepare a sample for carbohydrate analysis depends on the nature of the food being analyzed. Aqueous solutions, such as fruit juices, syrups and honey, usually require very little preparation prior to analysis. On the other hand, many foods contain carbohydrates that are physically associated or chemically bound to other components, *e.g.*, nuts, cereals, fruit, breads and vegetables. In these foods it is usually necessary to isolate the carbohydrate from the rest of the food before it can be analyzed. The precise method of carbohydrate isolation depends on the carbohydrate type, the food matrix type and the purpose of analysis, however, there are some procedures that are common to many isolation techniques. For example, foods are usually dried under vacuum (to prevent thermal degradation), ground to a fine powder (to enhance solvent extraction) and then defatted by solvent extraction.

One of the most commonly used methods of extracting low molecular weight carbohydrates from foods is to boil a defatted sample with an 80% alcohol solution. Monosaccharides and oligosaccharides are soluble in alcoholic solutions, whereas proteins, polysaccharides and dietary fiber are insoluble. The soluble components can be separated from the insoluble components by filtering the boiled solution and collecting the filtrate (the part which passes through the filter) and the retentante (the part retained by the filter). These two fractions can then be dried and weighed to determine their concentrations. In addition, to monosaccharides and oligosaccharides various other small molecules may also be present in the alcoholic extract that could interfere with the subsequent analysis *e.g.*, amino acids, organic acids, pigments, vitamins, minerals *etc.* It is usually necessary to remove these components prior to carrying out a carbohydrate analysis. This is commonly achieved by treating the solution with clarifying agents or by passing it through one or more ion-exchange resins.

- *Clarifying agents.* Water extracts of many foods contain substances that are colored or produce turbidity, and thus interfere with spectroscopic analysis or endpoint determinations. For this reason solutions are usually *clarified* prior to analysis. The most commonly used clarifying agents are heavy metal salts (such as lead acetate) which form insoluble complexes with interfering substances that can be removed by filtration or centrifugation. However, it is important that the clarifying agent does not precipitate any

of the carbohydrates from solution as this would cause an underestimation of the carbohydrate content.

- **Ion-exchange.** Many monosaccharides and oligosaccharides are polar non-charged molecules and can therefore be separated from charged molecules by passing samples through ion-exchange columns. By using a combination of a positively and a negatively charged column it is possible to remove most charged contaminants. Non-polar molecules can be removed by passing a solution through a column with a non-polar stationary phase. Thus proteins, amino acids, organic acids, minerals and hydrophobic compounds can be separated from the carbohydrates prior to analysis.

Prior to analysis, the alcohol can be removed from the solutions by evaporation under vacuum so that an aqueous solution of sugars remains.

Chromatographic and Electrophoretic methods

Chromatographic methods are the most powerful analytical techniques for the analysis of the *type* and *concentration* of monosaccharides and oligosaccharides in foods. Thin layer chromatography (TLC), Gas chromatography (GC) and High Performance Liquid chromatography (HPLC) are commonly used to separate and identify carbohydrates. Carbohydrates are separated on the basis of their differential adsorption characteristics by passing the solution to be analyzed through a column. Carbohydrates can be separated on the basis of their partition coefficients, polarities or sizes, depending on the type of column used. HPLC is currently the most important chromatographic method for analyzing  carbohydrates because it is capable of rapid, specific, sensitive and precise measurements. In addition, GC requires that the samples be volatile, which usually requires that they be derivitized, whereas in HPLC samples can often be analyzed directly. HPLC and GC are commonly used in conjunction with NMR or mass spectrometry so that the chemical structure of the molecules that make up the peaks can also be identified.

Carbohydrates can also be separated by electrophoresis after they have been derivitized to make them electrically charged, *e.g.*, by reaction with borates. A solution of the derivitized carbohydrates is applied to a gel and then a voltage is applied across it. The carbohydrates are then separated on the basis of their size: the smaller the size of a carbohydrate molecule, the faster it moves in an electrical field.

Chemical methods

A number of chemical methods used to determine monosaccharides and oligosaccharides are based on the fact that many of these substances are *reducing agents* that can react with other components to yield precipitates or colored complexes which can be quantified. The concentration of carbohydrate can be determined gravimetrically, spectrophotometrically or by titration. Non-reducing carbohydrates can be determined using the same methods if they are first hydrolyzed to make them reducing. It is possible to determine the concentration of both non-reducing and reducing sugars by carrying out an analysis for reducing sugars before and after hydrolyzation. Many different chemical methods are available for quantifying carbohydrates. Most of these can be divided into three categories: titration, gravimetric and colorimetric. An example of each of these different types is given below.

Titration Methods

The Lane-Eynon method is an example of a titration method of determining the concentration of reducing sugars in a sample. A burette is used to add the carbohydrate solution being analyzed to a flask containing a known amount of boiling copper sulfate solution and a methylene blue indicator. The reducing sugars in the carbohydrate solution react with the copper sulfate present in the flask. Once all the copper sulfate in solution has reacted, any further addition of reducing sugars causes the indicator to change from blue to white. The volume of sugar solution required to reach the end point is recorded. The reaction is not stoichiometric, which means that it is necessary to prepare a calibration curve by carrying out the experiment with a series of standard solutions of known carbohydrate concentration.

The disadvantages of this method are (i) the results depend on the precise reaction times, temperatures and reagent concentrations used and so these parameters must be carefully controlled; (ii) it cannot distinguish between different types of reducing sugar, and (iii) it cannot directly determine the concentration of non-reducing sugars, (iv) it is susceptible to interference from other types of molecules that act as reducing agents..

Gravimetric Methods

The Munson and Walker method is an example of a gravimetric method of determining the concentration of reducing sugars in a sample. Carbohydrates are oxidized in the presence of heat and an excess of copper sulfate and alkaline tartrate under carefully controlled conditions which leads to the formation of a copper oxide precipitate:

Module 14: Estimation of protein in food

Proteins are polymers of amino acids. Twenty different types of amino acids occur naturally in proteins. Proteins differ from each other according to the type, number and sequence of amino acids that make up the polypeptide backbone. As a result they have different molecular structures, nutritional attributes and physiochemical properties. Proteins are important constituents of foods for a number of different reasons. They are a major source of energy, as well as containing essential amino-acids, such as lysine, tryptophan, methionine, leucine, isoleucine and valine, which are essential to human health, but which the body cannot synthesize. Proteins are also the major structural components of many natural foods, often determining their overall texture, *e.g.*, tenderness of meat or fish products. Isolated proteins are often used in foods as ingredients because of their unique functional properties, *i.e.*, their ability to provide desirable appearance, texture or stability. Typically, proteins are used as gelling agents, emulsifiers, foaming agents and thickeners. Many food proteins are enzymes which are capable of enhancing the rate of certain biochemical reactions. These reactions can have either a favorable or detrimental effect on the overall properties of foods. Food analysts are interested in knowing the total concentration, type, molecular structure and functional properties of the proteins in foods.

Determination of Overall Protein Concentration

Kjeldahl method

The Kjeldahl method was developed in 1883 by a brewer called Johann Kjeldahl. A food is digested with a strong acid so that it releases nitrogen which can be determined by a suitable titration technique. The amount of protein present is then calculated from the nitrogen concentration of the food. The same basic approach is still used today, although a number of improvements have been made to speed up the process and to obtain more accurate measurements. It is usually considered to be *the* standard method of determining protein concentration. Because the Kjeldahl method does not measure the protein content directly a *conversion factor (F)* is needed to convert the measured nitrogen concentration to a protein concentration. A conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein) is used for many applications, however, this is only an average value, and each protein has a different conversion factor depending on its amino-acid composition. The Kjeldahl method can conveniently be divided into three steps: digestion, neutralization and titration.

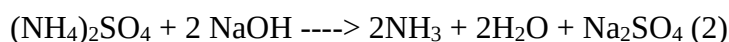
Principles

Digestion

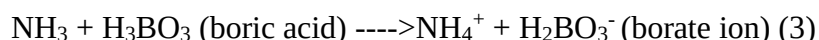
The food sample to be analyzed is weighed into a *digestion flask* and then digested by heating it in the presence of sulfuric acid (an oxidizing agent which digests the food), anhydrous sodium sulfate (to speed up the reaction by raising the boiling point) and a catalyst, such as copper, selenium, titanium, or mercury (to speed up the reaction). Digestion converts any nitrogen in the food (other than that which is in the form of nitrates or nitrites) into ammonia, and other organic matter to CO₂ and H₂O. Ammonia gas is not liberated in an acid solution because the ammonia is in the form of the ammonium ion (NH₄⁺) which binds to the sulfate ion (SO₄²⁻) and thus remains in solution.

Neutralization

After the digestion has been completed the digestion flask is connected to a *receiving flask* by a tube. The solution in the digestion flask is then made alkaline by addition of sodium hydroxide, which converts the ammonium sulfate into ammonia gas:

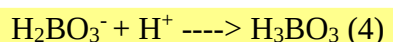


The ammonia gas that is formed is liberated from the solution and moves out of the digestion flask and into the receiving flask - which contains an excess of boric acid. The low pH of the solution in the receiving flask converts the ammonia gas into the ammonium ion, and simultaneously converts the boric acid to the borate ion:



Titration

The nitrogen content is then estimated by titration of the ammonium borate formed with standard sulfuric or hydrochloric acid, using a suitable indicator to determine the end-point of the reaction.



The concentration of hydrogen ions (in moles) required to reach the end-point is equivalent to the concentration of nitrogen that was in the original food (Equation 3). The following

equation can be used to determine the nitrogen concentration of a sample that weighs m grams using a xM HCl acid solution for the titration:

$$\% N = \frac{x \text{ moles}}{1000 \text{ cm}^3} \times \frac{(v_s - v_b) \text{ cm}^3}{m \text{ g}} \times \frac{14 \text{ g}}{\text{moles}} \times 100 \quad (5)$$

Where v_s and v_b are the titration volumes of the sample and blank, and 14g is the molecular weight of nitrogen N. A blank sample is usually ran at the same time as the material being analyzed to take into account any residual nitrogen which may be in the reagents used to carry out the analysis. Once the nitrogen content has been determined it is converted to a protein content using the appropriate conversion factor: %Protein = F %N.

Advantages and Disadvantages

Advantages. The Kjeldahl method is widely used internationally and is still the standard method for comparison against all other methods. Its universality, high precision and good reproducibility have made it the major method for the estimation of protein in foods.

Disadvantages. It does not give a measure of the true protein, since all nitrogen in foods is not in the form of protein. Different proteins need different correction factors because they have different amino acid sequences. The use of concentrated sulfuric acid at high temperatures poses a considerable hazard, as does the use of some of the possible catalysts. The technique is time consuming to carry-out.

Enhanced Dumas method

Recently, an automated instrumental technique has been developed which is capable of rapidly measuring the protein concentration of food samples. This technique is based on a method first described by a scientist called Dumas over a century and a half ago. It is beginning to compete with the Kjeldahl method as the standard method of analysis for proteins for some foodstuffs due to its rapidness.

General Principles

A sample of known mass is combusted in a high temperature (about 900 °C) chamber in the presence of oxygen. This leads to the release of CO₂, H₂O and N₂. The CO₂ and H₂O are removed by passing the gasses over special columns that absorb them. The nitrogen content is then measured by passing the remaining gasses through a column that

has a thermal conductivity detector at the end. The column helps separate the nitrogen from any residual CO₂ and H₂O that may have remained in the gas stream. The instrument is calibrated by analyzing a material that is pure and has a known nitrogen concentration, such as EDTA (= 9.59%N). Thus the signal from the thermal conductivity detector can be converted into a nitrogen content. As with the Kjeldahl method it is necessary to convert the concentration of nitrogen in a sample to the protein content, using suitable conversion factors which depend on the precise amino acid sequence of the protein.

Advantages and Disadvantages

Advantages: It is much faster than the Kjeldahl method (under 4 minutes per measurement, compared to 1-2 hours for Kjeldahl). It doesn't need toxic chemicals or catalysts. Many samples can be measured automatically. It is easy to use.

Disadvantages: High initial cost. It does not give a measure of the true protein, since all nitrogen in foods is not in the form of protein. Different proteins need different correction factors because they have different amino acid sequences. The small sample size makes it difficult to obtain a representative sample.

Module 15: Estimation of Fat in food

Lipids are one of the major constituents of foods, and are important in our diet for a number of reasons. They are a major source of energy and provide essential lipid nutrients. 

Nevertheless, over-consumption of certain lipid components can be detrimental to our health, *e.g.* cholesterol and saturated fats. In many foods the lipid component plays a major role in determining the overall physical characteristics, such as flavor, texture, mouthfeel and appearance. For this reason, it is difficult to develop low-fat alternatives of many foods, because once the fat is removed some of the most important physical characteristics are lost. Finally, many fats are prone to lipid oxidation, which leads to the formation of off-flavors and potentially harmful products.

Properties of Lipids in Foods

Lipids are usually defined as those components that are soluble in organic solvents (such as ether, hexane or chloroform), but are insoluble in water. This group of substances includes triacylglycerols, diacylglycerols, monoacylglycerols, free fatty acids, phospholipids, sterols, carotenoids and vitamins A and D. The lipid fraction of a fatty food therefore

contains a complex mixture of different types of molecule. Even so, triacylglycerols are the major component of most foods, typically making up more than 95 to 99% of the total lipids present. Triacylglycerols are esters of three fatty acids and a glycerol molecule. The fatty acids normally found in foods vary in chain length, degree of unsaturation and position on the glycerol molecule. Consequently, the triacylglycerol fraction itself consists of a complex mixture of different types of molecules. Each type of fat has a different profile of lipids present which determines the precise nature of its nutritional and physiochemical properties. The terms fat, oil and lipid are often used interchangeably by food scientists. Although sometimes the term *fat* is used to describe those lipids that are solid at the specified temperature, whereas the term *oil* is used to describe those lipids that are liquid at the specified temperature.

Sample Selection and Preservation

As with any analytical procedure, the validity of the results depends on proper sampling and preservation of the sample prior to analysis. Ideally, the composition of the sample analyzed should represent as closely as possible that of the food from which it was taken. The sample preparation required in lipid analysis depends on the type of food being analyzed (*e.g.* meat, milk, margarine, cookie, dairy cream), the nature of the lipid component (*e.g.* volatility, susceptibility to oxidation, physical state) and the type of analytical procedure used (*e.g.* solvent extraction, non-solvent extraction or instrumental). In order, to decide the most appropriate sample preparation procedure it is necessary to have knowledge of the physical structure and location of the principal lipids present in the food. Since each food is different it is necessary to use different procedures for each one. Official methods have been developed for specific types of foods that stipulate the precise sample preparation procedure that should be followed. In general, sample preparation should be carried out using an environment that minimizes any changes in the properties of the lipid fraction. If lipid oxidation is a problem it is important to preserve the sample by using a nitrogen atmosphere, cold temperature, low light or adding antioxidants. If the solid fat content or crystal structure is important it may be necessary to carefully control the temperature and handling of the sample.

Determination of Total Lipid Concentration

Introduction

It is important to be able to accurately determine the total fat content of foods for a number of reasons:

- Economic (not to give away expensive ingredients)
- Legal (to conform to standards of identity and nutritional labeling laws)
- Health (development of low fat foods)
- Quality (food properties depend on the total lipid content)
- Processing (processing conditions depend on the total lipid content)

The principle physicochemical characteristics of lipids (the "analyte") used to distinguish them from the other components in foods (the "matrix") are their solubility in organic solvents, immiscibility with water, physical characteristics (*e.g.*, relatively low density) and spectroscopic properties. The analytical techniques based on these principles can be conveniently categorized into three different types: (i) solvent extraction; (ii) non-solvent extraction and (iii) instrumental methods.

Solvent Extraction

The fact that lipids are soluble in organic solvents, but insoluble in water, provides the food analyst with a convenient method of separating the lipid components in foods from water soluble components, such as proteins, carbohydrates and minerals. In fact, solvent extraction techniques are one of the most commonly used methods of isolating lipids from foods and of determining the total lipid content of foods.

Sample Preparation

The preparation of a sample for solvent extraction usually involves a number of steps:

Drying sample. It is often necessary to dry samples prior to solvent extraction, because many organic solvents cannot easily penetrate into foods containing water, and therefore extraction would be inefficient.

Particle size reduction. Dried samples are usually finely ground prior to solvent extraction to produce a more homogeneous sample and to increase the surface area of lipid exposed to the solvent. Grinding is often carried out at low temperatures to reduce the tendency for lipid oxidation to occur.

Acid hydrolysis. Some foods contain lipids that are complexed with proteins (lipoproteins) or polysaccharides (glycolipids). To determine the concentration of these components it is necessary to break the bonds which hold the lipid and non-lipid components together prior to solvent extraction. Acid hydrolysis is commonly used to release bound lipids into easily extractable forms, *e.g.* a sample is digested by heating it for 1 hour in the presence of 3N HCl acid.

Solvent Selection. The *ideal* solvent for lipid extraction would completely extract all the lipid components from a food, while leaving all the other components behind. In practice, the efficiency of solvent extraction depends on the *polarity* of the lipids present compared to the polarity of the solvent. Polar lipids (such as glycolipids or phospholipids) are more soluble in polar solvents (such as alcohols), than in non-polar solvents (such as hexane). On the other hand, non-polar lipids (such as triacylglycerols) are more soluble in non-polar solvents than in polar ones. The fact that different lipids have different polarities means that it is impossible to select a single organic solvent to extract them all. Thus the total lipid content determined by solvent extraction depends on the nature of the organic solvent used to carry out the extraction: the total lipid content determined using one solvent may be different from that determined using another solvent. In addition to the above considerations, a solvent should also be inexpensive, have a relatively low boiling point (so that it can easily be removed by evaporation), be non-toxic and be nonflammable (for safety reasons). It is difficult to find a single solvent which meets all of these requirements. Ethyl ether and petroleum ether are the most commonly used solvents, but pentane and hexane are also used for some foods.

Batch Solvent Extraction

These methods are based on mixing the sample and the solvent in a suitable container, *e.g.*, a separatory funnel. The container is shaken vigorously and the organic solvent and aqueous phase are allowed to separate (either by gravity or centrifugation). The aqueous phase is then decanted off, and the concentration of lipid in the solvent is determined by evaporating the solvent and measuring the mass of lipid remaining: $\% \text{Lipid} = 100 (M_{\text{lipid}}/M_{\text{sample}})$. This procedure may have to be repeated a number of times to improve the efficiency of the extraction process. In this case the aqueous phase would undergo further extractions using fresh solvent, then all the solvent fractions would be collected together and the lipid determined by weighing after evaporation of solvent. The efficiency of the extraction of a

particular type of lipid by a particular type of solvent can be quantified by an equilibrium partition coefficient, $K = c_{\text{solvent}}/c_{\text{aqueous}}$, where c_{solvent} and c_{aqueous} are the concentration of lipid in the solvent and aqueous phase, respectively. The higher the partition coefficient the more efficient the extraction process.

Semi-Continuous Solvent Extraction

Semi-continuous solvent extraction methods are commonly used to increase the efficiency of lipid extraction from foods. The Soxhlet method is the most commonly used example of a semi-continuous method. In the Soxhlet method a sample is dried, ground into small particles and placed in a porous thimble. The thimble is placed in an extraction chamber, which is suspended above a flask containing the solvent and below a condenser. The flask is heated and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. Eventually, the solvent builds up in the extraction chamber and completely surrounds the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. As the solvent passes through the sample it extracts the lipids and carries them into the flask. The lipids then remain in the flask because of their low volatility. At the end of the extraction process, which typically lasts a few hours, the flask containing the solvent and lipid is removed, the solvent is evaporated and the mass of lipid remaining is measured (M_{lipid}). The percentage of lipid in the initial sample (M_{sample}) can then be calculated: $\% \text{Lipid} = 100 (M_{\text{lipid}}/M_{\text{sample}})$. A number of instrument manufacturers have designed modified versions of the Soxhlet method that can be used to determine the total lipid content more easily and rapidly (e.g. Soxtec).

Continuous Solvent Extraction

The Goldfish method is similar to the Soxhlet method except that the extraction chamber is designed so that the solvent just trickles through the sample rather than building up around it. This reduces the amount of time required to carry out the extraction, but it has the disadvantage that *channeling* of the solvent can occur, i.e., the solvent may preferentially take certain routes through the sample and therefore the extraction is inefficient. This is not a problem in the Soxhlet method because the sample is always surrounded by solvent.

Accelerated Solvent Extraction

The efficiency of solvent extraction can be increased by carrying it out at a higher temperature and pressure than are normally used. The effectiveness of a solvent at extracting lipids from a food increases as its temperature increases, but the pressure must also be increased to keep the solvent in the liquid state. This reduces the amount of solvent required to carry out the analysis, which is beneficial from a cost and environmental standpoint. Special instruments are available to carry out solvent extraction at elevated temperatures and pressures.

Module 16: Estimation of crude fiber in food

Analysis of Fibers

Over the past twenty years or so nutritionists have become aware of the importance of fibre in the healthy diet. Liberal consumption of fiber helps protect against colon cancer, cardiovascular disease and constipation. Adequate intake of dietary fiber is therefore beneficial to good health. Dietary fibre is defined as plant polysaccharides that are indigestible by humans, plus lignin. The major components of dietary fiber are cellulose, hemicellulose, pectin, hydrocolloids and lignin. Some types of starch, known as *resistant starch*, are also indigestible by human beings and may be analysed as dietary fiber. The basis of many fiber analysis techniques is therefore to develop a procedure that mimics the processes that occur in the human digestive system.

Major Components of Dietary Fibre

Cell Wall Polysaccharides

Cellulose occurs in all plants as the principal structural component of the cell walls, and is usually associated with various hemicelluloses and lignin. The type and extent of these associations determines the characteristic textural properties of many edible plant materials. Cellulose is a long linear homopolysaccharide of glucose, typically having up to 10,000 glucose subunits. Cellulose molecules aggregate to form microfibrils that provide strength and rigidity in plant cell walls. Hemicelluloses are a heterogeneous group of branched heteropolysaccharides that contain a number of different sugars in their backbone and side-chains. By definition hemicelluloses are soluble in dilute alkali solutions, but insoluble in water. Pectins are another form of heteropolysaccharides found in cell walls that are rich in uronic acids, soluble in hot water and that are capable of forming gels.

Non Cell Wall Polysaccharides

This group of substances are also indigestible carbohydrates, but they are not derived from the cell walls of plants. Non-cell wall polysaccharides include hydrocolloids such as guar and locust bean gum, gum arabic, agar, alginates and caragenans which are commonly used in foods as gelling agents, stabilizers and thickeners.

Lignin

Lignin is a non-carbohydrate polymer that consists of about 40 aromatic subunits which are covalently linked. It is usually associated with cellulose and hemicelluloses in plant cell-walls.

Common Procedures in Sample Preparation and Analysis

There are a number of procedures that are commonly used in many of the methods for dietary fiber analysis:

- *Lipid removal.* The food sample to be analyzed is therefore dried, ground to a fine powder and then the lipids are removed by solvent extraction.
- *Protein removal.* Proteins are usually broken down and solubilized using enzymes, strong acid or strong alkali solutions. The resulting amino acids are then separated from insoluble fiber by filtration or from total fiber by selective precipitation of the fiber with ethanol solutions.
- *Starch removal.* Semi-crystalline starch is gelatinized by heating in the presence of water, and then the starch is broken down and solubilized by specific enzymes, strong acid or strong alkali. The glucose is then separated from insoluble fiber by filtration or separated from total fiber by selective precipitation of the fiber with ethanol solutions.
- *Selective precipitation of fibers.* Dietary fibers can be separated from other components in aqueous solutions by adding different concentrations of ethanol to cause selective precipitation. The solubility of monosaccharides, oligosaccharides and polysaccharides depends on the ethanol concentration. *Water:* monosaccharides, oligosaccharides, some polysaccharides and amino acids are soluble; other polysaccharides and fiber are insoluble. *80% ethanol solutions:* monosaccharides, oligosaccharides and amino acids are soluble; polysaccharides and fibers are insoluble. For this reason,

concentrated ethanol solutions are often used to selectively precipitate fibers from other components.

- **Fiber analysis.** The fiber content of a food can be determined either *gravimetrically* by weighing the mass of an insoluble fiber fraction isolated from a sample or *chemically* by breaking down the fiber into its constituent monosaccharides and measuring their concentration using the methods described previously.

Gravimetric Methods

Crude Fiber Method

The crude fiber method gives an estimate of indigestible fiber in foods. It is determined by sequential extraction of a defatted sample with 1.25% H₂SO₄ and 1.25% NaOH. The insoluble residue is collected by filtration, dried, weighed and ashed to correct for mineral contamination of the fiber residue. Crude fiber measures cellulose and lignin in the sample, but does not determine hemicelluloses, pectins and hydrocolloids, because they are digested by the alkali and acid and are therefore not collected. For this reason many food scientists believe that its use should be discontinued. Nevertheless, it is a fairly simple method to carry out and is the official AOAC method for a number of different foodstuffs.

Total, insoluble and soluble fiber method

The basic principle of this method is to isolate the fraction of interest by selective precipitation and then to determine its mass by weighing. A gelatinized sample of dry, defatted food is enzymatically digested with α-amylase, amyloglucosidase and protease to break down the starch and protein components. The total fiber content of the sample is determined by adding 95% ethanol to the solution to precipitate all the fiber. The solution is then filtered and the fiber is collected, dried and weighed. Alternatively, the water-soluble and water-insoluble fiber components can be determined by filtering the enzymatically digested sample. This leaves the *soluble* fiber in the filtrate solution, and the *insoluble* fiber trapped in the filter. The insoluble component is collected from the filter, dried and weighed. The soluble component is precipitated from solution by adding 95% alcohol to the filtrate, and is then collected by filtration, dried and weighed. The protein and ash content of the various fractions are determined so as to correct for any of these substances which might remain in the fiber:

Fiber = residue weight - weight of (protein + ash).

This method has been officially sanctioned by the AOAC and is widely used in the food industry to determine the fiber content of a variety of foods. Its main disadvantage is that it tends to overestimate the fiber content of foods containing high concentrations of simple sugars, *e.g.*, dried fruits, possibly because they get trapped in the precipitates formed when the ethanol is added.

Lecture 2

FATS AND OILS**Module 17: Fats and oils**

In both animal and plant foods, three groups of naturally occurring organic compounds are very important oils and fats, carbohydrates and proteins. These are essential nutrients which sustain life. Fats and oils have a simple molecular structure. Oils and fats belong to a naturally occurring substances called **lipids**. The common characteristics of lipids are:

1. They are soluble in organic solvents (ether, acetone etc.)
2. They are insoluble in water
3. Most of them are derivatives of fatty acids

Some important examples of lipids which are derivatives of fatty acids are oils, fats, phospholipids and waxes. **Steroids** which are also lipids, are an exception in that these are not derivatives of fatty acids. Cholesterol, a steroid is an important constituent of body tissues and is present in animal foods. Vitamin D and bile acids are other important steroids, which are related to cholesterol.

In every day use, the group oils and fats has a definite meaning. It includes such familiar substances as:

Vegetable oils Groundnut, sesame (gingelly), mustard, coconut, safflower, coconut, corn, cotton seed, soyabean and palm oil.

Animal fats Butter, ghee and cream from milk, lard from hogs and tallow from cattle.

Manufactured fats Vanaspati and margarine.

Some plants store fat in the seeds, for example, oil seeds and nuts. Animals secrete fat in the milk, which is extracted as cream and butter and later clarified to yield ghee. Animals store fat in adipose tissues from which it can be extracted, e.g., lard from hogs and tallow from cattle.

Most cereals, vegetable and fruits contain very little fat. The only exceptions are the grain corn, and the fruit palm, which contain sufficient fat to permit commercial production.

Both oils and fats are extracted from vegetable and animal foods by various processes. Thus, these are processed foods, and their quality is affected by the process used to extract these. Fats have been used for a much longer time in man's dietary, than oils, which gained commercial importance only at the end of the nineteenth century.

A gramme of oil or fat supplies nine calories in contrast to starchy foods, which provide only four calories per gramme. They are prized for the flavour and richness they impart to foods. Oils and fats provide 10 to 30 per cent of our daily energy intake.

Oils and fats are similar in composition, but physically, fats are solid at normal temperatures (18–25°C), whereas oils are liquids.

Fats and oils are widely distributed in nature and are found in almost every natural food. Oilseeds and nuts are rich sources of oils and are used in the commercial manufacturing of oils. Corn, olives and fruit palm are also used as sources for oil extraction. Whole grain cereals and legumes contain 1 to 6 per cent of fat. Even fruits and vegetables contain between 0.1 and 1 per cent of total fat.

Animal foods, milk and its products, eggs, fowl, fish and meat are natural sources of fat in our diets. Oils and fats are added in food preparation as spreads, shortening, as flavour enhancers and as seasonings. They are also used as a medium of cooking in shallow and deep fat frying of foods.

Even when no oil or fat is added to the diet, the natural fat in the foods provides 10 to 12 per cent of the total energy intake

Module 18 : Composition and Classification of Fats and Oils

Composition and Classification

Oils and fats are composed of the elements carbon, hydrogen and oxygen. Fats are built up by linking together a number of individual fatty acids with glycerol.

The kind of glyceride formed is indicated by the prefix attached to the word glyceride. Thus, the union of one molecule of glycerol and one molecule of fatty acid forms a *Monoglyceride* (mono-one); in the process one molecule of water is freed. If two fatty acids are attached to

glycerol, it is called a *Diglyceride* (di-two), and if three fatty acids are attached to glycerol, it is a *Triglyceride* (tri-three).

In a mixed glyceride, more than one kind of fatty acid is present. When three fatty acids in a triglyceride are of the same kind, the fat is a *Simple Triglyceride*. If the fatty acids are different, the fat is a *Mixed Glyceride*. Edible fats are complex mixtures of mixed triglycerides and small amount of other associated substances occurring naturally in plants and animals. This may account for the wide variation in the flavour and consistency of food fats.

Fatty acids are composed entirely of carbon, hydrogen and oxygen atoms. They are found in all simple and compound lipids. Some common fatty acids are palmitic, stearic, oleic and linoleic acid. Fatty acids differ from one another in their chain length (the number of carbon atoms in each molecule) and the degree of saturation. There are short chain fatty acids (with a chain length of 10 or fewer carbon atoms), examples of which include acetic acid found in vinegar and butyric and caproic acid in butter. Long chain fatty acids have a chain length of 12 to 18 carbon atoms and include palmitic and stearic acid found in lard and beef tallow respectively. Oleic acid and linoleic acid (18 carbon atoms) are also long chain fatty acids. They are found in olive and corn oils respectively.

Fatty acids may be saturated or unsaturated. Certain fatty acids contain as many hydrogen atoms as the carbon chain can hold. They are called *saturated fatty acids*; of which stearic acid is an example. Other fatty acids have only one double bond linkage (two hydrogen atoms missing) in the carbon chain. They are referred to as monounsaturated fatty acid, e.g., oleic acid.

A third group the polyunsaturated fatty acids, may have two, three, four or more double bond linkages in their carbon chain. Vegetable oils contain several polyunsaturated fatty acids, of which linoleic is essential for human beings. Naturally occurring unsaturated fatty acids have a low melting point and are liquid at normal temperatures. Oils have a large amount of olein (a triglyceride of oleic acid—18 carbon unsaturated fatty acid) and hence are liquid at ordinary temperature.

Module 19: Nutritive Value and Fats and Oils

Nutritive Value and Digestibility

Fats and oils are concentrated sources of energy. Each gramme of pure fat or oil supplies nine kilocalories. Fats and oils have other functions in the body besides supplying energy. They carry fat soluble vitamins A, D, E and K into the body and assist in the absorption of these vitamins. Some vegetable oils contain an essential fatty acid, which is necessary for normal body functions. Essential fatty acid is not synthesized in the body. Fats impart special flavour and texture to our foods, thus increasing the palatability. Fats are also valuable for the satiety value that they give to meals. They are slow in leaving the stomach and hence may delay the recurrence of hunger pangs. Fats that are ordinarily consumed as constituents of common foods do not differ greatly in digestibility being utilized to the extent of 95–98 per cent.

Digestion is the process which prepares food for assimilation by the body. The digestion of fat starts in the small intestine. When the food enters the first part of the small intestine called the duodenum, the gall bladder is stimulated to release some *bile* and the pancreas a fat splitting enzyme called *lipase*. These fluids enter the duodenum through a common duct. Bile neutralizes the acidity of the food mass and provides the alkaline pH necessary for the action of the pancreatic enzymes. It emulsifies the fat and increases their surface area which helps the lipase hydrolyze part of the fat to glycerol and fatty acids and the rest to mono- and di-glycerides. The bile salts disperse the fatty acids and glycerol into small units called *micelles* in the small intestine so that they are easily absorbed through the intestinal walls. The remaining partly hydrolyzed products viz., mono- and di-glycerides are broken down completely to fatty acids and glycerol by the intestinal lipase and are also absorbed, after being converted to micelles.

Module 20: Physical Properties of Fats and Oils

Physical and Chemical Properties

The following are some of the physical properties of fat which play an important role in food preparation:

Melting Point All food fats are mixtures of triglycerides, and therefore, do not have a sharp melting point, but melt over a range of temperatures.

Creaming of Fats Solid fats like butter and margarine can be creamed or made soft and fluffy by the incorporation of air. Fat and sugar are usually creamed together in the preparation of cakes.

Plasticity of Fats Fats are mouldable and can be creamed to exhibit plasticity. Such fats do not have the ability to flow at room temperature and are thus solid fats. The spreading quality of butter is the result of its plastic nature. Plastic fats are composed of a mixture of triglycerides and not of one kind of a molecule. They, therefore, do not have a sharp melting point and are plastic over a fairly wide range of temperature.

Emulsification The specific gravity of oils and fats is about 0.9, which indicates that they are lighter than water. Though insoluble in water, they can form an emulsion with water when beaten up with it to form tiny globules in the presence of suitable emulsifying agent. Butter is an emulsion, so also is cream. The presence of minute amounts of milk protein helps to stabilise these emulsions. Lecithin, a phospholipid from egg yolk helps to stabilise mayonnaise, a salad dressing made from vegetable oil. Emulsification of fats is a necessary step in a number of products such as cakes, ice cream and other frozen desserts.

Smoke Point The smoke point is the temperature at which a fat or oil gives off a thin bluish smoke. Foods that are fried are added to the hot oil just before it reaches its smoke point. Fats and oils with low molecular weight fatty acids (those with a short chain length) have low smoke point. Normally, oils that are selected for deep fat frying are those, which have a high smoke point. If oils with low smoke points are used for deep fat frying, then the foodstuff is fried at a lower temperature and thus will take a longer time to acquire the stage of doneness. In this case, the exposure of the foodstuff to the oil is increased thereby increasing its oil absorption.

Chemical properties of fats (such as iodine value, acid number and saponification number) are useful in that they have been widely used in the identification of different kinds of fats and oils, and in the detection of adulteration of refined oils with other oils that are cheaper and of poorer quality.

Iodine Number measures the degree of unsaturation in the oil and thus gives an estimate of the total amount of unsaturated fatty acids present.

One molecule of iodine is needed to saturate each double bond. Iodine value is expressed as the number of grams of iodine required to saturate 100 grams of oil.

Reichert- Meissel Number (RM Number) : The Reichert-Meissel number is a measure of the volatile water soluble fatty acids present in the fat. It is an important parameter to detect adulteration of butter, especially with coconut oil. The RM number is defined as the number of mls of 0.1N alkali (e.g., KOH) needed to neutralize the volatile fatty acids in a 5 g sample of fat. The volatile acids will be those from butyric to myristic acid. (C4 to C14). The RM test measures the amount of butyric, caproic, caprylic and capric acids present. The RM value of butter is between 24 and 34 (variation is due to season, nutrition and stage of lactation), which is higher than other edible oils.

Saponification Number: is defined as the number of mg of potassium hydroxide needed to saponify 1 g of fat or oil. The fat containing lower molecular weight fatty acids will have a high saponification number. Butter which contains high percentage of butyric acid has the highest saponification number, i.e., 17.

Module 21: Refining of Crude Oils

Refining of Crude Oils

About 80 years back, oils used to be extracted from oilseeds and nuts in small pressure extraction units. The extracted oil was used in the human diet and the oilseed concentrate was used as cattle feed. Now most of the oils used in human diet are refined.

In the refining process the impurities in oils are removed. The impurities in the oils include moisture, free fatty acids, colouring pigments, resins, gums and sometimes vitamins. The colour, flavour, odour and clarity as also the shelf-life of the oil are affected by impurities. Hence, the impurities are removed by refining to increase the shelf-life and acceptability of oils. The refining process consists of the following five steps:

1. **Degumming** Some impurities in the crude oil form gums in the presence of water. Such impurities are removed by adding water to the warm oil and centrifuging it to remove the denser gum particles. Thus, a clarified oil is obtained as an upper layer, which is siphoned off.

2. *Neutralising* All crude oils contain some free fatty acids formed due to spoilage. The oil is neutralized with caustic soda solution and the insoluble soaps formed from the free fatty acids, which settle at the bottom are removed.

3. *Washing and Drying* The last traces of soap from the oil are removed by washing. The lower aqueous soap layer is run off and the oil layer is dried under vacuum. The oil thus obtained has a yellowish colour and a distinct odour. To remove these, the oil is bleached and deodourised.

4. *Bleaching* To absorb the colouring matter from the oil, adsorbing substances such as activated carbon and fuller's earth are added to the warmed oil. The mixture is stirred with maintenance of partial vacuum. After, all the coloured matter is adsorbed, the mixture is put through filter presses to get a clear colourless oil.

5. *Deodourising* Finally the oil is deodourised by injecting steam with agitation to remove all odorous material as vapour. The deodourised oil is packed as such or blended with other oils and packed. The refined oil has to be stored under an inert gas such as nitrogen or vacuum packed to prevent oxidation.

Module 22: Different Uses/Products of Fats and Oils

Role in Cookery

The role of different types of fats and oils in cookery is largely based on their composition and properties. Thus, liquid fats or oils with a high smoke point are used for deep-fat frying purposes and likewise, solid fats like butter and margarine are used as shortening and tenderizing agents in foods.

Fats are used in food preparation

- as a medium of cooking.
- as a shortening as in *chakali*, *puri*, *shankarpala*, biscuits, pastry and cakes.
- to add richness and flavour as in *shira*, *halwa*, seasoning of vegetables and

salads.

As a Medium of Cooking

Fat and oils have a high boiling point as compared to water. Therefore, foods get cooked in fat in shorter time than when cooked in water. Fried foods, such as *Wafers* and *Chivda*, have a crisp texture and a delectable flavour. The high temperature used in frying destroys

harmful bacteria, thus making the food safe for consumption. Some fat is absorbed by the food and the calorific value of the food is increased when it is cooked in fat or oil.

As a Shortening

In many preparations, such as cakes, biscuits *chakali* and *chirote*, fats or oils are added to improve the texture. The fat covers the surface of the flour particles and prevents the sticking of particles together. Many factors such as the nature of the fat or oil, the amount added, the temperature, presence of other ingredients, manipulation and the extent of mixing, affect the shortening power.

As a Seasoning

Fats and oils are used to season most food preparations. In sweet preparations, fats, such as butter, ghee, vanaspati are used, as they have mild flavour, which blends with the sweet preparation.

Module 23: Storage and Shelf life of Fats and Oils

Changes in Fats during Storage:

Fats and oils undergo certain undesirable changes during storage, which result in spoilage. The major kind of spoilage is known as *rancidity*. Rancidity implies development of undesirable odour and flavour in fats and oils. It occurs in a number of foods and is not restricted to pure fats and oils or foods with high fat content. In fact, the spoilage of foods containing very small percentage of fat such as cereals, flours, infant foods is brought about by change in the fat fraction.

Flavour Reversion The fats undergo a peculiar change before the onset of rancidity. The characteristic flavour is lost and the fat or oil has a flat taste and a greasy feel on the tongue. This is known as flavour reversion and precedes rancidity changes.

Rancidity Spoilage of fats results in off flavour and renders the fat inedible. These changes are known as rancidity of fats. Fats and oils can get rancid by the action of

- air (oxidation),
- water (hydrolysis) and
- enzymes (enzymatic breakdown).

Let us look at these in detail:

Hydrolysis is the decomposition of fats (triglyceride molecules) to glycerol and free fatty acids. Presence of moisture, microorganisms and the enzyme lipase hastens the hydrolytic breakdown. This kind of spoilage is known as **hydrolytic rancidity**.

The unpleasant odour and flavour of rancid fats is due to the release of free fatty acids of low molecular weight. For example, the butyric acid produced in the hydrolysis of butter is responsible for the rancid odour of spoiled butter. Even when a small amount of butyric acid is released, the butter has a disagreeable flavour and odour which indicates the onset of rancidity.

Oils containing combined fatty acids with more than 14 carbon atoms do not develop hydrolytic rancidity as the free acids are flavourless and odourless. Oils should be stored in completely dry, airtight containers to prevent hydrolytic rancidity.

Oxidation of unsaturated fats leads to oxidative rancidity. Thus, oils or fats containing more double bonds (unsaturation) are more likely to develop oxidative rancidity than those with few double bonds. This oxidation is a chain reaction initiated by the production of free radicals.

Addition of small quantities of antioxidants suppresses the production of these free radicals and improves the shelf-life of fats and oils. Some of the antioxidants used in fats and oils are vitamin E (tocopherol), butylated hydroxy toluene (BHT) and permitted gallates.

Enzymatic breakdown normally accompanies hydrolysis as indicated above.

Prevention of Fat Spoilage

Storage of fats and oils so as to minimise possibility of spoilage is a very important aspect. 

The following points must be noted to prevent spoilage of fats:

Keep fats and oils in dry, tightly covered containers to ensure exclusion of air and moisture. Keep the container sealed until needed. Keep fat in a container having a narrow opening to prevent undue exposure. Store in a cool, dry place away from cooking area, where the temperature and humidity fluctuations are not great.

Addition of antioxidants, such as tocopherols, and other phenolic compounds such as BHA, BHT, or propyl gallate are used to retard rancidity in commercial fatty products.

Lecture 3

DAIRY PRODUCTS**Module 24: Introduction to Dairy Products****Milk and Milk Products**

Milk has a very special place in the traditional food. It is an essential part of our morning's cup of tea or coffee. Curd and butter milk are made from milk. Milk is also used to make popular sweets such as *pedhas*, *barfi* and a variety of *halwas*. A number of desserts from milk made for special feasts include *kheer*, *shreekhand*, *rasgullas*, *gulab jamuns* etc.

Composition of Milk

Milk from different animals is used as food, but in sub-continent, buffalo and cow are the two species which are most important for the commercial production of milk and milk products.

Milk is a complex food, which contains more than 100 components. Most of these components are suspended in water and thus milk is a colloidal solution and is opaque.

The major components of milk are water, protein, fat, the sugar lactose and minerals. The composition of milk varies with species. Buffalo's milk contains twice as much fat as cow's milk.

Milk fat contains some volatile fatty acids (e.g., caproic and butyric acid). These are released when butter turns rancid. Their presence is noticeable in rancid butter due to their bad smell.

Lactose is the sugar present in milk. It is present in the milk serum. Milk is an excellent source of the minerals calcium and phosphorus. The minerals in milk are present partly in solution, partly in suspension and some as components of proteins and fats. For example, most of the phosphorus is suspended in the form of calcium phosphate, but a little is combined with casein and another trace is found in combination with the fat. Milk contains all the vitamins known to be beneficial to human nutrition.

Module 25 : Microbiology of Milk

Milk is a highly nutritious media for bacteria and can spoil easily. Initially milk may have few organisms, but bacteria can enter milk during milking process and handling

Refrigeration improved milk industry, but still other improvements needed to be done to reduce No's of non pathogenic bacteria.

Non Pathogens in milk

Air borne contaminants: Pseudomonas, Flavobacterium, Alcaligenes, coliforms, micrococcus, fungi

Psychrotrophs :grow at 5°C and less and make up a large number of the microbes in milk, they are natural soil inhabitants and wide spread.

Spoilage of refrigerated milk consists usually of bitter, rancid, fruity flavours

Sources of Pathogens in Milk:

Animal itself: Mycobacterium bovis, Brucella species, Coxiella burnetii, Campylobacter sp.

Humans: Salmonella, Shigella

Environment: Bacillus anthracis, Clostridium perfringens, Clostridium botulinum

Module 26: Fluid Milk and its handling

Milk has a very special place in the diet of everyone including infant sole feeding to adults daily life food habits it plays a very important role in nutrition and variety of food products. 

For example, It is an essential part of our mornings cup of tea or coffee or other dairy products. Milk is also used to make popular desserts for special feasts. Therefore, it is important for us to understand its handling.

Care of Milk at home:

Fresh clean milk has a delicious rich taste. However, it is highly perishable food commodity and have only a shelf life of few hours. In order to extend its shelf life it must be handled carefully. It is important to ensure that the taste of milk is retained during production and storage. It is very necessary to receive and store milk in a clean container in the home. Raw milk sours on storage due to the action of lactic acid producing bacteria,

especially in summers. Milk should therefore be boiled as soon as it is received in the home to prevent spoilage. Further, it should be covered and stored in a cool place/ refrigerated.

Effect of Heat on Milk:

Milk is boiled prior to use. A number of changes occur in the milk due to heat. The changes affect the color, flavor and viscosity of milk. A light brown tinge develops by reaction of milk protein with reducing sugars such as lactose, glucose and fructose. Processed milk may develop a burnt taste which is undesirable for organoleptic properties of milk. The dispersion of calcium phosphate in milk decreases, when milk is heated and a part of it is precipitated at the bottom along with the coagulated lactalbumin. Volatile elements such as iodine tend to be lost when milk is heated.

Scum Formation

Scum forms when milk is heated. As the temperature of heating increases, a scum forms at the top, and it can be removed. But as soon as it is removed, another layer of scum forms. The milk boils over due to the scum formed. The scum is a tough, leathery, insoluble layer. The scum is forced

Module 27: Processing of Milk

Milk may be given various treatments like heating, concentrating, drying or altering its pH to obtain a number of different products. The objective of such treatments is to preserve milk and add variety to our meals. This is done by:

- exposing milk to high temperatures at which microorganisms are killed,
- binding or reducing the water present in milk and thus making moisture unavailable to micro-organisms,
- by increasing the acidity of milk to a level that does not permit growth of spoilage microorganisms, and yet imparts a desirable sour flavour to the product.
- Some of the processing methods employed and the common milk products available are discussed below.

Pasteurization of milk is a process which consists of heating milk to a certain temperature, for a definite time to ensure destruction of pathogenic bacteria, which are likely to be present.

There are three methods used to pasteurize milk.

Holding Method Milk is heated to 62.7°C and held at that temperature for 30 minutes.

Higher Temperature Short Time Method (HTST)—Milk is heated to 71.6°C at least for 15 seconds.

Ultra High Temperature Process Milk is heated to a temperature of 89–90°C or more for 1 second or less.

In all three processes, milk is immediately cooled to 10°C or lower and held at that temperature. Since cold storage facilities are not commonly available in most homes, milk is routinely boiled prior to use in Sub-continent, which results in improving the shelf-life of milk. Therefore, pasteurization does not have the same significance here from the health point of view as in the western countries.

Concentrated Milks These include *khoa*, evaporated milk, sweetened condensed milk and dried milk made from both whole and skimmed milk. Varying amounts of water are removed to make the concentrated milk products. These products have a longer shelf-life and some can be reconstituted to their original form. The dispersion of calcium phosphate in milk decreases, when milk is heated and a part of it is precipitated at the bottom along with the coagulated lactalbumin. Volatile elements such as iodine tend to be lost when milk is heated.

Module 28: Other Dairy Products

Yoghurt:

Yoghurt or curd is very famous product in our culture and it is used in variety of forms. It ranks second to milk in the extent of consumption. Milk is boiled and cooled to about 50°C and a teaspoon of curd from an earlier batch of curd is added and mixed thoroughly. The lactic acid bacteria present in this sample of curd curdle the milk. They utilize the lactose in milk and break it down to lactic acid. The formation of lactic acid increases the acidity of milk. When the pH reaches 4.6, the milk protein, casein, coagulates as curd.

Since the process is gradual, the milk serum is bound in the mass of coagulated proteins.

The optimum temperature for the formation of curd is 35–40°C. The time needed for curd formation varies from 8–12 hours depending on atmospheric temperature. Once made, curd

keeps well at refrigerator temperature of 2–3 days. It is used as dressing on salads made from fresh vegetables.

Preparation of curd is one method of prolonging the shelf-life of milk by several days. Soured milk does not readily undergo proteolysis and other undesirable changes. Curds can be held at refrigerator temperature for several days without loss of acceptability. It is reported that the riboflavin and thiamin content increases during curd formation. It is also reported that fermented milk inhibits the growth of *Bacillus typhosus*, *Bacillus dysenteriae*, and *Vibrio cholerae* to a certain extent.

Paneer is prepared by addition of lemon juice or citric acid to hot milk and precipitating the casein. The liquid released in this process is known as *whey*, which contains most of the soluble nutrients from milk. Paneer contains about 18 per cent protein and is a good source of it.

Cheese The milk is subjected to several process steps to produce cheese. The milk is held at about 27°C in vats and a lactic acid culture is added. When the milk gets acidic, rennet is added to it and the milk is allowed to coagulate. The curd formed is cut into strips and heated to about 37°C with stirring to separate the whey. The whey is drained. Salt is mixed with the curd and it is pressed to remove further amounts of whey. The cheese formed is coated with paraffin to prevent loss of moisture and allowed to ripen at low temperature. Cheese contains about 24 per cent protein and is thus a concentrated source of protein.

Buttermilk Buttermilk is made by adding water to curd and churning to remove fat in the form of butter. The energy value is thus reduced due to fat removal. The concentration of the other nutrients is reduced in proportion to the dilution. It is known to re-establish intestinal flora after an attack of diarrhoea.

Module 29: Detection of proteases in Milk

Proteases are enzymes that degrade proteins. Raw milk may have proteolytic activity from endogenous or indigenous origin. Wherever they come from, proteases can cause unpleasant flavours and odours in milk.

Several bacteria present in raw milk can produce proteases. Among them, those from psychrotrophic microorganisms (*Bacillus subtilis*), which produce enzymes under refrigeration, cause the most serious problems. It

secretes a thermo-resistant protease which may cause proteolysis in pasteurized or sterilized milk.

Sample preparation

Milk sample need to be chosen that is required for analysis. The standard protease from *Bacillus subtilis* is needed and required concentrations are made for calibration curve.

Enzyme determination

Reagents

Disodium hydrogenphosphate,

Sodium dihydrogenphosphate

Trichloroacetic acid

Solutions

Prepare a pH 7.2 buffer solution by mixing 36 ml of sodium hydrogenphosphate (5 mM) and 14 ml of sodium dihydrogenphosphate (5 mM) and diluting with distilled water to 1000 ml. This buffer is used to prepare a 1% azocasein solution. A 5% aqueous solution of trichloroacetic acid was also required.

Determination of protease activity

- The assay for proteolytic activity involves combining 1 ml of the azocasein solution with 100 ml of the sample which contains the enzyme.

- The contents of the tubes are mixed and incubated at 35.5 degree C for 15 min.

The reaction is stopped by the addition of 2 ml of 5% TCA.

- The absorbance of the supernatant is recorded at 345 nm in a UV/Visible spectrophotometer.

- Activity calculated against standard curve.

Module 30: Detection of amylases in milk

Amylase activity in milk is a critical quality parameter. The amylase activity in milk is often referred as diastatic power and refers to the production of reducing substances by the action of alpha & beta amylases. The measurement involves digestion of the soluble matter with a

malt infusion (extract) and following increase in reducing substances by measuring reduction of Fehling Solution. Specifically measuring alpha amylase activity (dextrinizing activity) is more complicated and is based on using a limit dextrin as substrate.

- Limit dextrin is prepared by action of beta amylase (free of alpha amylase activity).
- The beta amylase clips maltose units off the nonreducing end of the starch molecule until an alpha-1,6-brach point is encountered.
- The resulting product is a beta limit dextrin that serves as the substrate for the endo cleaving alpha amylase.
- A malt infusion is added to the previously prepared limit dextrin substrate and aliquots removed periodically to a solution of dilute iodine.
- The alpha amylase activity is measured by changed color of the starch iodine complex in the presence of excess beta amylase used to prepare the limit dextrin.
- The color is compared to a colored disc on a comparator.
- This is continued until the color is matched to a color on a comparator.
- The time to reach that color is dextrinizing time and is a measure of alpha amylase activity, a shorter time representing a more active preparation.

Module 31: Determination of iodine number of fats

Principle:

The iodine number is a measure of degree of unsaturation, that is, number of carbon double bonds in relation to the amount of fat or oil.

Iodine value is defined as the grams of iodine absorbed per 100 gram sample. The higher the amount of unsaturation the more iodine is absorbed. A quantity of fat or oil dissolved in solvent is reacted with a measured amount of iodine or some other halogen. Halogen addition to double bonds takes place.

Procedure:

- A solution of potassium iodide is added to reduce excess ICl to free iodine.
- The liberated iodine is then titrated with a standardized solution of sodium thiosulfate using a starch indicator, and the iodine value is calculated.

$$\text{Iodine Value} = \frac{(B-S) \times N \times 126.9}{W} \times 100$$

W

Where Iodine Value= g iodine absorbed per 100 g.

B= volume of titrant (ml) for blank

S= volume of titrant (ml) for sample

N= normality of $\text{Na}_2\text{S}_2\text{O}_3$ (mol/1000ml)

126.9= MW of iodine (g/mol)

W= sample mass (g)

Calculated iodine value is obtained from fatty acid composition using below equation for triacylglycerol.

Lecture 4

MEAT**Module 32:** Introduction to Meat**Meat**

Meat is one of the animal protein foods used in diet. Meat protein has high biological value. Meat is a good source of proteins and fat. The proportion of nutrients in meat depend upon the kind of animal, the species and the type of cut. The protein content of meat decreases with an increase in fat content. The average protein content of meat ranges from 16 to 23 per cent and the average fat content ranges from 10 to 40 per cent. Meat is a good source of phosphorus, iron and some trace elements. Unless the cooking water is discarded, minerals and water soluble vitamins are not lost to a great extent. Meat provides us vitamin B-complex and some vitamin A, depending on the cut. The meat of sheep, which is under 12 months of age, is sold as *lamb*. After the age of 12 months, it is called *mutton*

Module 33: Structure of Muscles**Structure of the Muscle**

Muscle Tissue Muscle is a complex structure. The smallest unit of a muscle is a *muscle fibre*. Many fibres are joined by connective tissue into bundles. These bundles are called *fasciculi*. The fasciculi together with fat deposits are covered with a thick membrane and attached to the bone.

Connective Tissue These are the tissues, which bind meat fibres together. Meat muscle is connected to the bone by means of a connective tissue. Collagen and elastin are two types of connective tissues. During cooking, collagen is softened and converted to gelatin. Elastin does not become soft during cooking. Some parts of the animal have greater amount of connective tissues, where as others have a lower amount of the tissue. The part of the animal which is exercised more contains greater amount of the connective tissues e.g., the leg. The greater the amount of connective tissue the less tender is the cut of meat.

Fatty tissues These are made up of connective tissues with embedded fat cells. Fat is deposited under the skin, around the glands, organs and between and within the muscle

fibres. Fat distribution in lean part of meat is called *marbling*. A meat which is well marbled is desired by consumers.

Bones The appearance of the bone is an indicator of the part of the animal from which the cut of meat is taken. Bones are either long or short. Long bones are hollow and contain yellow marrow. Other bones, which contain red marrow, are spongy inside.

Pigments Myoglobin is the pigment in meat that gives the characteristic red colour to meat. The greater the amount of myoglobin, the darker the colour of meat. As the animal ages the amount of myoglobin increases. Raw meat when allowed to stand in the refrigerator, changes its colour from red to brownish red. If myoglobin is heated the colour changes from red to brown.

Module 34: Post mortem Changes in Meat

Postmortem Changes

When the animal is slaughtered, certain changes take place. The meat starts to stiffen. This change is called *rigor mortis*. Along with this change, other metabolic changes also take place. More lactic acid is formed, which lowers the pH. The muscle fibre swells with fluid and become hard. As soon as the animal is slaughtered, the circulatory system stops functioning but the enzymes continue to remain active. Oxidation of glycogen brings about production of lactic acid. The amount of glycogen stored in a particular animal decides the amount of lactic acid produced after slaughter. A lower glycogen storage produces sticky and gummy meat which is undesirable. Stiff muscles of the animal start softening after holding for some time. This is called *aging*. Tenderness in the meat increases with holding time. Holding conditions should be carefully controlled. Uncontrolled conditions may allow putrefactive bacteria to grow. Controlled conditions of holding are:

- Temperature of holding should have a range of (34–36°F)
- Carbon dioxide in the atmosphere
- 70 per cent humidity

This helps keep the meat in a good condition for 3 to 6 weeks. After this aging process, the meat is ready for sale. This process of aging is also called ripening of meat.

Module 35: Preservation of Meat

Meat is a highly perishable products and soon becomes unfit to eat and possibly dangerous to health through microbial growth, chemical change and breakdown by endogenous enzymes.

Chilling/refrigeration

slows or limit the spoilage rate at temperature below the optimal range can inhibit the microbial growth, enzymatic as well as chemical reactions. Storage of fresh meat is done at a refrigeration temperature of 2 to 5°C. Chilling is critical for hygiene, safety, shelf life, appearance and nutritional quality.

Curing Many methods are used. Meat is treated with salt, sugar, sodium nitrate and spices to give different flavours. Smoke is used some times to give a desirable flavour and to improve the keeping quality of meat.

Freezing This is a common method of preserving meat. Nutrient loss is least during freezing. The one undesirable effect, freezing has on meat is that, the meat when cooked, is less juicy. This method is commonly used for storing cooked meat.

Canning Meat can also be preserved by this method. Higher temperatures which are used during canning bring about some undesirable changes in flavour and texture.

Radiation and Sterilization This is a new method of preservation of meats. The meat is sterilized and bacteria present killed. According to some people, this meat is not palatable.

Module 36: Cooking and Effect of Heat on Meats

- Cooking of meat or application of heat brings major changes into meat.
- These include colour and texture changes
- Food safety is achieved by reducing bacterial load.
- The method of cooking depends on the type of meat.
- Dry heat methods like roasting, broiling, pan frying are used for tender cuts or young animals.
- Moist heat methods like pressure cooking, closed pan cooking, steaming are used for less tender cuts.

- Most common cooking methods appear to be roasting, broiling and pan-frying.

Effect of Heat on Meat

- Thiamin in meat is partially lost during cooking.
- If the food is heated for a long time, the loss is extensive.
- If water or juices, in which the meat is cooked, are discarded, then the loss of the

vitamin increases.

Lecture 5

CEREAL PRODUCTS**Module 37: Cereal Foods: Introduction**

On a worldwide basis, rice is the most important cereal, being produced for human food in the largest amount, while in the United States, corn is produced in the largest amount, although it is used for animal food and other products as well as for human food. The grain grown in the largest quantity for human food use is wheat.

For most food uses in cereals, the bran and the germ are removed: the bran, because it is indigestible by humans and because of its adverse effect on the appearance and on some functional properties of flour, and the germ, because of its high oil content, which may subsequently become rancid. The germ is used to produce oil (e.g., corn oil). The bran goes mainly to feed animals. However, with dietary guidelines recommending more fiber, a growing amount of bran is being used in the production of breakfast cereals, bakery products, and other human foods.

The first ready-to-eat cereals were produced just before the turn of the twentieth century, with flaked and puffed cereals following within a decade. Ready-to-eat cereals, made from the endosperm of wheat, corn, rice, and oats, are convenient, nutritious, and they come in a very large variety of forms, textures, and tastes. The processing of cereals into breakfast commodities was started in the United States and is still largely carried out in the United States, with considerable quantities being exported throughout the world. The most popular of the breakfast cereals are those that are ready to eat. These are formed or puffed and oven-baked

Module 38: Composition and Structure

Cereal grains are composed of three parts, i.e. Bran, Germ and Endosperm. Bran is outer chaffy coat that covers the kernel. Endosperm is the large central portion. Whereas germ is a small structure at the lower end of the endosperm.

Bran : Eliminated when grains are harvested, Constitutes 5% of the kernel, Discarded during milling . High fiber and mineral content, Good source of thiamin and riboflavin

Aleurone layer: Located just underneath the bran, Rich in protein, phosphorus and thiamin. Some fat, Makes up 8% of the whole kernel, Lost in the milling process

Endosperm: Constitutes about 84-85% of the kernel, Contains most of the starch and protein Little fiber and mineral matter, Traces of fat, Low vitamin content

Germ: Makes up 2-3% of whole kernel, Rich in fat, protein, minerals and vitamins, Serves as a store of nutrients for the seed, Some of it is lost during milling

Module 39: Nutritional Importance of Wheat Crop

Wheat

Whole wheat, consisting of about 13% protein, can contribute considerably to the diet. The flour made from the whole wheat is higher in biological value than white flour (made from the endosperm only). Wheat is the most popular cereal grain for the production of bread and cakes and other pastries. Wheat produces a white flour. In addition, the unique properties of wheat protein alone can produce bread doughs of the strength and elasticity required to produce low-density bread and pastries of desirable texture and flavor.

There are many varieties of wheat. They may be classified as hard red winter wheats, hard spring wheats, soft red winter wheats, white wheats, and durum wheats. Winter wheats are planted in the fall and harvested in the late spring or early summer. Spring wheats are planted in the spring and harvested in the late summer. Hard wheats are higher in protein content and produce more elastic doughs than soft wheats. Therefore, hard wheats are used for breads, and soft wheats are used for cakes. Durum wheats are used most for alimentary pastes (spaghetti, macaroni, etc.) and for the thickening of canned soups.

Wheat is harvested by combines that cut the stalk, remove and collect the seed, and either return the straw to the soil, to be plowed under with the stubble and thus provide humus, or compress and bale it for future uses such as for litter or ensilage. Wheat may be bagged in jute sacks and stored in warehouses, or it may be stored in bulk in elevators. The latter method provides the best protection against rodent and insect infestation. The moisture content of bulk-stored wheat should not be higher than 14.5% and that of sack-stored wheat not higher than 16%. Otherwise, microorganisms may grow and cause heating and spoilage. When it is necessary to lower the moisture content of wheat, it may be dried in bins by blowing hot air (not higher in temperature than 175°F (79.4°C) across the bins.

In preparing wheat for milling, the wheat is blown into hopper scales that record the quantity of uncleaned wheat. Some of the coarser impurities are removed by this process. The grain then passes over a series of coarse and fine sieves that further remove contaminating materials, including chaff and straw. With the wheat still in the dry state, stones may be removed by passing wheat over short openings that allow the heavier stones to fall out of the mass and be trapped. The wheat is next passed over discs or cylinders containing indented surfaces that remove seeds shorter or longer than wheat, following a pass through a magnetic separator to remove any metals present. The next cleaning process is dry scouring to remove adhering dirt. The wheat is then washed in water, a process that both removes dirt and adds 2% to 3% water to the grain. The added water is necessary to provide desirable conditions for milling. A stone trap is included in the washer. Excess water is removed by centrifugation. A second wet cleaning with a light brushing action is ordinarily used, followed by aspiration (blowing air through the grain), which is the final cleaning operation. The grain is then carried into a bin from which it is fed to the milling operation. This bin is located on the top floor of the flour mill, the grain having been elevated to this position during the various cleaning operations.

In milling, grain is fed automatically through scale hoppers that regulate the flow of the seeds at rates corresponding to those of the following operations: milling may be carried out by passing the grain through a series of corrugated rotating toward each other, which remove chunks of the endosperm from the bran.

Module 40: Maize

Maize:

Many types of corn are grown in the United States. Sweet corn is produced as a vegetable and eaten fresh, canned, or frozen. Popcorn is also used as a food. However, the type of corn most utilized in the United States and considered as a grain rather than a vegetable is field corn. There are a number of varieties of corn usually classified as starchy or waxy, depending on the characteristics of the carbohydrate present. The development of hybrid strains has improved the yields of field corn. This corn is lower in protein than wheat, and, like all vegetable proteins, including wheat, is deficient in some amino acids and so does not provide a complete protein for humans. Corn is especially deficient in the amino acid lysine, but a variety of high-lysine corn has been developed that may eventually have a great impact on human nutrition in some parts of the world.

Ears of field corn are harvested by a machine that strips the matured ears from the stalks. If harvested in wet weather, corn may have to be dried before it is stored. Usually, it is allowed to dry on the stalk in the field, is harvested, and is stored in small roofed bins or silos with metal or wire mesh walls. Much of the corn storage is done on the farm, as most of the corn crop is used as feed for animals. Stalks and leaves may be harvested, chopped, and placed in piles or in silos to form ensilage for animal feed. Stalks and leaves may also be chopped and returned to the soil for humus.

Corn milled for flour (corn meal) is cleaned, as is wheat, then moistened to a water content of 21 %. The germ is removed mechanically. The endosperm is then dried to a moisture content of 15%, passed through crushing rolls, and sifted to remove the bran.

With use of sieves, milled corn is separated into grits (largest-sized particles) and meals and flours (smallest-sized particles).

Most of the corn crop is used for animal feed, but considerable amounts are used to produce cornstarch, corn syrup, high-fructose corn syrup, and other various sugar derivatives.

Various types of sweeteners are made from cornstarch, as starch consists of a long straight or branched chain of glucose molecules that may be broken down to short chains of glucose molecules (dextrins), to maltose (two molecules of glucose), or to glucose (dextrose).

Corn is also used to produce popcorn. The variety used is a specific one. When the dried kernels are heated, internal moisture creates a vapor pressure owing to the rise in temperature, and when the pressure is sufficient, the hard outer shell is burst and the pressurized grain is expanded. Essentially, popcorn is puffed cereal.

Module 41: Rice

Some quick-cooking rice is produced by precooking the kernels and redrying. This process provides for the preparation of rice for human consumption by merely bringing the water used for rehydration to the boiling point and allowing the mixture to stand for short periods. Some puffed rice cereal is produced by heating the rice to a temperature above the boiling point of water in closed containers and suddenly releasing the pressure, which causes the kernel to increase in size, as the water vaporizes, allowing it to escape from the interior to the outside.

About one-third of the rice produced in the United States is used by the coffee brewing industry. This consists mostly of broken kernels, but some whole grain rice is also used for this purpose. Rice flour is produced, and most of this is used by those who are allergic to wheat flour. Rice flour may be used, too, for the preparation of white sauces, especially for prepared frozen-food products, as certain types of rice flour produce sauces that do not curdle and weep (separation of liquid from the sauce) when frozen and defrosted.

Rice bran has been used for cereal and baked goods and its water-soluble fiber is said to have cholesterol-lowering effects similar to those suggested with oat bran. Rice kernels may be enriched, as is wheat flour, by mixing with powder containing vitamins and minerals. This powder sticks to the surface of the kernels. The enrichment materials may then be coated with a waterproof, edible film to protect them from being washed off.

The protein of rice is comparable to that of wheat in composition, although rice is lower in total protein content than wheat. Neither of these grains contain a complete protein, that is, the proteins do not contain sufficient amounts of certain amino acids to provide for the requirements of the human, although the biological value of rice protein is reportedly equal to or superior to that of wheat protein.

Rice Rice is the major food of many people living in Asia, and is the most widely used of all cereals next to wheat. The average production of rice in the year 1980–1981 was 1.34 tonnes per hectare.

Rice is available as milled, unpolished, polished, parboiled, flaked and puffed rice. Milling removes the bran, aleurone layer and some of the germ. Polishing further removes more than half the mineral matter and most of the vitamins. By a special process, grains of rice may be parboiled before milling.

Module 42: Oats

Oats, one of the popular nutritious present-day cereals, was once regarded as useful for feeding only cattle. Oats can grow in colder and wetter climates than can wheat. Oats are harvested much in the same manner as is wheat. The moisture content at the time of harvest should not be higher than 13%.

Milling of oat kernels requires that they first be washed and cleaned and then dried in a rotary kiln or pan drier to a moisture content of about 12%. They are then hulled by impact,

the seeds being thrown from a rotating disc against a rubber ring that splits off the hull and leaves the groat mostly intact. After the hulls are removed by passing the product through sieves, the oats are steam heated and passed between rollers to produce rolled oats, or they are cut into pieces about one-third of the original size and then steamed and rolled to produce quick-cooking rolled oats. Small amounts of oatmeal may be produced by grinding the steamed oats. Steaming facilitates cooking and inactivates enzymes that, if not inactivated, may cause bitter flavors to develop. Oat flour may be produced for use as an ingredient of bread or a thickener for soups. If made from unheated oats, lipolytic enzymes may remain and there may be a problem with the development of rancidity.

Oat bran has become a very popular item and much of this is due to the research that links its water-soluble fiber with reduction of blood cholesterol. Many articles have been published such as those by James Anderson of the University of Kentucky in the American Journal of Clinical Nutrition (1981) and by Jeremiah Stamler of Northwestern University School of Medicine in the Journal of the American Dietetic Association (1986) which show that consumption of oat bran can lower blood cholesterol (Anderson said that those who ate 100 g of oat bran a day lowered their cholesterol by 13% and Stamler said it dropped 3% if 35 to 40 g of oat bran was consumed daily). Frank Sacks of Harvard Medical School in the New England Journal of Medicine (1990) found that oat bran didn't lower the cholesterol level any more than refined wheat flour with a low bran content. He suggested here that just the replacement of foods with high levels of saturated fat and cholesterol with those low in these components will lower the blood cholesterol.

It is agreed by the vast majority that this replacement of fatty foods with lower fat varieties is beneficial. The American Dietetic Association and American Heart Association have recommended a decrease of fat to less than 30% of calories consumed and an increase of fiber in the diet. The Institute of Food Technologists (IFT) in 1989 cautioned against the overuse of such fibers. If fiber is greatly increased in a low-fat diet, consumers may not retain and adequately use numerous minerals required by the body. The IFT report said: "Recommendations for stepping up fiber in the diet-fiber supplements, oat or other types of fiber-should take into account a person's mineral intake and all other nutrient consequences for the best outcome."

Module 43: Barley

Barley products do not bake as well as wheat products; thus, barley, containing little or no gluten, is not as popular as wheat when there is an option. However, barley has the advantage of growing in climates too cold and in soils too poor to grow wheat, and, in addition to being a hardy grain, its growth requires a shorter time than does that of wheat.

Some barley is produced in the United States. Spring and winter varieties are planted as in the case of wheat. In the United States, barley is used as feed for cattle and poultry, for the production of malt used in brewing, and as an ingredient of soups. Small amounts of barley flour are also produced.

For producing malt, the grain is soaked in water for several days or until the moisture content reaches approximately 50%. It is then removed from the steep tank and placed in containers where air at 65 to 70°F (18.3 to 21.1°C) can be drawn through it over a period of approximately 1 week. This allows the barley to germinate or sprout. The sprouted barley is then kiln dried over a period of 24 hours. Drying is begun at a low temperature that is gradually raised as drying proceeds. The purpose of malting barley is to produce enzymes that will hydrolyze starch to maltose, a sugar that can be utilized by yeasts to produce ethyl alcohol and carbon dioxide. Non-diastatic malt (will not hydrolyze starch) may be produced for its flavor components. Therefore, in drying the sprouted barley, the temperature must not be raised to the point where the starch-splitting enzymes, produced during sprouting, will be inactivated. It would appear, however, that temperatures are raised to the point where some of the sugars present in the sprouted barley are caramelized, hence the dark brown color of malt. Malt is used in the brewing industry for converting the starches present in barley, rye, rice, corn, or other grains to maltose, which can be utilized by yeasts.

Producing beer is an interesting process. The science of fermentation or zymurgy is utilized here. First, as mentioned previously, the barley is allowed to germinate to accumulate starch-digesting enzymes (amylases). Next, the partially germinated kernels of barley malt are dried to stop enzyme activity (but not to permanently inactivate them) and to develop color and some flavor. In this process, called mashing, the malt is mashed in warm water and another grain such as corn, rye, or rice may be added and the enzymes are reactivated by the gentle temperature and moisture and act to reduce the long-chained carbohydrates to sugars (glucose). The resulting sweet liquid produced by this enzymatic action is called wort. With beer and related beverages, hops are added here for flavor. This

process, called brewing, takes place in the brew kettle at elevated temperatures and the enzymes are now permanently inactivated.

Module 44: Other Cereal Grains

Sorghum

Sorghums, comprising four general classes (sweet sorghum, broom corn, grass sorghum, and grain sorghum), are grown in southern sections of the Great Plains and in parts of the Southwest. Some varieties of the grain sorghum class yield glutinous starch, similar to that of corn. During World War II, sorghum was used as a substitute for tapioca, because the importation of tapioca was impeded by the war situation. The deterrent to the use of grain sorghum for the production of starch is the pigmentation of the grain's pericarp, which complicates the production of a white starch. However, enough progress has been made in the development of desirable sorghums to warrant the consideration of sorghum for the production of starch in the future.

Buckwheat

Buckwheat is not a true cereal grain. All the cereal grains belong to the botanical family Gramineae, whereas buckwheat belongs to the family Polygonaceae. However, from a use standpoint, it is considered to be a cereal food. Although it is a minor crop in the United States, only the Soviet Union and France produce more buckwheat than the United States. It is grown mainly in New York, Pennsylvania, Michigan, Maine, and Ohio. Of the few varieties used, the Silver hull is used mainly for producing flour because of the higher yield of the endosperm. Buckwheat is dried to about 12% moisture, cleaned, graded by size, and milled similarly to wheat. Most of the flour is used for making pancakes.

Cottonseeds, Soybeans, and Peanuts

Although cottonseeds come from plants of the family Malvaceae, and soybeans and peanuts from plants belonging to the family Leguminosae, it should be mentioned that they have been used to produce edible flours and other food ingredients.

Cottonseeds are used the least in flour production because they contain the toxic pigment gossypol. The pigment glands that contain gossypol can be removed by a process that involves disintegration of the seeds in hexane and separation of the glands by centrifugation. Heating also destroys the toxin and when heat is applied, the flavor of the seed is improved.

Soybeans have been studied extensively and many soy byproducts have been produced. These include soy "meats" from the dehulled bran, oil, soy flour, and grits (about 50% protein). The grits can be washed at a pH of 4.5 and dried to produce soybean concentrate (70% protein). Grits can also be dissolved in alkali, filtered, and have acid added to a pH of 4.5. This can now be concentrated further to a simple protein isolate (90% to 95% protein). The flour can also be wet, extruded, and dried to textured vegetable protein (50% to 55% protein). The simple protein isolate can be modified further into a range of isolates, spun protein, or structured protein isolates.

Some of the extruded, dried soy protein doughs, on rehydration and cooking, have a very similar texture to that of meat and are being used in combination with meats in mixtures. They add protein, little fat, and are less costly than meat. Dried, flavored textured protein produced such as imitation bacon bits are quite popular also.

A problem exists with soybean in that the raw beans contain a trypsin inhibitor. This interferes with normal growth of animals and humans but it can be inactivated by the heat of cooking or by heat in processing.

Peanuts can also be processed into flour, protein concentrates, and protein isolates but their use in human foods is limited. Peanuts have lower amounts of lysine than do soybeans. Most of the world's peanuts are used for oil (about 67%). In the United States, however, about one-half of the crop is made into peanut butter. Peanuts have a high moisture content at harvest and are susceptible to mold spoilage. If the molds grow, they may produce aflatoxins as metabolites. Strict government controls govern peanuts and if levels of aflatoxins reach 15 parts per billion (ppb) in the raw peanut or 20 ppb in the finished product they must be rejected. This could be devastating economically to the peanut growers, so peanuts are stored under conditions to control mold growth and they are carefully inspected.

Millet

Millet is used for food in Asia and, to some extent, in Europe. In many parts of Europe, it is used for hay, as it is used in the United States. Some varieties are used as food seeds for caged birds and poultry.

Module 45: Processed Cereal Products

A number of processed products are made from cereals and pulses. These include wheat products such as cracked wheat, semolina (*rawa*), atta, maida, rice flakes, puffed rice etc. These are made by grinding to varying degrees of fineness (various particle sizes) or by roasting and pounding, or any other method. These processes increase the surface area of the product exposed to atmosphere, decrease the preparation time and also reduce the shelf-life of these products. While the whole grains have a shelf-life of a year or more, the shelf-life of these processed products may vary from two weeks to a few months.

Broken Wheat or Dalia is whole wheat coarsely ground into large particles. As the losses during milling are very little, it is a very nutritious food. Good quality is indicated by sweet taste and an absence of sour, mouldy odour and flavour. It spoils very quickly in storage, due to insect infestation. It can be cooked as such, made into porridge.

Semolina, Suji or Rawa is available in varied sizes. The fine grain varieties are used for the preparation of halwas, while the large grain varieties are suitable for preparation of *porridges* etc.

These are selected on the basis of uniformity of size, freedom from insect infestation, freedom from oxidised or mouldy odour, grit and bran.

Maida is white finely extracted wheat flour. It is free from bran and has a lower content of protein, iron and *B*-vitamins. It has a lower shelf-life than semolina, as the large surface area permits faster rate of spoilage. Good quality maida is free from insect infestation, bad odours, and lump formation.

Processed Rice Products include rice flake and rice puffs. Rice flakes are made after soaking the paddy in hot water, parching it by roasting and then flattening it by force while it is hot to form flakes. It retains a large part of the iron and *B*-vitamin of the aleurone layer. The roasting helps to toast the grain, resulting in partial cooking of the grain. It needs very little to prepare and is used as a snack. It should be free from bran, broken particles, fragments of the seed coat, insects, stones, trash and bad odour.

Rice Puff 's are another ready-to-eat rice product. It is selected for crispness, freedom from stones, seed coats, sand and dirt.

Lecture 6

FOOD SPOILAGE**Module 46: Food Spoilage: Introduction**

Biodeterioration is defined as *any undesirable change in the property of a material caused*

*by the vital activities of organisms.*¹ It is applicable to many materials e.g. food, wood, 

paper, leather, fuels, cosmetics, building materials and building structures. Biodeterioration may be as a result of the metabolic processes of one of many micro-organisms or it may be caused by insect, rodent or bird damage. An incredibly broad and diverse field, all biodeterioration has as a common theme that it affects materials and substances that we need and value, and that it can largely be controlled by proper understanding of the materials and the possible spoilage organisms and mechanisms.

Biodeterioration is also specifically different from biodegradation in that the changes are 'undesirable'. Biodegradation occurs when complex materials are broken down by micro-organisms to form simple end-products. Within a biological ecosystem, there are micro-organisms that produce a host of enzymes that can biodegrade natural as well as some synthetic products; this is very important for maintaining the stability of the ecosystem and is extremely important for water purification and sewage treatment, and is widely used in the food industry. The main differences between biodeterioration and biodegradation are the undesirability and uncontrollability of the former

Chemical biodeterioration

There are two modes of chemical biodeterioration. Both have a similar result, i.e. the material becomes spoilt, damaged or unsafe, but the cause or biochemistry of the two is quite different:

1. Biochemical assimilatory biodeterioration – the organism uses the material as food i.e. an energy source.

2. Biochemical dissimilatory biodeterioration – the chemical change in the food is as a result of waste products from the organisms in question.

Physical biodeterioration

Mechanical biodeterioration – this occurs when the material is physically disrupted/damaged by the growth or activities of the organisms

Soiling/fouling – with this kind of biodeterioration the material or product is not necessarily unsafe, but as its appearance has been compromised, it is rendered unacceptable. The building up of biofilms on the surface of a material can affect the performance of that material.

Living organisms can be divided on the basis of their nutritional requirements into autotrophs and heterotrophs. Autotrophic organisms see all inorganic materials as a potential source of nutrients, while heterotrophic organisms can only use organic matter. The organisms responsible for biodeterioration of food are usually chemoheterotrophs, however it is important to realize that even the packaging that the food is stored in and the warehouses themselves, can be a source of nutrients for some micro-organisms, and it is therefore important to control the humidity, temperature and duration of storage of food, as far as possible.

Module 47: Types of Microorganisms involved in food spoilage:

Living organisms that can cause biodeterioration are referred to as *bio-deteriogens*.² Animals, insects and higher plants can be easily identified by visual observation and by examining their morphological and physiological characteristics. Organisms like bacteria, fungi and algae are less easy to identify and need to be isolated to be examined. Growth of these organisms under laboratory conditions is often difficult and specialized methods using fluorescent dyes and antibodies or examination using a scanning electron microscope must be used. In some instances, identification can only be made using DNA techniques.

Module 48: Bacterial food Spoilage

Bacteria are a large diverse group of microscopic, prokaryotic, unicellular organisms. They can be of various shapes (spherical, rod-like or spiral) and may be motile or non-motile. They include both autotrophic and heterotrophic species, and can be aerobic or anaerobic, and many species can thrive under either condition. They have relatively simple nutritional needs, and are easily adaptable and can readily change to suit their environment.

Food Spoiler bacteria classified on the basis of their activity or the end product / effect they produce after spoilage.

- Lipolysis bacteria
- Pigment former bacteria
- Gas former Bacteria

Lipolysis is the breakdown of fats and other lipids by hydrolysis to release Fatty Acids

Bacteria which cause lipolysis is called lipolysis bacteria e.g. Lactobacillus, leuconostoc

These bacteria make food containing fats rancid and food give off odour and smell and become spoil.

Sometimes discoloration of food occurs which lowers its aesthetic look and makes it unpleasant to consume this is done by Pigment former bacteria

Examples:-

1. Flavobacterium
2. Micrococcus
3. Serratia

Sometimes a canned product swells due to production of gas in it by gas former bacteria

e.g. Beverage can swells due to the activity of Clostridium Botulinum

Examples:-

1. Lactobacillus
2. Leuconostoc
3. Proteus

Despite of spoiling activities some bacteria have beneficial effects for use

Example:-

LAB (Lactic Acid Bacteria) produce lactic acid which is used as starter culture for the production of yoghurt from Milk

Proper care should be taken to prevent food spoiling bacteria to spoil food

Module 49: Fungal food Spoilage

Fungi are a large group of small chemoheterotrophic organisms. They do not contain chlorophyll and therefore cannot make their own food by using sunlight. They are, however extremely adaptable and can utilize almost any organic material. Their growth is characterized by unicellular or multicellular filamentous hyphae, which can often be the cause of physical biodeterioration. Moulds are filamentous fungi that rapidly grow in a mass that may cover several centimetres in a day.

Moulds multiply by means of ascospores, zygosporos or conidia. The ascospores of some moulds are particularly significant in food spoilage as they are heat resistant. Some moulds found associated with food spoilage are:

- *Botrytis* moulds, which cause grey mould rot: this condition affects many fruit and vegetable crops and products (e.g. grapes, strawberries, tomatoes, cauliflowers, pumpkins, cucumbers, sweet potatoes and many more). The fungus grows, causing the decay of the fruit or vegetable, and appears as a prominent grey mould.

- *Rhizopus stolonifer* moulds, which produce pectinases that causes soft rot, which makes vegetables soft and mushy.

- *Aspergillus* moulds, which have been implicated in the spoilage of a large number of foods including bacon, bread, peanuts, fish, etc. They produce mycotoxins.

- *Byssochlamys* moulds, which can cause spoilage in canned fruit as a result of their heat-resistant ascospores and the pectinases that they produce. They produce mycotoxins.

- *Fusarium* moulds, which have extensive mycelium that have tinges of brown, red, purple and pink. They cause brown rot in citrus fruit and on pineapples and can grow on cereal crops. They produce mycotoxins.

- *Penicillium* moulds, which typically spoil fruits causing blue and bluegreen coloration. They produce many different mycotoxins.

Module 50: Factors that effect Microbial growth

There are many complex reactions and conditions that either inhibit or encourage microbial growth. The availability of oxygen, the temperature (hot or cold), light and other radiation, moisture and dryness, the activity of natural enzymes and the amount of spoilage micro-organisms that are present will all affect the growth of spoilage organisms.

Factor affecting Microbial growth are:

- pH
- Moisture
- Humidity of environment
- Temperature
- Availability of Oxygen

Module 51: Effect of pH

Micro-organisms are sensitive of pH

Best growth at neutral pH 7

Below pH 4 only few micro-organism can grow

pH < 4.6 (no spore forming bacteria can grow)

Bacteria are more sensitive to pH as compared to Mould and yeast

Module 52: Effect of Moisture

Micro-organisms growth greatly influence by the availability of moisture. If there is high moisture in a food then there are great chances of microbial growth so proper knowledge of relationship between moisture is needed in order to control microbial growth.

Water Activity:-

Water activity (a_w) is a measure of the water that is available to micro-organisms

Pure water a_w (1.0)

Fresh fruits a_w (0.97)

Bacteria cannot grow at a_w (<0.91)

Yeast and moulds need less a_w to grow

Lowest mould growth reported at a_w at (0.61)

Classification of food on the basis of water content :-

1) Perishable Foods :- water content (80-95 %) e.g. Milk, Meat, Tomato

2) Semi Perishable Foods :- water content (60-80 %) e.g. Onion , Potato, Apple

3) Stable Foods :- water content (10-15%) e.g. Cereals

Module 53: Effect of Temperature

Storage temperature can be considered the most important factor that affects biodegradation 

of food; however, the relative humidity and availability of oxygen must also be controlled. Micro-organisms have been reported to grow over a wide temperature range; the lowest reported is -34°C and the highest is 90°C . All micro-organisms do, however, have an optimum temperature as well as a range in which they will grow. This preference for temperature forms the basis of dividing micro-organisms into groups.

- Psychrotrophs have an optimum from 20 to 30°C , but can grow at or below 7°C .

- Mesophiles have an optimum of 30 – 40°C , but can grow between 20 and 45°C .

- Thermophiles grow optimally between 55 and 65°C , but can grow at a temperature as low as 45°C .

Just as moulds are able to grow over a wide range of pH values and moisture conditions, they can also tolerate a wider temperature range than bacteria. Many moulds can grow in the refrigerator. Yeasts are not usually found growing in the thermophilic temperature range, but prefer psychrotrophic and mesophilic temperatures.

While lower storage temperature generally slows down microbial growth, it is not suitable to store all foods in the freezer (-18°C) or even the refrigerator ($<8^{\circ}\text{C}$), as they lose texture and other desirable features.

Lecture 7

FOOD BORNE DISEASE**Module 46: Food Borne Disease: Introduction**

While food is necessary for sustaining life, it could also be a cause of illness. There is a general misconception that if a food is 'natural', it must be 'safe'. Unfortunately the fact that many toxins occur in natural plant foods, falsifies this naïve view. Most of these endogenous toxins are in plant foods and a few in animal foods.

Module 47: Plant Toxins

Solanine of potatoes is one of the best known plant toxins. It is a steroid which occurs in potatoes and other members of solanaceae family (e.g., aubergine) and the highly poisonous nightshades. Normally potatoes contain 2–15 mg per 100 g (fresh weight). When potatoes are exposed to light and turn green, the level of solanine can be as high as 100 mg per 100 g. It is mostly concentrated under the skin. Potato sprouts may contain even higher amounts. Solanine can cause abdominal pain and diarrhoea, if ingested in large amounts. Solanine is an inhibitor of the enzyme acetyl choline esterase, which is a key component of the nervous system. Ingestion of solanine have been reported to lead to signs of neurological damage. As there is a general public awareness of the health hazards of eating green potatoes, the incidence of potato poisoning is low. Solanine is **not lost** during normal cooking as it is insoluble in water and is heat stable.

Caffeine is a purine alkaloid. **Theobromine** is another important member of this group. These occur in tea, coffee, cocoa and cola beverages, which are regarded as stimulants. But there are three good reasons for treating these as toxins. Firstly, these could never be regarded as nutrients, secondly they are addictive in nature and thirdly their physiological effects are dependent on the amount ingested.

Phenylethylamine found in chocolate brings on migraine headache in susceptible persons. Phenylethylamine and **serotonin** are vasopressor amines, which occur in plant foods, or are formed during fermentation in cheese and wine. Constriction of blood vessels, especially in brain is the usual effect of these amines. Hence there is a need to minimise the intake of these.

Hydrogen cyanide released from glycosides in foods can be toxic. When there is tissue damage of tapioca during harvest or preparation for cooking hydrogen cyanide is formed from amygdalin, a glucoside in tapioca. Therefore, tapioca varieties, rich in cyanogenic glucosides need to be fermented in preparation to release HCN as a volatile gas and thus make it safe for consumption, as is done in West Africa in preparation of “Garri”, a staple of that region. In spite of this, chronic cyanide poisoning occurs in tapioca eating persons, due to habitual low level intake of cyanides. One of the common diseases in such conditions can lead to neurological degeneration and a form of blindness. Lima beans also contain generous amounts of glycosides. It is difficult to cook lima beans so as to eliminate their toxicity; so bean varieties with low levels of glucosides are being produced by breeding. Besides cyanogens, legumes contain *inhibitors* of trypsin and chymotrypsin. Peas, beans, soybeans and groundnuts contain protease inhibitors (the proteases are trypsin and chymotrypsin). The inhibitors in most legumes are inactivated by cooking except in soybeans, which have to be autoclaved to achieve the same. Some plants when consumed can cause food poisoning. Certain varieties of mushrooms are very poisonous and could even be fatal if consumed. Snakeroot poisoning could result from drinking milk from cows that have fed on this weed.

Lectins or **haemoglutinins** present in kidney beans are also toxins. The name haemoglutinin indicates that these toxins bind to the surface of red blood cells and cause them to clump. These are destroyed during cooking. There is massive breakdown of lectins when beans are germinated.

The vogue of eating raw foods poses a potential hazard, especially if the diet consists of *only* raw foods. The lectins are toxic in the region of 0.5 mg/kg.

Myristicin, a toxin occurs in significant amounts in nutmeg and smaller amounts in black pepper, carrots and celery. 10 g of nutmeg contain enough myristicin to produce initial euphoria, hallucinations and narcosis, just like a heavy dose of alcohol. Since nutmeg is used in very small amounts as a flavouring only, it only induces sleep. But it is not advisable to eat nutmeg flavoured foods in pregnancy.

Module 48: Animal Toxins

Tetrodotoxin is a toxin, which occurs in the organs (liver, ovaries) of the puffer fish. The muscles and testes of puffer fish is a popular delicacy in Japan. As the minimum lethal dose

of tetrodotoxin lies between 1.5 and 4.0 mg. Great skill is needed by food handlers to separate the deadly parts of fish from edible ones. Expert cooks have to be strictly licensed, but fatalities occur regularly.

The toxin blocks movement of sodium across the membranes of nerve fibres, disrupting transmission of nerve impulses. A number of nervous symptoms develop leading to total paralysis and respiratory failure. Death occurs within 6 to 24 hours. No effective treatment has been found so far.

Other toxins find their way into shellfish such as mussels, cockles, clams and scallops due to a type of plankton, at certain times of the year. Some of these planktons are red coloured and proliferate in coastal waters. When these occur, these are called *red tides*. Hence, coastal communities have avoided fishing during red tides. But in cooler parts such as Alaska and Scandinavia, the planktons are not coloured. Hence, routine checks are carried out on toxin levels of shellfish in these regions. Sea food such as mussels and clams sometimes contain a poisonous alkaloid and could cause food poisoning symptoms.

A different type of poisoning occurs when fish from the family *Scrombridae* (including tuna, sardines, mackerel) are held above 10°C for some hours, due to formation of high levels of histamine from tryptophan in the muscle. This reaction is catalysed by the decarboxylase produced by bacteria present in the fish. High levels of histamine (100 mg/100 g) are produced before the putrefaction occurs. Susceptible persons suffer from headaches, palpitations, gastrointestinal upsets, skin flushes, and erythema. The symptoms are relieved by taking antihistamine drugs. The residents of the areas where such poisonous plants and animals are found usually know about the hazard and guard against it.

Mycotoxins

Toxins produced by moulds are known as *mycotoxins*. There are over 150 mould species, which produce toxins when grown on foods. Of these, two important ones are **ergot** and **aflatoxin**.

Ergotism is a disease known since the Middle Ages when it occurred in epidemic proportions in Europe due to infected rye which was used as a staple food. When rye and other cereals are infected by the mould *Claviceps purpurea* or *ergot*, at one stage in its life cycle, it produces hard, purplish black masses of dormant cells called *sclerotia*.

These have the same size as a cereal grain. If the sclerotia are not removed by sorting before milling, these are milled with the cereal into flour. The sclerotia contain 20 different toxic alkaloids, *ergotamine* being the most abundant one. The effects of these alkaloids on the body are not well understood, but depend on the kinds and amounts produced by the moulds present. Symptoms include burning pains in hands and feet, loss of sensation in the limbs, followed by gangrenous withering, blackening and loss of the limbs. Simultaneously, there is mental derangement and gastrointestinal failure. Finally the victim dies.

The disease is rare now due to reduction of infected plants with the use of fungicides, application of modern drying methods to prevent post harvest growth of microorganisms and the use of mechanical grain cleaning methods, which separate heavy grains from the light sclerotia formed.

Of the three genera *Fusarium*, *Penicillium* and *Aspergillus*, which infect foods, especially cereals, *Aspergillus* is the most dangerous one.

A. flavus produces aflatoxins, which are carcinogens. **Aflatoxin** B₁ is one of the most potent liver carcinogens known. Use of mouldy cereals containing 0.2 to 20 mg per kg aflatoxin have led to many fatalities in the tropics. The level of aflatoxin in staple foodstuffs has been found to be positively correlated to incidence of liver cancer.

Groundnuts and their products from the third world countries are potential sources of aflatoxin in the diet. When these contaminated feeds are used as cattle feed 1% of the aflatoxin is found in milk but not much comes through in the meat.

Module 49: Agricultural Residues

Residues of pesticides which are toxic may enter the food when used in agriculture for killing weeds, insects and microflora. **DDT** a pesticide used for killing mosquitoes has been found in many foods in proportions far exceeding the safety threshold. Some of the other pesticide residues found in food include **dieldrin**, **lindane** (**BHC**) and **malathion**, which are used for spraying on fruits and vegetables to control insects and microflora. Many of these pesticides have been banned. But their use for many years has left an appreciable amount in the soil, which find their way through the crops into human diet. A second source of residue are **sterols**, used for increasing growth in animals raised for meat. Residues of these sterols may be present in meat of these animals. Some of these sterols have been shown to be carcinogenic.

The final type of agricultural residue are **antibiotics**. For example, **penicillin** is used to treat cows with mastitis. Milk from treated cows must be discarded, but it may not be. The penicillin in milk can cause allergic reaction in sensitive persons. It will also interfere with manufacture of curd and cheese. **Antibiotics** are also used as growth promoters in animal feed. Animals fed antibiotic may develop resistant strains of bacteria, which may infect humans and cause untreatable disease. Therefore, the unscrupulous use of antibiotics in animals needs to be prohibited.

Module 50: Poisoning by Chemicals

Toxic metals may reach our food from a number of sources. The most important sources include water used in food processing and cooking, soil on poorly washed vegetables, water pipes (lead), equipment, containers and utensils used for processing, storage and cooking of food.

Lead the use of lead piping and tank in water supply can lead to a higher level of lead, if water is soft. Lead contamination of beverages is restricted to illicitly produced alcoholic drinks, as also poorly glazed pottery used to store acidic beverages. Use of lead-based solder can be a source of lead contamination in canning. As most foods are subject to regulation of lead content, the likelihood of lead poisoning is not very common.

However, symptoms of chronic lead poisoning such as anaemia, poor I.Q. performance etc. may be found in children from congested city environment, living in dwellings that have peeling, deteriorated lead surfaces. They may eat paint chips, breathe air-borne dust particles released from paint before remodeling. In addition, food and water contribute to daily lead intake. The lead pigment used to print labels on soft plastic food packaging can also be a source, especially as families reuse the bag for food storage.

The tetraethyl lead in exhaust fumes may be an additional source of lead. For example, studies in U.S.A. showed that absorption of lead in children is eight times greater than adults and tends to accumulate in their bodies. The brain-damaging effects of lead poisoning in young children is associated with anaemia, fatigue, poor attention span and learning ability. Hence, there is a need for a vigorous plan of prevention to eradicate this serious problem.

Mercury Inorganic mercury compounds reach our food from two sources – cereal seed grains treated with antifungal mercury compounds, meant for planting, being mistakenly

used as food and industrial pollution of coast water contaminating fish and other seafoods with alkyl mercury compounds.

The symptoms of mercury poisoning are variable but all point to damage to the central nervous system. In all situations, children, being vulnerable, are more sensitive to the exposure.

Arsenic is widely distributed in nature and may contaminate chemicals used in food processing and thus finds its way into food. For example, the use of sodium phosphate contaminated with arsenic trioxide as stabilizer in milk powder led to the poisoning of 12,000 infants in Japan, who were fed the formula made from that milk powder; at least 120 died.

Therefore, it is important to check for arsenic contamination in food additives.

Cadmium Contamination of foods from cadmium can occur from three sources. The first is use of contaminated water (e.g., water from mine operation as occurred in Japan in 1960) for the irrigation of paddy. Second is use of cadmium plated components in food processing machinery. The third is zinc plating or galvanizing of containers used to store acid foods. Usually zinc contains some cadmium, which may get dissolved with zinc and enter food products.

All these metal contaminants **lead**, **mercury**, **arsenic** and **cadmium** are bivalent, similar in some ways to nutrient mineral like iron, calcium and zinc. If the diet is low in bivalent nutrient metals (calcium, iron) the contaminants may enter the vulnerable systems (nerves, kidney, bone marrow), accumulate and disturb their function. There is a need to monitor the environmental contamination of foods by these metals.

Module 51: Poisoning by Microorganisms

There are two chief kinds of food poisoning caused by bacteria:

Botulism caused by the presence of toxin in the food produced by *Clostridium botulinum*

Staphylococcus poisoning caused by a toxin produced in the food by *Staphylococcus aureus*.

Botulism *Clostridium botulinum*, is a spore forming, anaerobic microorganism found in the soil. Foods such as corn, beans, peas, meat, fish are likely to be contaminated with the

spores of this organism. If these contaminated foods are not given adequate heat treatment during canning, these spores survive and the *Clostridium botulinum* multiplies in the can, as it is an anaerobic organism. During the process of growth, some strains of this organism produce a toxin in the food, which is a potent poison. If the food containing the toxin, is consumed without heating, it can cause paralysis of involuntary muscles and may ultimately lead to death due to respiratory failure. This effect is known as **botulism**. The toxin is, however, destroyed on heating. Any suspected food should, therefore, be boiled for at least 15 minutes before it is consumed. The incidence of botulism is, however, very rare as all commercially heated processed foods manufactured by reputable companies are always given approved heat treatment which is enough to kill pathogenic bacteria that may be present in the food. It is likely to occur if neutral foods are inadequately processed at home and consumed by families.

Staphylococcus Food Poisoning The causative organism of staphylococcus poisoning is *Staphylococcus aureus*. Staphylococcus contamination of the food may either be from human or animal sources. The nasal passage of many human beings, especially those with sinus infection contains many such *staphylococci*. Similarly, boils and infected wounds are potential sources. Cows affected by a disease called *mastitis* could discharge *staphylococci* into the milk. Environmental conditions such as temperature (37°C), presence of an abundance of protein and starch, are conducive to growth of *staphylococci*. Some strains of *Staphylococcus aureus* can produce an enterotoxin. Enterotoxin is a toxin produced by an organism outside the cell-wall and can thus be produced in the food even when the microorganism is living. In contrast, endotoxins are produced inside the cell-wall and can only permeate the food or the body when the organism is killed.

Foods containing **staphylococcal enterotoxin** when consumed can cause salivation, nausea, vomiting, abdominal cramps and diarrhoea. Recovery takes about a day or two and mortality is very low. The time between the consumption of the food and the appearance of symptoms can range from 1–6 hours.

Precautions should thus be taken to prevent entry of *staphylococci* into foods if such food poisoning is to be avoided. Employees suffering from *staphylococcal* infections such as colds, boils should not be allowed to handle foods. Refrigeration of foods immediately after preparation prevents growth and formation of enterotoxin by *staphylococci*. Pasteurisation kills all the *staphylococci* that may be present in foods.

Module 52: Food Infections

In contrast to food poisoning, food infection can be caused by organisms growing in food in large numbers. One such organism which has been known to cause symptoms very similar to the *staphylococcal* poisoning seen earlier is the *salmonella bacillus*. The various species of *Salmonellae* vary in their degree of infectiveness. Some like *Salmonellae enteritidis* can cause infection when a few organisms (about a million or so) are consumed whereas with other less infective species such as *Salmonellae pullorum* hundreds of millions of the organism would have to be ingested before they can cause infection.

The incubation period of *salmonella* infections is longer than that for staphylococcal poisoning, the period being usually between 12 and 24 hours.

The symptoms observed in **salmonellosis** are nausea, vomiting, abdominal pain and diarrhoea. The recovery is usually uneventful even though it may take two to three days. Some of the persons attacked by the organism after being cured become carriers of the microorganism.

People can be prevented from being infected by the disease by: (i) avoiding foods which are contaminated by the organism through diseased human beings and animals, e.g., eggs, which are broken in transit are also prone to contamination by the organism (ii) by preventing the growth of the organism by adequate refrigeration after the food has been prepared, and (iii) by pasteurising the food at such temperatures and times to kill all the pathogens present.

Other Infections: Besides salmonellae, other organisms such as the *streptococci* can also cause infections by growing in sufficient numbers in the food.

Module 53: Sequence of Events in Food borne Disease

For a foodborne disease to occur, several events have to happen in sequence. An understanding of these sequences is helpful in investigating the cause (the source and means of transmission) of a foodborne disease. It also helps in recognizing how the sequence can be broken in order to stop a foodborne disease. Initially, there has to be a source of a pathogen. Next, the pathogen has to contaminate a food. Consumption of the food contaminated with a pathogenic virus or parasites may lead to viral or parasitic infection. For bacterial pathogens (and toxicogenic molds) the contaminated food has to support

growth and be exposed for a certain period of time at a suitable temperature to enable the pathogens to grow. However for some potent pathogens (such as *Esc. coli* O157:H7) growth may not be necessary to cause a foodborne infection. For intoxication, the growth should reach a sufficient level to produce enough toxins so that when the food is consumed, the individual develops the symptoms. For bacterial infection, viable cells of a pathogen need to be consumed in sufficient numbers, which vary greatly with pathogens, to survive stomach acidity, establish in the digestive tract, and cause illness. In case of toxicoinfection, viable cells should be consumed either in very high numbers (for those that cannot multiply in the digestive tract, such as *Clo. peifringens*) or in reasonable numbers (for those that can multiply in the digestive tract, such as *Vibrio cholerae*) so that toxins released by them in the digestive tract can produce the symptoms.

Module 54: Detection of E.Coli in Drinking Water

1. Application

The Most Probable Number (MPN) method is applicable to the enumeration of coliforms, faecal coliforms and aerogenic *Escherichia coli* in foods, food ingredients and water, including contact water from food manufacturing plants.

Note: This method is **not** intended to be used to isolate and enumerate *E. coli* serotypes associated with human illness, particularly the enterohemorrhagic serotype O157:H7. Many of the pathogenic serotypes do not give a positive faecal coliform reaction and therefore would not be detected and recovered by this method. 

2. Description

The MPN procedure involves a multiple tube fermentation technique where three or more decimal dilutions of the sample are inoculated into tubes of broth medium and incubated at a specific temperature and for a specific time. The method is progressive; i.e., first determining the presence of coliforms in the tubes, then determining if these tubes also contain faecal coliforms, and then confirming whether *E. coli* is present. Based on the number of tubes indicating the presence / absence of the three groups of organisms, the most probable number present can be estimated from a standard statistical MPN table. The method has been shown to produce satisfactory results with naturally-contaminated foods and water for the detection of coliforms, faecal coliforms and aerogenic *E. coli*.

3. Principle

The terms “coliform” and “faecal coliform” have no taxonomic validity and, therefore, are only meaningful when expressed in terms of the analytical test parameters of medium, time and temperature of incubation.

Coliforms, faecal coliforms, and *E. coli* are considered “indicator organisms.”

The presence of “indicator organisms” in foods processed for safety may indicate one of the following possibilities: 1. inadequate processing and/or post-processing contamination; and/or 2. microbial growth. The presence of faecal coliforms and *E. coli* may indicate faecal contamination; however, it must be understood that these microorganisms can survive and multiply in a variety of non-intestinal environments, including the processing plant. When assessing the presence of “indicator organisms” in a sample, **one must assess the results against the tolerance limits specified by government standards or guidelines, health agencies, or a laboratory’s in-house specifications,** keeping in mind that established standards and guidelines are specifically linked to the method used to develop these standards.

The presence of coliforms, faecal coliforms and aerogenic *E. coli* in food and water may be determined by means of the MPN procedure. Briefly, this method involves serially diluting out the target organisms in the sample, in 5-replicate aliquots, to extinction. The probable level of the target organisms is then statistically estimated from an MPN table.

Gas production is used as an indication of ability to ferment lactose from LST broth (presumptive coliform test); gas production from BGLB broth is considered confirmation of coliform presence; gas production at 44.5 or 45° C from EC broth is used as confirmation of faecal coliform presence; and appearance of typical nucleated, dark-centred colonies with or without metallic sheen when positive EC broths are streaked onto L-EMB agar are indicative of *E. coli*. The typical colonies on L-EMB agar must be confirmed by further biochemical tests to prove the presence of *E. coli*.

4. Materials and special equipment

The media listed below (1 to 8) are commercially available and are to be prepared and sterilized according to the manufacturer's instructions.

Note: If the analyst uses any variations of the media listed here (either product that is commercially available or made from scratch), it is the responsibility of the analyst or Laboratory Supervisor to ensure equivalency. 

- 1) Peptone Water (0.1% and 0.5%)
- 2) Aqueous Sodium Citrate (2.0%), tempered to 40-45°C
- 3) Lauryl Sulfate Tryptose (LST) broth
- 4) Brilliant Green Lactose 2% Bile (BGLB) broth
- 5) *Escherichia coli* (EC) broth or EC broth with MUG (4-methylumbelliferyl- β -D-glucuronide)
- 6) Levine's Eosin Methylene Blue (L-EMB) agar or Endo agar
- 7) MacConkey agar
- 8) Nutrient Agar (NA) or other non-selective agar
- 9) Covered water baths, with circulating system to maintain temperature of 44.5°C and 45°C. Water level should be above the medium in immersed tubes.

Thermometer, calibrated and traceable

Incubator, 35°C.

Stomacher, blender or equivalent.

Control cultures (use ATCC cultures or equivalent): positive control(s): *E. coli* that is known to produce gas at 44.5 / 45° C and is capable of fermenting lactose to produce typical reactions on L-EMB agar; if using EC-MUG, a strain that is known to produce β -glucuronidase EMB / IMViC negative control: *Enterobacter aerogenes* or an equivalent gram negative rod that does not produce “positive” reactions on EMB and is indole-negative, methyl red-negative, Voges-Proskauer-positive, and citrate positive. MPN broths negative control: *Salmonella berta* or an equivalent gram negative rod that is gas-negative in MPN broths and in the secondary EC broth

NOTE: Some strains of *E. aerogenes* will give false-positive reactions in the MPN broths (LST, BGLB and EC broths) by producing a small gas bubble. Therefore, use *S. berta* or an

equivalent culture for these broths and *E. aerogenes* or an equivalent culture for EMB agar and IMViC tests.

1 pH meter capable of distinguishing to 0.1 pH units within the range of pH 5.0 to 8.0 or pH paper capable of distinguishing from 0.3 to 0.5 pH units, within the same range.

Supplies needed for confirmation (commercially available): The following supplies may be needed for confirmation; use A or B. The choice of further identification schemes may require alternate media A. IMViC media and reagents:

2 Tryptone (or tryptophane) broth Indole reagents (available commercially)

3 Buffered Glucose broth Voges-Proskauer test reagents (available commercially) Methyl red solution

4 Simmon's Citrate (SC) agar

B. Rapid Identification Kits or Systems (such as API, Vitek or equivalent)

5. Procedure

Each sample unit may be analyzed individually or the analytical units may be combined where requirements of the applicable sampling plan can be met. Carry out the test in accordance with the following instructions:

Handling of Sample Units

In the laboratory prior to analysis, except for shelf-stable foods, keep sample units refrigerated (0-5°C) or frozen, depending on the nature of the product. Thaw frozen samples in a refrigerator, or under time and temperature conditions which prevent microbial growth or death.

Analyze sample units as soon as possible after their receipt in the laboratory. Shellfish must be analyzed within 24 hours of collection.

Preparation for Analysis

Have ready sterile peptone water.

Clean the surface of the working area with a suitable disinfectant.

Arrange LST broth tubes in rows of five and mark them identifying the sample unit and the dilution to be inoculated.

Preparation of Sample, Initial Set-up and Reporting- Raw and Processed Shellfish

For all shellfish, always use 0.5% peptone water for all dilutions.

Include only live animals in the sample for unfrozen shellfish. Select 10 or more animals to obtain a minimum of 200 g of meat and liquor.

Scrape off all extraneous growth and loose material from the shell and scrub the shellfish (including the crevices at the juncture of the shells) with a sterile stiff brush under running water of potable quality. Do not use faucets equipped with aerators. Drain shellfish in a clean container or on clean towels.

Disinfect hands (soap and water, rinse with potable water then rinse with 70% alcohol) or gloves (dipped in iodophore solution or other suitable disinfectant then rinsed with potable water) prior to shucking shellfish. Alternatively, use disposable gloves disinfected with 70% alcohol. A protective mail glove may be worn under the disposable glove to prevent accidental injury. Using a sterile shucking knife, open the shellfish through the bill, not hinge, and collect meats and liquor into a sterile container.

Weigh at least 200 g of shellfish and liquor into a tared blender jar and add an equal amount of 0.5% peptone water. Blend for 1 - 2 minutes. Blended homogenate represents a 1 in 2 dilution.

To obtain a 1 in 10 dilution, add 20 g of the homogenate to 80 g of peptone water and shake. Shake dilutions 25 times through a 1-foot (30 cm) arc in approximately 7 seconds.

Prepare succeeding decimal dilutions as required using a separate sterile pipette for making each transfer.

Shake all dilutions immediately prior to making transfers to ensure uniform distribution of the microorganisms present.

Immediately (i.e., within 2 minutes after blending) prepare the dilutions from the ground sample and then proceed to inoculate into tubes. Inoculate each of separate sets of five tubes of LST broth with each dilution to be tested, according to the scheme in as follows:

Inoculate shellfish samples into LST: 10 mL of a 1 in 10 dilution into each of 5 tubes of double strength LST, 1 mL of 1 in 10 dilution into each of 5 tubes of single strength LST, and 1 mL of 1 in 100 dilution to each of 5 tubes of single strength LST.

Follow incubation of LST and confirmation steps for coliforms, faecal coliforms and *E. coli* as required, and record results as MPN per 100 g of shellfish.

Preparation of Sample, Initial Set-up and Reporting - Water

Inoculate each of separate sets of five tubes of LST broth with each dilution to be tested, as follows.

Inoculate each of the five tubes of 10 mL double strength LST broth (first row) with 10 mL of the undiluted water sample. Inoculate each of the five tubes of 10 mL single strength LST broth (second row) with 1 mL undiluted water. Inoculate each of the five tubes of 10 mL single strength LST broth (third row) with 0.1 mL of undiluted water.

Follow incubation of LST and confirmation steps for coliforms, faecal coliforms and *E. coli* as required, and record results as MPN per 100 mL of water

Preparation of Sample, Initial Set-up and Reporting - All other commodities

To ensure a truly representative analytical unit, agitate liquids or free flowing materials until the contents are homogeneous. If the sample unit is a solid, obtain the analytical unit by taking a portion from several locations within the sample unit. To reduce the workload, the analytical units may be combined for analysis. It is recommended that a composite contain not more than 500 g.

Prepare a 1 in 10 dilution of the food by aseptically blending 11 (10) g or mL (the analytical unit) into 99 (90) mL of the required diluent, as indicated in Tables I and II. If five sub-samples are composited for analysis, aseptically blend 50 g or mL into 450 mL of the required diluent.

For fish products an alternative method may be used. Weigh 100 g fish products and add 300 mL of 0.1% peptone water. Blend for 2 minutes. Blended homogenate represents a 1 in 4 dilution. Weigh 40 g of homogenate into 60 mL of 0.1 % peptone to obtain a 1 in 10 dilution. Pipette into LST as in 5.3.9 and express results as MPN/100g.

With products that require blending, blend or stomach for the minimum time required to produce a homogeneous suspension and to avoid overheating, blending time should not exceed 2.5 min. When blending foods that tend to foam, use blender at low speed and remove aliquot from below liquid/foam interface.

Check pH of the food suspension. If the pH is outside the range of 5.5-7.5, adjust pH to 7.0 with sterile 1N NaOH or 1N HCl.

Allow the food homogenate (1 in 10 dilution) of dry foods to stand at room temperature for 15 min. In all other instances, continue the analysis without this delay.

Prepare succeeding decimal dilutions as required using a separate sterile pipette for making each transfer. Shake dilutions 25 times through a 1-foot (30 cm) arc in approximately 7 seconds.

Shake all dilutions immediately prior to making transfers to ensure uniform distribution of the microorganisms present.

Inoculate each of separate sets of five tubes of LST broth with each dilution to be tested.

Inoculate each of the five tubes of 10 mL single strength LST broth with 1 mL of the 10^{-1} dilution. Inoculate each of the five tubes of succeeding rows of single strength LST with 1 mL additional dilutions.

Follow incubation of LST and confirmation steps for coliforms, faecal coliforms and *E. coli* as required. Compute MPN per g (mL) of food (per 100 g of shellfish or fish products or per 100mL of water) convert the number of gas-positive tubes to MPN values.

Incubation of LST

In order to verify growth conditions in the elevated temperature water baths, inoculate one LST broth tube with the MPN broths positive control and one LST broth tube with the MPN negative control, for each bath used. Transfer into all media used at different stages of the procedure. Set up an uninoculated tube of medium corresponding to each step in the procedure as a media control.

Mix inoculum and medium by gently shaking or rotating the tubes, but avoid entrapping air in the gas vials.

FOOD PRESERVATION

Module 63: Food preservation methods

Food preservation can be defined as the science, which deals with the prevention of decay or spoilage of food, thus allowing it to be stored in a fit condition for future use. The process used varies with the length of storage intended. It may be as simple as boiling milk so that it may keep for 24 hours or pickling of mango or lemon where the intended period of storage may be as long as a year.

Importance of Food Preservation

Food supply has to keep pace with the needs of the population. There is always a shortage of food in developing countries because of the demands of the increasing population. Increasing food production to meet this shortage results in wastage due to inadequate facilities available for storage and preservation. It is therefore, important to improve and expand facilities for the storage and preservation of food. Preservation of food helps in:

- increasing the shelf-life of foods thus increasing the supply.
- making the seasonal food available throughout the year.
- adding variety to the diet.
- saving time by reducing preparation time and energy.

Preservation increases availability of foods, thus improving the nutrition of the people. Availability of seasonal foods throughout the year also helps in stabilizing prices of such foods.

Food Spoilage

Food spoils, due to deteriorative changes that occur in it, that make it inedible or harmful. Foods change from the time of harvest, catch or slaughter. These changes may result in making the foods unfit for human beings.

There are several causes of food spoilage. These are

1. Growth of microorganisms, which bring about undesirable changes.
2. Action of enzymes present in the food.

3. Oxidative reactions in the food.
4. Mechanical damage to the food (e.g., bruising of apples, bananas, mangoes, tomatoes).
5. Damage due to pests (e.g., insects and rodents).

Foods vary greatly in the length of time for which they can be held in their natural form without spoilage. For purposes of food preservation, foods are classified as perishable, semi-perishable and non-perishable. Perishable foods such as milk, meat, sea foods and many fruits and vegetables begin to deteriorate almost immediately after harvest if not preserved. These foods have high moisture content and are highly susceptible to spoilage.

Module 64: Need for food preservation

Why Preservation?

Another important question is *why* food needs to be preserved. The main reasons for food preservation are to overcome inappropriate planning in agriculture, produce value-added products, and provide variation in diet [20]. The agricultural industry produces raw food materials in different sectors. Inadequate management or improper planning in agricultural production can be overcome by avoiding inappropriate areas, times, and amounts of raw food materials as well as by increasing storage life using simple methods of preservation. Value-added food products can give better-quality foods in terms of improved nutritional, functional, convenience, and sensory properties. Consumer demand for healthier and more convenient foods also affects the way food is preserved. Eating should be pleasurable to the consumer, and not boring. People like to eat wide varieties of foods with different tastes and flavors. Variation in the diet is important, particularly in underdeveloped countries to reduce reliance on a specific type of grain (i.e., rice or wheat). In food preservation, the important points that need to be considered are

- The desired level of quality
- The preservation length
- The group for whom the products are preserved

After storage of a preserved food for a certain period, one or more of its quality attributes may reach an undesirable state. Quality is an elusive ever-changing concept. In general, it is defined as the degree of fitness for use or the condition indicated by the satisfaction level of consumers. When food has deteriorated to such an extent that it is considered unsuitable for

consumption, it is said to have reached the end of its shelf life. In studying the shelf life of foods, it is important to measure the rate of change of a given quality attribute. In all cases, safety is the first attribute, followed by other quality. The product quality attributes can be quite varied, such as appearance, sensory, or microbial characteristics. Loss of quality is highly dependent on types of food and composition, formulation (for manufactured foods), packaging, and storage conditions. Quality loss can be minimized at any stage of food harvesting, processing, distribution, and storage. When preservation fails, the consequences range broadly from minor deterioration, such as color loss, to food becoming extremely hazardous

Module 65: Blanching

Blanching serves a variety of functions, one of the main ones being to destroy enzymic activity in vegetables and some fruits, prior to further processing. As such, it is not intended as a sole method of preservation but as a pre-treatment which is normally carried out between the preparation of the raw material and later operations (particularly heat sterilization, dehydration and freezing. Blanching is also combined with peeling and/or cleaning of food, to achieve savings in energy consumption, space and equipment costs.

A few processed vegetables, for example onions and green peppers, do not require blanching to prevent enzyme activity during storage, but the majority suffer considerable loss in quality if blanching is omitted or if they are under-blanching. To achieve adequate enzyme inactivation, food is heated rapidly to a pre-set temperature, held for a pre-set time and then cooled rapidly to near ambient temperatures. The factors which influence blanching time are:

1. type of fruit or vegetable
2. size of the pieces of food
3. blanching temperature
4. method of heating.

The theory of unsteady-state heat transfer by conduction and convection, which is used to calculate blanching time.

The maximum processing temperature in freezing and dehydration is insufficient to inactivate enzymes. If the food is not blanched, undesirable changes in sensory characteristics and nutritional properties take place during storage. In canning, the time

taken to reach sterilising temperatures, particularly in large cans, may be sufficient to allow enzyme activity to take place. It is therefore necessary to blanch foods prior to these preservation operations. Under-blanching may cause more damage to food than the absence of blanching does, because heat, which is sufficient to disrupt tissues and release enzymes, but not inactivate them, causes accelerated damage by mixing the enzymes and substrates. In addition, only some enzymes may be destroyed which causes increased activity of others and accelerated deterioration.

The heat resistance of enzymes is characterised by D and z values. Enzymes which cause a loss of eating and nutritional qualities in vegetables and fruits include lipoxygenase, polyphenoloxidase, polygalacturonase and chlorophyllase. Two heat-resistant enzymes which are found in most vegetables are catalase and peroxidase. Although they do not cause deterioration during storage, they are used as marker enzymes to determine the success of blanching. Peroxidase is the more heat resistant of the two, so the absence of residual peroxidase activity would indicate that other less heat-resistant enzymes are also destroyed. The factors that control the rate of heating at the centre of the product can be summarised as:

- the temperature of the heating medium
- the convective heat transfer coefficient
- the size and shape of the pieces of food
- the thermal conductivity of the food.

Blanching reduces the numbers of contaminating micro-organisms on the surface of foods and hence assists in subsequent preservation operations. This is particularly important in heat sterilisation, as the time and temperature of processing are designed to achieve a specified reduction in cell numbers. If blanching is inadequate, a larger number of micro-organisms are present initially and this may result in a larger number of spoiled containers after processing. Freezing and drying do not substantially reduce the number of micro-organisms in unblanched foods and these are able to grow on thawing or rehydration.

Blanching also softens vegetable tissues to facilitate filling into containers and removes air from intercellular spaces which increases the density of food and assists in the formation of a head-space vacuum in cans

Module 66: Pasteurization

Pasteurisation is a relatively mild heat treatment, in which food is heated to below 100°C. In low acid foods (pH 4.5, for example milk) it is used to minimise possible health hazards from pathogenic micro-organisms and to extend the shelf life of foods for several days. In acidic foods (pH 4.5, for example bottled fruit) it is used to extend the shelf life for several months by destruction of spoilage micro-organisms (yeasts or moulds) and/or enzyme inactivation. In both types of food, minimal changes are caused to the sensory characteristics or nutritive value.

Processing containers of food, either which have a naturally low pH (for example fruit pieces) or in which the pH is artificially lowered (for example pickles) is similar to canning. It is often termed *pasteurisation* to indicate the mild heat treatment employed.

Module 67: Heat Sterilization

Heat sterilisation is the unit operation in which foods are heated at a sufficiently high temperature and for a sufficiently long time to destroy microbial and enzyme activity. As a result, sterilised foods have a shelf life in excess of six months at ambient temperatures. The severe heat treatment during the older process of in-container sterilisation (canning) may produce substantial changes in nutritional and sensory qualities of foods. Developments in processing technology therefore aim to reduce the damage to nutrients and sensory components, by either reducing the time of processing in containers or processing foods before packaging (aseptic processing).

In-container sterilization

- The length of time required to sterilize a food is influenced by:
- the heat resistance of micro-organisms or enzymes likely to be present in the food
- the heating conditions
- the pH of the food
- the size of the container
- the physical state of the food.

In order to determine the process time for a given food, it is necessary to have information about both the heat resistance of micro-organisms, particularly heat resistant spores, or enzymes that are likely to be present and the rate of heat penetration into the food.

Module 68: UHT Process

Ultra-high temperature processing (UHT), ultra-heat treatment, or ultra-pasteurization is a food processing technology that sterilizes liquid food, chiefly milk, by heating it above 135 °C (275 °F) the temperature required to kill spores in milk – for 1 to 2 seconds.

UHT process would heat the product instantly to the required temperature, hold it at that temperature to achieve sterility and cool it instantly to filling temperature.

Module 69: Effect of Heating on foods:

Effect on Colour:

- In meats the red oxymyoglobin pigment is converted to brown metmyoglobin, and purplish myoglobin is converted to red–brown myohaemichromogen
- Maillard browning and caramelisation also contribute to the colour of sterilised meats.
- Chlorophyll is converted to pheophytin and anthocyanins are degraded to brown pigments.
- Iron or tin react with anthocyanins to form a purple pigment, or when colourless leucoanthocyanins form pink anthocyanin in pears and quinces.
- In sterilised milk slight colour changes are due to caramelisation, Maillard browning and changes in the reflectivity of casein micelles.

Flavor and Aroma

- In canned meats there are complex changes, like pyrolysis, deamination and decarboxylation of amino acids, degradation, Maillard reactions and caramelisation of carbohydrates to furfural and hydroxymethylfurfural, and oxidation and decarboxylation of lipids.

- In fruits and vegetables, changes are due to complex reactions which involve the degradation, recombination and volatilisation of aldehydes, ketones, sugars, lactones, amino acids and organic acids
- Interactions between these components produce more than 600 flavour compounds in ten chemical classes.
- In milk the development of a cooked flavour is due to denaturation of whey proteins to form hydrogen sulphide and the formation of lactones and methyl ketones from lipids.
- In aseptically sterilised foods the changes are better retained.

Texture or Viscosity

- In canned meats, changes in texture are caused by coagulation and a loss of water holding capacity of proteins, which produces shrinkage and stiffening of muscle tissues.
- Softening is caused by hydrolysis of collagen, solubilisation of the resulting gelatin, and melting and dispersion of fats through the product.
- In fruits and vegetables, softening is caused by hydrolysis of pectic materials, gelatinisation of starches and partial solubilisation of hemicelluloses.

Nutritional Value

- Canning causes the hydrolysis of carbohydrates and lipid but the nutritional value is not affected.
- Proteins are coagulated and, in canned meats, losses of amino acids are 10–20%.
- Negligible vitamin losses in aseptically processed milk and lipids, carbohydrates and minerals are virtually unaffected..

Module 70: Evaporation

In common with other unit operations that are intended to separate components of foods, evaporation and distillation aim to separate specific components to increase the value of the food. In both types of operation, separation is achieved by exploiting differences in the

vapor pressure (volatility) of the components and using heat to remove one or more from the bulk of the food.

Evaporation

Evaporation, or concentration by boiling, is the partial removal of water from liquid foods by boiling off water vapour. It increases the solids content of a food and hence preserves it by a reduction in water activity. Evaporation is used to pre-concentrate foods (for example fruit juice, milk and coffee) prior to drying, freezing or sterilization and hence to reduce their weight and volume. This saves energy in subsequent operations and reduces storage, transport and distribution costs. There is also greater convenience for the consumer (for example fruit drinks for dilution, concentrated soups, tomato or garlic pastes, sugar) or for the manufacturer (for example liquid pectin, fruit concentrates for use in ice cream or baked goods). Changes to food quality that result from the relatively severe heat treatment are minimised by the design and operation of the equipment. Evaporation is more expensive in energy consumption than other methods of concentration (membrane concentration and freeze concentration but a higher degree of concentration can be achieved).

Theory

During evaporation, sensible heat is transferred from steam to the food, to raise the temperature to its boiling point. Latent heat of vaporisation is then supplied by the steam to form bubbles of vapour, which leave the surface of the boiling liquid. The rate of evaporation is determined by both the rate of heat transfer into the food and the rate of

Module 71: Distillation

Although common in the chemical industry, distillation in food processing is mostly confined to the production of alcoholic spirits and separation of volatile flavour and aroma compounds (for example, production of essential oils by steam distillation). When a food  that contains components having different degrees of volatility is heated, those that have a higher vapour pressure (more volatile components) are separated first. These are termed the 'distillate' and components that have a lower volatility are termed 'bottoms' or residues. Although batch distillation (in 'pot stills') remains in use in some whisky and other spirit distilleries, most industrial distillation operations use more economical continuous distillation columns. Feed liquor flows continuously through the column and as it is heated, volatiles are produced and separated at the top of the column as distillate and the residue is

separated at the base. In order to enhance the separation of these components and equilibrium conditions between the liquid and vapour phases, a proportion of the distillate is added back to the top of the column (reflux) and a portion of the bottoms is vapourised in a reboiler and added to the bottom of the column. Columns are filled with either a packing material (typically ceramic, plastic or metal rings) or fitted with perforated trays, both of which increase the contact between liquid and vapour phases. A more recent development is the use of a 'spinning cone column' to remove volatile components from liquids. It is used to recover flavours from beer, coffee, tomato products and fruit juices, to produce low-alcohol wines and beers and to remove off-flavours. The equipment consists of a column containing a series of rotating inverted cones, which are intermeshed with stationary cones attached to the column wall. Steam or nitrogen is supplied to the base of the column and the feed liquor enters at the top. Thin turbulent films are produced over the large surface area of the cones and rapid separation takes place. The gas passes out of the top of the column and volatile aroma compounds are condensed and collected. Because separation is achieved by mechanical energy, rather than heat, there is less damage to flavours and lower energy consumption. The equipment is also considerably smaller than a packed column having an equivalent throughput.

Module 72: Dehydration

Dehydration (or drying) is defined as 'the application of heat under controlled conditions to remove the majority of the water normally present in a food by evaporation' or in the case of freeze drying by sublimation). This definition excludes other unit operations which remove water from foods (for example mechanical separations and membrane concentration, evaporation and baking as these normally remove much less water than dehydration).

The main purpose of dehydration is to extend the shelf life of foods by a reduction in water activity. This inhibits microbial growth and enzyme activity, but the processing temperature is usually insufficient to cause their inactivation. Therefore any increase in moisture content during storage, for example due to faulty packaging, will result in rapid spoilage. The reduction in weight and bulk of food reduces transport and storage costs. For some types of food, dehydration provides a convenient product for the consumer or more easily handled ingredients for food processors. Drying causes deterioration of both the eating quality and the nutritional value of the food. The design and operation of dehydration equipment aim to minimise these changes by selection of appropriate drying conditions for individual foods.

Examples of commercially important dried foods are coffee, milk, raisins, sultanas and other fruits, pasta, flours (including bakery mixes), beans, pulses, nuts, breakfast cereals, tea and spices. Examples of important dried ingredients that are used by manufacturers include egg powder, flavourings and colourings, lactose, sucrose or fructose powder, enzymes and yeasts.

Dehydration involves the simultaneous application of heat and removal of moisture from foods. Except for osmotic dehydration, in which foods are soaked in concentrated solutions of sugar or salt to remove water using the difference in osmotic pressure as the driving force for moisture transfer. This method is used to produce 'crystallised' or sugared fruits and with salt it is used in some countries as a pre-treatment for fish and vegetables before drying.

Module 73: Use of Low Temperature

Chilling:

Chilling is the unit operation in which the temperature of a food is reduced to between -1°C and 8°C . It is used to reduce the rate of biochemical and microbiological changes, and hence to extend the shelf life of fresh and processed foods. It causes minimal changes to sensory characteristics and nutritional properties of foods and, as a result, chilled foods are perceived by consumers as being convenient, easy to prepare, high quality and 'healthy', 'natural' and 'fresh'.

Chilling is often used in combination with other unit operations (for example fermentation or pasteurisation) to extend the shelf life of mildly processed foods. There is a greater preservative effect when chilling is combined with control of the composition of the storage atmosphere than that found using either unit operation alone.

FRESH FOODS

Chilling reduces the rate of enzymic and microbiological change and retards respiration of fresh foods. The factors that control the shelf life of fresh crops in chill storage include:

- the type of food and variety or cultivar
- the part of the crop selected (the fastest growing parts have the highest metabolic rates)

- and the shortest storage lives.
- the condition of the food at harvest.
- the temperature of harvest, storage, distribution and retail display
- the relative humidity of the storage atmosphere, which influences dehydration losses.

Mechanical Refrigerators:

Mechanical refrigerators have four basic elements:

- an evaporator,
- a compressor,
- a condenser and
- an expansion valve

constructed from copper as the low thermal conductivity allows high rates of heat transfer and high thermal efficiencies.

Cryogenic Refrigerators

- A cryogen is a refrigerant that changes phase by absorbing latent heat to cool the food
- Cryogenic chillers use solid carbon dioxide, liquid carbon dioxide or liquid nitrogen.
- Solid carbon dioxide removes latent heat of sublimation and liquid cryogen remove latent heat of vaporization.

Control of Storage Conditions

- In all stores it is important to maintain an adequate circulation of air using fans, to control the temperature, relative humidity or atmospheric composition.
- Foods are therefore stacked in ways that enable air to circulate freely around all sides.

Module 74: Use of food Preservatives

The practice of preserving food by the addition of chemical is quite old, ordinary table salt (sodium chloride) having been used as a preservative for centuries. It might be surprising to think of a naturally occurring substance as a chemical preservative, but many chemical substances used in the preservation of foods occur naturally. When they are used with the proper intent, they can be used to preserve foods that cannot be easily preserved by other means. They should not be used as a substitute for sanitation and proper handling procedures. Sometimes chemicals are used together with other processes, such as holding at refrigerator temperatures above freezing. To preserve food, it is necessary either to destroy all of the spoilage microorganisms that contaminate it or to create and maintain conditions that prevent the microbes from carrying out their ordinary life processes. Although preservation is aimed mainly at microbial spoilage, it must be remembered that there are other types of spoilage factors, such as oxidation.

Although foods can be sterilized (such as by heat processing) and contained in such a way as to prevent contamination by microbes during storage, it still is often necessary in some cases to forego sterilization, thus making it necessary to take other steps to prevent microbial degradation of the food. Foods can be protected against microbial attack for long periods (months to years) by holding them at temperatures below freezing. They can be preserved for shorter periods (several days) by holding them in ice or in a refrigerator at temperatures in the range 32 to 40°F (0 to 7.8°C). Foods can also be preserved by altering them to make them incapable of supporting microbial growth. Drying is an example of this type of preservation. Foods must also be preserved against color and texture changes. Quite often it is either impossible or undesirable to employ conventional preservation methods, and a large variety of food additives is available for use, alone or in combination with other additives or with mild forms of conventional processes, to preserve foods. Usually, chemical preservatives are used in concentrations of 0.1% or less. Sodium diacetate and sodium or calcium propionate are used in breads to prevent mold growth and the development of bacteria that may produce a slimy material known as rope. Sorbic acid and its salts may be used in bakery products, cheeses, syrups, and pie fillings to prevent mold growth. Sulfur dioxide is used to prevent browning in certain dried fruits and to prevent wild yeast growth in wines used to make vinegar. Benzoic acid and sodium benzoate may be used to inhibit mold and bacterial growth in some fruit juices, oleomargarines, pickles, and condiments. It should also be noted that benzoic acid is a natural component of cranberries.

Salt is an excellent microbe inhibitor, mainly as a result of its suppression of the water activity of the material to which it is added. Its effectiveness is enhanced when the food is also dried or smoked or both. Smoking also imparts a partial preservative effect. Weak acids, such as sorbic acid, or salts of weak acids, benzoates, propionates, nitrites, certain chelating agents (chemicals that tie up metals and prevent the catalytic action of metals), and other chemical additives are effective preservatives. Natural spices also have antimicrobial properties. Antibiotics have been used as food additives and are still used to preserve animal feeds and human foods in some countries. Their use in human foods is banned in the United States and in some other countries.

Because many antimicrobial agents are generally toxic to humans, their use must be regulated not to exceed established levels beyond which they are hazardous to human health.

Module 75: Use of High Pressure and Ultrasound

When high pressures, up to 1000 MPa (10 000 bar), are applied to packages of food that are submerged in a liquid, the pressure is distributed instantly and uniformly throughout the food (i.e. it is 'isostatic'). The high pressure causes destruction of micro-organisms. In general, bacteria in the log phase of growth are more barosensitive (sensitive to high pressures) than cells in the stationary, dormant or death phases. Moderately high pressures (300–600 MPa) cause vegetative microbial cells to be killed or inactivated. Typically, a pressure of 350 MPa applied for 30 min or 400 MPa applied for 5 min will cause a ten-fold reduction in vegetative cells of bacteria, yeasts or moulds.

Processing by Ultrasounds:

Ultrasound waves are similar to sound waves but have a frequency that is above 16 kHz and cannot be detected by the human ear. In nature, bats and dolphins use low intensity ultrasound to locate prey, and some marine animals use high-intensity pulses of ultrasound to stun their prey.

When ultrasonic waves hit the surface of a material, they generate a force. If the force is perpendicular to the surface, it results in a compression wave that moves through the food, whereas if the force is parallel to the surface it produces a shearing wave. Both types of wave become attenuated as they move through the food. Ultrasound produces very rapid localised changes in pressure and temperature that cause shear disruption, 'cavitation'

(creation of bubbles in liquid foods), thinning of cell membranes, localised heating and free radical production, which have a lethal effect on micro-organisms.

Application to processing

The shearing and compression effects of ultrasound cause denaturation of proteins that result in reduced enzyme activity, although short bursts of ultrasound may increase enzyme activity, possibly by breaking down large molecular structures and making the enzymes more accessible for reactions with substrates. The effects of ultrasound on meat proteins produce tenderisation in meat tissues after prolonged exposure, and the release of myofibrillar proteins that in meat products result in improved water binding capacity, tenderness and cohesiveness.

Module 76: Electricity, radiation and Microwaves

Dielectric (microwave and radio frequency) energy and infrared (or radiant) energy are two forms of electromagnetic energy. They are both transmitted as waves, which penetrate food and are then absorbed and converted to heat. In contrast, ohmic (or resistance) heating uses the electrical resistance of foods to directly convert electricity to heat. Foods can be heated by either direct or indirect methods: dielectric and ohmic heating are direct methods in which heat is generated within the product, whereas infrared heating is an indirect method that relies on heat that is generated externally being applied to the surface of the food mostly by radiation, but also by convection and to a lesser extent, conduction. The main differences between dielectric, ohmic and infrared energy can be summarised as follows:

1. Dielectric energy induces molecular friction in water molecules to produce heat, whereas ohmic heating is due to the electrical resistance of a food and infrared energy is simply absorbed and converted to heat.
2. Dielectric heating is determined in part by the moisture content of the food, whereas the extent of heating by radiant energy depends on the surface characteristics and colour of the food and ohmic heating depends on the electrical resistance of the food.
3. Dielectric and ohmic heating are used to preserve foods, whereas infrared radiation is mostly used to alter the eating qualities by changing the surface colour, flavour and aroma.

4. Commercially, microwaves and radio frequency energy are produced at specified frequency bands that are allocated to prevent interference with radio transmissions, whereas radiant heat is less controlled and has a wider range of frequencies.

Ohmic heating uses mains frequency electricity.

5. The depth of penetration into a food is directly related to frequency; the lower frequency dielectric energy penetrates more deeply than radiant energy. In contrast, ohmic heating penetrates throughout the food instantly.

6. The thermal conductivity of the food is a limiting factor in infrared heating, whereas it is not so important in dielectric and ohmic heating.

Dielectric heating

The majority of foods contain a substantial proportion of water. The molecular structure of water consists of a negatively charged oxygen atom, separated from positively charged hydrogen atoms and this forms an electric dipole. When a microwave or radio frequency electric field is applied to a food, dipoles in the water and in some ionic components such as salt, attempt to orient themselves to the field (in a similar way to a compass in a magnetic field). Since the rapidly oscillating electric field changes from positive to negative and back again several million times per second, the dipoles attempt to follow and these rapid reversals create frictional heat. The increase in temperature of water molecules heats surrounding components of the food by conduction and/or convection.

Because of their widespread domestic use, some popular notions have arisen that microwaves ‘heat from the inside out’. What in fact occurs is that outer parts receive the same energy as inner parts, but the surface loses its heat faster to the surroundings by evaporative cooling. It is the distribution of water and salt within a food that has the major effect on the amount of heating (although differences also occur in the rate of heating as a result of the shape of the food, at its edges etc.).

Ohmic heating

Also termed ‘resistance heating’ or ‘electroheating’, this is a more recent development in which an alternating electric current is passed through a food, and the electrical resistance of the food causes the power to be translated directly into heat. As the food is an electrical

component of the heater, it is essential that its electrical properties (its resistance) are matched to the capacity of the heater.\

Module 77: Detection of yeast and mould in dairy and bakery products:

Enumeration of yeasts and moulds in foods

1. Application

This method is applicable to the enumeration of viable yeasts and moulds in foods and food ingredients. It may also be used to confirm the viability of apparent yeast and mould material scraped from food plant equipment and the manufacturing environment.

2. Principle

In the past, acidified media were used to enumerate yeasts and moulds in foods. Such media are now recognized as inferior to antibiotic supplemented media that are formulated to suppress bacterial colony development, enhance resuscitation of injured fungi, and minimize precipitation of food particles.

A medium, containing (a) adequate nutrients for growth of most yeasts and moulds and (b) antibiotics for inhibition of most bacteria, is inoculated with a given quantity of the product or with scrapings from equipment or the manufacturing environment. It is incubated at 22-25°C for 3-5 days. Colonies appearing on the medium are then counted and/or examined. The method described here is a "general purpose" method and may not be suitable for detection of yeasts and moulds adapted to certain foods, e.g., foods of very low water activity.

Defination of terms

Scrapings: Suspected yeast and mould material scraped from food plant equipment and the manufacturing environment.

Xerophilic: Moulds capable of growing at reduced water activity (a_w). (Yeasts preferring reduced a_w are also sometimes referred to as xerophilic.)

Osmophilic: Yeasts preferring reduced a_w for growth.

4. Materials and special equipment

The following media and reagents (1-8) are commercially available and are to be prepared and sterilized according to the manufacturer's instructions. and reference 7.3 for the formula of individual media.

Note: If the analyst uses any variations of the media listed here (either product that is commercially available or made from scratch), it is the responsibility of the analyst or Laboratory Supervisor to ensure equivalency.

Enumeration of yeasts and moulds in foods (not specified below)

These agars are suitable for foods where the aW is above 0.95, such as fresh foods (fruit, vegetables, meat and dairy).

- 1) Dichloran rose bengal chloramphenicol agar (DRBC)
- 2) Plate count agar with chloramphenicol (PCA-C)
- 3) Potato dextrose agar with chloramphenicol (PDA-C)
- 4) Potato dextrose salt agar with chloramphenicol (PDSA-C) (for analysis of 'spreader' moulds)

Enumeration of xerophilic yeasts and moulds in grains, flours, nuts, and spices

- 5) Dichloran-glycerol DG 18 agar (DG-18)

Enumeration of xerophilic yeasts and moulds in jams, jellies, fruit concentrates, and dried fruits

- 6) 20% sucrose (diluent additive for osmophiles, see 6.3.1)
- 7) Malt extract agar containing 50% (w/w) sucrose

Other:

- 8) Peptone water (0.1%) (PW)
- 9) 2% sodium citrate tempered to 45°C (diluent for high fat foods, such as cheese) (optional)
- 10) 1N HCl and 1N NaOH

11) Gram stain solutions

12) Stomacher, blender or equivalent

13) pH meter or paper capable of distinguishing to 0.3 to 0.5 pH units within a range of 5.0 to 8.0

14) Light microscope

15) Colony counting device (optional)

16) Incubator (darkened) capable of maintaining 22 to 25°C, 55°C waterbath (and 45°C waterbath if sodium citrate is to be used).

Procedure

Each sample unit shall be analyzed individually. The test shall be carried out in accordance with the following instructions:

Handling of Sample Units and Scrapings

- During storage and transport, the following shall apply: with the exception of shelf-stable products, keep the sample units refrigerated (0-5°C). Sample units of frozen products shall be kept frozen.
- Thaw frozen samples in a refrigerator or under time and temperature conditions which prevent microbial growth or death.
- Analyze the sample units as soon as possible after receipt at the laboratory.

Preparation of Medium

- Prepare the appropriate media for the analysis being carried out.

NOTE: DRBC agar should not be exposed to light, since photo-degradation of rose Bengal produces compounds that are toxic to fungi.

- Temper melted agar in a 55°C waterbath, ensuring that the water level is 1 cm above the level of the medium in the bottles.
- Clean surface of working area with a suitable disinfectant.
- Mark clearly the duplicate petri plates identifying sample, sample unit, dilution and date of inoculation.

Preparation of Dilutions

- Prepare 0.1% peptone water as diluent. An appropriate solute, such as 20%

sucrose, should be added to the diluent when enumerating osmophiles in foods such as syrups and fruit juice concentrates. In addition, a 2% solution of sodium citrate, pre-warmed to 45°C, can be used as diluent for high-fat foods such as cheese.

- To ensure a representative analytical portion, agitate liquid or free flowing materials until the contents are homogeneous. If the sample unit is a solid, obtain the analytical unit by taking a portion from several locations within the sample unit.

- Some degree of soaking may be beneficial for the recovery of yeasts and moulds from dried or intermediate-moisture foods. Soaking may allow for the repair of sub-lethally damaged cells (resuscitation). Rehydrate dried foods for 1 h with an equal amount of distilled water or peptone water and store at room temperature.

- Prepare a 1:10 dilution of the food by aseptically blending 25 g or mL (the analytical unit) into 225 mL of the required diluent. If a sample size other than 25 g or mL is used, maintain the 1:10 sample to dilution ratio, such as 11 (10) g or mL into 99 (90) mL.

NOTE: Weight or volume in brackets indicates alternate procedure for making dilutions. 

- Stomach, blend or shake according to the type of food.

Blend or stomach for the minimum time required to produce a homogeneous suspension. To prevent over-heating, blending time should not exceed 2.5 min. With foods that tend to foam, use blender at low speed and remove aliquot from below liquid/foam interface.

- Verify the pH of the suspension. If the pH is not between 5.5 and 7.5, adjust the pH to 7.0 with a sterile solution of 1N NaOH or 1N HCl.

- If the 1:10 dilution is prepared in a dilution bottle, it should be mixed by shaking the bottle 25 times through a 30 cm arc in approximately 7 sec.

- Prepare succeeding decimal dilutions as required, using a separate sterile pipette for making each transfer.
- Because mould propagules may settle out within a few minutes, it is important to shake all dilutions immediately prior to making transfers to ensure uniform distribution of the microorganisms present.

Plating

Agitate each dilution bottle to resuspend material that may have settled out during preparation.

Moulds should be enumerated by a surface spread-plate technique rather than with pour plates. This technique provides maximal exposure of the cells to atmospheric oxygen and avoids heat stress from molten agar. Agar spread plates should be dried overnight before being inoculated. Spread 0.1 mL onto duplicate plates

For determination of viability of suspected yeast and mould material from food plant equipment and the manufacturing environment, aseptically tease the scrapings apart and distribute the pieces over the surface of solidified medium.

Incubation

Incubate plates undisturbed in an upright position at 22 to 25°C for 3-5 days. Incubate plates in the dark. Normally, count colonies on plates after 5 days. Examine on the third day and if mould colonies are numerous, count them and then count again on the fifth day, if possible. Handle the plates as little as possible when counting on day 3 so spores will not be dislodged, which may result in secondary growth

Counting Colonies and Examining Growth

- Count colonies, distinguishing, if required, yeast colonies from mould colonies, according to their colonial morphology. Microscopic examination with crystal violet stained smears may be necessary to distinguish yeast colonies from some bacterial colonies that may look like yeast.
- If possible, select plates with 10-150 colonies. Determine the identity of pin-point colonies microscopically.

Lecture 9

FERMENTED FOODS**Module 79: Fermented Foods**

Currently, more than 3500 different fermented foods are consumed by humans worldwide; many are ethnic and produced in small quantities to meet the needs of a group in a particular region. Some are, at present, produced commercially, and only a few are produced by large commercial producers. Production by large producers is now on the rise.

At present, there is interest in consumption of many types of fermented foods other than cheese, bread, pickles, and alcoholic beverages. One reason for this increase is consumer interest in natural and healthy foods, which fermented foods have been thought to satisfy. Even countries in which many types of fermented foods have been consumed for a long time but mostly produced in small volumes, have started commercially producing some products in large volumes. It is anticipated that in the future, consumption of many fermented foods will increase worldwide. I

Module 80: microbiology of fermented products

Many desirable species and strains of bacteria, yeasts, and molds are associated with fermentation of foods. Depending on a product, fermentation may be achieved by a single predominating species and strain. However, in most fermentations, a mixed population of several bacterial species and strains, or even bacteria and yeasts or bacteria and molds, is involved. When a fermentation process involves a mixed population, the members should not be antagonistic toward one another rather, they should preferably be synergistic. Maximum growth of a desirable microorganism and optimum fermentation rate are dependent on environmental parameters such as nutrients, temperature of incubation, oxidation-reduction potential, and pH. In the fermentation process, if the different species in a mixed population need different environmental conditions (e.g., temperature of growth), a compromise is made to facilitate growth of all the species at a moderate rate. Depending on a raw or starting material and a specific need, carbohydrates (dextrose in meat fermentation), salts, citrate, and other nutrients are supplemented. In some natural fermentation, several species may be involved for the final desirable characteristics of the product. However, instead of growing at the same time, they appear in sequence, with the consequence that a particular species predominates at a certain stage during fermentation.

But analyzing the final product to isolate the species involved in fermentation of such a food *does not* give the right picture. Instead, samples should be analyzed at intervals to determine predominant types at different times and to know the sequences in their appearance. Finally, some minor flora (secondary flora) can be present in a very low level in the raw material and the final product and might not be detected during regular analysis. However, they may have important contributions for the desirable characteristics, particularly some unique aroma, of the product.

Module 81: General Methods of productions

The production of a fermented product has two related, yet separate, aspects, one involving the importance of metabolic activities of microorganisms during fermentation and storage of the product and the other involving the parameters used during processing and storage of the product.

Fermentation involves exposing the raw or starting food materials to conditions that favor growth and metabolism of specific and desirable microorganisms. As the desirable microorganisms grow, they utilize some nutrients and produce some end products. These end products, along with the unmetabolized components of the starting materials, constitute the fermented foods having desirable acceptance qualities, many of which are attributed to the metabolic end products.

Raw Materials:

A large number of raw materials from plant and animal sources are used to produce fermented foods. These include milk (from cows, buffalo, sheep, goats, and mares), meat (beef, pork, lamb, goat, and fowl), fish (many types), eggs (chicken and duck), vegetables and vegetable juices, many fruits and fruit juices, cereal grains, tubers, lentils, beans, and seeds. Some are used in combination.

Module 82: Fermentation Process

Foods can be fermented in three different ways, based on the sources of the desirable microorganisms: natural fermentation, back slopping, and controlled fermentation.

Natural Fermentation

Many raw materials used in fermentation (usually not heat treated) contain both desirable and associated microorganisms. The conditions of incubation are set to favor rapid growth of the desirable types and no or slow growth of the associated (many are undesirable) types. A product produced by natural fermentation can have some desirable aroma resulting from the metabolism of the associated flora. However, because the natural microbial flora in the raw materials may not always be the same, it is difficult to produce a product with consistent characteristics over a long period of time. Also, chances of product failure because of growth of undesirable flora and foodborne diseases by the pathogens are high.

Back Slopping

In this method, some products from a successful fermentation are added to the starting materials, and conditions are set to facilitate the growth of the microorganisms coming from the previous product. This is still practiced in the production of many ethnic products in small volumes. Retention of characteristics over a long period may be difficult because of changes in microbial types. Chances of product failure and foodborne diseases are also high. Starting materials (may be heat treated) are inoculated with a high population (10^6 cells/ml or more) of a pure culture of single or mixed strains or species of microorganisms (starter culture). Incubation conditions are set for the optimum growth of the starter cultures. Large volumes can be produced with consistent and predictable characteristics each day. Generally, there are less chances of product failure and foodborne diseases. However, there may be no growth of desirable secondary flora. As a result, a product may not have some delicate flavor characteristics.

Composition and Quality

Growth of desirable microorganisms and the quality of a fermented dairy product are influenced by the composition and quality of the milk used in a fermentation process. Cow's milk contains 3.2% protein, 4.8% lactose, 3.9% lipids, 0.9% minerals, traces of vitamins, and ca. 0.2% water. Among the proteins, casein in colloidal suspension as calcium caseinate is present in higher amounts than the other two soluble proteins, albumin and globulin. Lactose is the main carbohydrate and is present in solution, and lipids are dispersed as globules of different sizes in emulsion (fat in water). Minerals are present in solution and as colloid with casein. Water-soluble vitamins are present in aqueous phase, whereas fat-soluble vitamins are present with the lipids. The solid components (ca. 12.8%) are designated as total solids.

(TS), and TS without lipids is designated as solid-not-fat (SNP; ca. 8.9%). The whey contains principally the water-soluble components, some fat, and water.

The growth of desirable microorganisms can be adversely affected by several components that are either naturally present or have entered in the milk as contaminants. The natural antimicrobials are agglutinins and the lactoperoxidase-isothiocynate system. The agglutinins can induce clumping of starter-culture cells and slow their growth and metabolism. The lactoperoxidase-isothiocynate system can inhibit starter cultures. Antimicrobials can cause problems only when raw milk is used, because both are destroyed by heating milk. Milk can also contain antibiotics, either used in the feed or used to treat animals for some infections, such as mastitis. Their presence can also affect the growth of starter cultures. Some milk can contain heat-stable proteases and lipases produced by some psychrotropic bacteria, such as *Pseudomonas* species, during refrigerated storage of raw milk before pasteurization. These enzymes remain stable after heating and can cause product defects (low yield of cheese, proteolysis, and rancidity). Before milk is used for fermentation, these aspects need to be considered.

Module 83: Fermented Dairy food products

Fermented Milk Products

Many types of fermented milk products are produced in different parts of the world. A few are produced by controlled fermentation, and the microbial types and their respective contributions are known. In many others, fermented either naturally or by back slopping, the microbial profiles and their contribution are not exactly known. Many types of lactic acid bacteria and some yeasts are found to predominate microbial flora in these products,^{3A} some of which are listed:

Buttermilk. Made with *Lactococcus* species without or with *Leuconostoc cremoris*; some can have biovar diacetylactis in place of *Leu. cremoris* (such as ymer in Denmark), whereas some can have aropy variant of *Lactococcus* species (langfil in Norway) or mold (*Geotrichum candidum* in villi in Finland).

Yogurt. Made with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*; some types can also have added *Lab. acidophilus*, *casei*, *rhamnosus*, and

Bifidobacterium spp.; some may also have *LactOCOCCllS* species and *Lab. plantarum* and lactose-fermenting yeasts.

Acidophilus Milk. Made with *Lab. acidophilus*.

Bifidus Milk. Made with *Bifidobacterium* spp.

Yakult. Made with *Lab. casei*; may contain *Bifidobacterium* spp.

Kefir. Made from *Lab. kefir* (several species of yeasts along with *Leuconostoc*, *Lactobacillus*, and *Lactococcus* spp.).

Kumiss. Made from *Lab. delbrueckii* subsp. *bulgaricus* and yeasts.

Among these, cultured buttermilk and yogurt are discussed here.

Module 84: Microbiology of Yogurt

Plain yogurt has a semisolid mass due to coagulation of milk (skim, low~ or full fat) by starter-culture bacteria. It has a sharp acid taste with a flavor similar to walnuts and a smooth mouth feel. The flavor is due to the combined effects of acetaldehyde, lactate, diacetyl, and acetate, but 90% of the flavor is due to acetaldehyde.

Many types of yogurt are available in the market; for example, plain yogurt, fruit yogurt, Flavored and colored yogurt, blended yogurt, sweetened yogurt, heated yogurt, frozen yogurt, dried yogurt, low-lactose yogurt, and carbonated yogurt.

Processing

Yogurt is generally fermented in batches, but a continuous method has also been developed. The batch process for a low-fat (2%) plain yogurt is as follows:

1. Homogenized milk (12% TS) + stabilizer (1 %). The stabilizer is added to give desired gel structure.
2. Heated to 185°F (85°C) for 30 min. and cooled to 110°F (43.3°C). Heating helps destroy vegetative microbes and slightly destabilize casein for good gel formation.
3. Starter added, incubated at 110°F (29.5°C) to pH 4.8 for ca. 6 h, acidity ca. 0.9%. Starter used as either direct vat set (frozen) or bulk culture (2-3%).

4. Quickly cooled to 85°F in ca. 30 min to slow down further starter growth and acid production, especially by *Lactobacillus* species, agitated, and pumped to filler machine.
5. Packaged in containers, and cooled by forced air to 40°F (4.4°C). Final cooling by forced air results in a rapid drop in temperature to stop the growth of starters.
6. Held for 24 h; pH drops to 4.3.

Starters (Controlled Fermentation)

Frozen concentrates or direct vat set starters can be used. Normally, *Lab delbrueckii* ssp. *bulgaricus* and *Str. thermophilus* are used. Some processors also combine these two with other species, such as *Lab. acidophilus* and *Bifidobacterium* spp., *Lab. rhamnosus*, or *Lab. casei*. However, in general, they do not compete well in growth with the two yogurt starters. Therefore, they are added in high numbers after fermentation and before packaging. They may not survive well when present in yogurt with the regular yogurt starter cultures.

For a good product, the two starter species should be added at a *Streptococcus:Lactobacillus* cell ratio of 1:1; in the final product, the ratio should not exceed 3:2. However, *Lactobacillus* cells are more susceptible to freezing and freeze-drying. In a frozen concentrate starter for use as

Module 85: Fermented products: cheese

Cheeses are made by coagulating the casein in milk with lactic acid produced by lactic acid bacteria, without or with the enzyme rennin, followed by collecting the casein for further processing, which may include ripening.⁶ The process was probably accidentally discovered in the Middle East ca. 7000 B.C. from the coagulation of milk stored in a calf stomach by lactic acid produced by lactic acid bacteria (and probably rennin in the stomach). At present, many varieties of cheeses are made worldwide, which probably use more than 20% of the total milk produced. In the United States, the total production of different varieties of cheese in 1982 was 4.4 billion pounds (2 billion Kg) and, in 1987, this increased to 5.3 billion pounds (2.4 billion Kg). Because of the world-wide increase in cheese consumption, cheese production will continue to increase not only in the United States, but also in other countries, especially in dairy-rich countries such as Europe and New Zealand.

Cheese varieties have been grouped in different ways. Examples of several varieties based on starter cultures used and some important secondary flora are listed here.

Unripened Cheese

Soft

Cottage cheese with starters *Lac. lactis* ssp. *lactis* and *cremoris* and *Leuconostoc mesenteroides* ssp. *cremoris*.

Mozzarella cheese with starters *Stl. thermophilus* and *Lab. delbrueckii* ssp. *bulgaricus*.

Ripened Cheese (Soft) Brie cheese with starter *Lac. lactis* ssp.; *Penicillium* sp. and yeasts are secondary flora.

(Semihard) Gouda cheese with starters *Lac. lactis* ssp. and *Leuconostoc* spp.; dairy *Propionibacterium* may be secondary flora., Blue cheese with starter *Lac. lactis* ssp., *Leuconostoc* spp.; *Penicillium roquefortii*, yeasts, and micrococci are secondary flora.

(Hard): Cheddar cheese with starters *Lac. lactis* ssp.; some lactobacilli and pediococci (and probably enterococci) are secondary (or associative) flora.

Swiss cheese with starters *Str. thermophilus*, *Lab. helveticus*, and dairy *Propionibacterium* spp.; enterococci can be secondary (or associative) flora.

Only cottage, Cheddar, Swiss, and blue cheeses are further discussed to understand the microbiological aspects of unripened and ripened cheeses.

MICROBIOLOGY OF COTTAGE CHEESE

Cottage cheese is made from low-fat or skim milk and has a soft texture with ca. 80% moisture. It is unripened and has a buttery aroma due to diacetyl (along with lactic acid and little acetaldehyde).

Processing (from Skim Milk)

Pasteurized, cooled to 70°F (22.2°C), starter added, and incubated for 12 h at pH 4.7.

Firm curd set, cut in cubes, and cooked at 125°F (51.7°C) for 50 min or more. Whey drained off, stirred to remove more to get dry curd.

Salted, creamed, and preservative added.

Packaged and refrigerated.

Module 86: Fermented Meat Products

Types

Fermented meat products are produced by first mixing meat, fat, salt, sugar, curing agents, and spices; filling the mixture in a casing; and fermenting it either naturally or by adding (during mixing) selected starter-culture bacteria.³ The acids produced by the starters during fermentation and the curing agents used help control the growth of pathogenic and spoilage bacteria that might be present in the meat. Depending on the type, the fermented products may be dried to reduce A_w or smoked or heated to ensure the safety and shelf life of the products.

Meat fermentation probably originated in the Mediterranean countries and later spread to European countries and North America. In the United States, semidry sausages are most popular, although some dry sausages are also produced. Following fermentation, semidry sausages are heated (also sometimes smoked) before consumption. For dry sausages, following cooking, the products are dried to reduce the A_w . Even now, fairly large amounts of fermented sausages in the United States are produced by natural fermentation, especially those produced by small processors. However, more processors now use selected starter cultures and controlled fermentation. Commercial starter cultures are available as both frozen and freeze-dried concentrates for direct inoculation in the meat mixture. Semidry and dry sausages include many types, such as pepperoni, Genoa salami, hard salami, summer sausage, beef sticks, beef logs, thuringer, cervelat, and Italian salami. Most are made with beef and pork, but in recent years, some have been made with meat from chicken and turkey. The microbiology of semidry sausages is described here.

MICROBIOLOGY OF SEMIDRY SAUSAGES

Characteristics

Semidry sausages include summer sausage, thuringer, and semidry salami. The average composition is ca. 30% fat, 20% protein, 3% minerals (salts), and 47% water. They have a tangy taste with a desirable flavor imparted by the combined effect of lactate, acetate, and diacetyl, and some breakdown components from proteolysis and lipolysis. The use of spices

also contributes to the flavor. Those containing nitrite have a pinkish color in contrast to the grayish color in products without it.

Processing

- Meat, salts, glucose, cure, spices, and starter mixed uniformly.
- Stuffed in casings, fermented at 85-110°F (29.4-43.3°C) with 80--90% relative humidity.
- Incubated until the pH drops to ca. 5.2-4.6, cooked to 140° P (60°C) internal temperature, and cooled to 50°F.
- Stored at 40-50°P (4.4-10°C) for 3-4 days, vacuum-packaged, and consumed directly.
- Cures contain nitrite to give a final concentration of ca. 100 ppm. Fermentation can be carried out in a smokehouse. Fermentation time is usually 8-12 h, during which the pH is dropped to desired level.

Module 87: Microbiology of semidry sausage

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Processing

1. Meat, salts, glucose, cure, spices, and starter mixed uniformly.
2. Stuffed in casings, fermented at 85-110°F (29.4-43.3°C) with 80--90% relative humidity.
3. Incubated until the pH drops to ca. 5.2-4.6, cooked to 140°P (60°C) internal temperature, and cooled to 50°F.
4. Stored at 40-50°P (4.4-10°C) for 3-4 days, vacuum-packaged, and consumed directly.
5. Cures contain nitrite to give a final concentration of ca. 100 ppm. Fermentation can be carried out in a smokehouse. Fermentation time is usually 8-12 h, during which the pH is dropped to desired level.

Starters (Controlled or Natural Fermentation)

In controlled fermentation, frozen or dried concentrates are used directly at 10^6 - 7×10^7 cfu/g mix. Starters should not be mixed with salt, cure, or spices as it can kill injured cells. Instead, they should be thawed and immediately put into the meat. Starters vary, depending on the fermentation temperature and final pH of the product desired. For high temperature and low pH, *Pediococcus acidilactici* strains are preferred; for low temperature and high pH, *Lab. plantarum* strains are preferred. *Ped. pentosaceus* strains can be used under both conditions. Some starters can have both *Pediococcus* and *Lactobacillus* species. In addition, selected *Micrococcus* spp. or *Sta. carnosus* strains are added as secondary flora for their beneficial effects on desired product color. In naturally fermented sausages, *Lab. sake*, *Lab. cillivatus*, and *Leuconostoc* spp. present in raw materials are important starter bacteria, especially when fermentation is set at lower temperatures (60-70°F [15.6-21.1 °C]) for several days and the final pH reached is not below 5.0.

Growth

Because the raw meat used may contain pathogens and spoilage bacteria, it is extremely important that starter culture grows rapidly and produces acid in large amounts to reduce pH from the initial 5.7 to ca. 5.3 very quickly to retard their growth. This can be achieved by adding large numbers of active starter cells, adding dextrose to the mix, and setting the temperature of fermentation optimum for the starters used. The optimum growth temperatures for *Ped. acidilactici*, *Ped. pentosaceus*. And *Lab. plantarum* are ca. 40, 35, and 30°C (104, 95, and 86°F). respectively. *Micrococcus* spp. and *Sta. carnosus* grow well at ca. 32.2°C (90°F). Cooking to an internal temperature of 60°C (140°F) kills *Lab. plantarum* and probably *Ped. pentosaceus*, but probably not *Ped. acidilactici*, *Micrococcus*, or *Sta. carnosus*. However, low pH and low A_w prevent their growth in the finished products.

Biochemistry

Both pediococci are homolactic fermentors and metabolize glucose to mainly lactic acid (DL-forms), with small amounts of acetate and diacetyl. *Lab. plantarum*, being facultatively heterofermentative, metabolizes glucose to principally lactic acid (DL); however, it can also produce substantial amounts of acetate, ethanol, and diacetyl. Strains of all three species can produce H_2O_2 , which can discolor the product by oxidizing myoglobin during fermentation. *Micrococcus* spp. or *Sta. carnosus* have catalase that can destroy H_2O_2 .

If nitrate is used in place of nitrite in cure, these bacteria can produce nitrite and help develop the agreeable pinkish color of the product. If the products are cured or stored for long periods of time, some of the intracellular enzymes of the lysed cells of starters are able to cause proteolysis and lipolysis and produce biologically active amines (such as histamine).

Module 88: Fermented Vegetable Products

Fermented Vegetable Products

Almost all vegetables can be fermented through natural processes, because they harbor many types of lactic acid bacteria. Worldwide, many types of vegetables are fermented, mostly in small volumes. However, some are produced commercially. Vegetable fermentation originated in the early years of human civilization and even now is widely used by many cultures. Examples of some fermented products and vegetables used currently for fermentation are sauerkraut (from cabbage), olives, cucumbers, carrots, celery, beans, peas, corn, okra, tomatoes, cauliflower, peppers, onions, citron, beets, turnips, radishes, chard, Brussels sprouts, and their blends. Most are produced by natural fermentation; however, some, such as cucumbers, are currently produced in limited amounts by controlled fermentation. Production of sauerkraut by natural fermentation is described here as an example.

Module 89: Saurkraut

Sauerkraut is produced by fermenting shredded cabbage. The product has a sour taste with a clean acid flavor.

Processing

1. Cabbage cleaned, trimmed, and shredded fine and uniform.
2. Packaged tight to exclude air in vat, and layered with salt (2.25%).
3. Top covered to exclude air, and fermented at 18°C (65°F) for 2 months.

Fine shredding helps the sugars (3-6%) come out of cabbage cells. Tight packaging helps create an anaerobic condition, thus preventing the growth of aerobes. Salt stimulates growth of some lactic acid bacteria, and discourages the growth of some undesirable bacteria and pectinase (in cabbage) action. The top is covered to exclude air and prevent growth of some aerobes. Fermentation at 18°C (65°F) discourages the rapid growth of some undesirable

bacteria (facultative anaerobic or anaerobic), but encourages the growth of desirable lactic acid bacteria. Natural inhibitors in cabbage also discourage the growth of undesirable Gram-negative and Gram-positive bacteria.

Module 90: Pickles

Blends of different vegetables (Reddish, Carrot, Peas, Lemon etc.) which are fermented (Lactic Acid Fermentation) to have special aroma , flavor and increased shelf life are called vegetable pickles

Process of Vegetable Pickling :-

- 1) Selection of Qualitatively good vegetables
- 2) Preparation of vegetables (Cutting, Slicing , Dicing , Shredding etc. and addition of salt)
- 3) Fermentation :-

Anaerobic Fermentation mostly by LAB

PRODUCTION OF CHEESE**Module 91: Production of Cheddar and related Hard cheese**

Cheddar cheese is classified as a hard cheese, ranging in color from nearly white (particularly if made from goat or sheep milk) to yellow to orange. Standards of identity for Cheddar include 39% moisture and 50% fat on a dry basis. Low-sodium Cheddar cheese contains not more than 96 mg of sodium per 454 g finished food.

Production of Cheddar and Related Hard Cheeses

Good cheese requires high-quality milk and carefully selected starter cultures. However, additional ingredients are often utilized to enhance visual appeal (annatto), coagulation properties (calcium chloride or enzymes), and flavor development (adjunct cultures or enzymes) to make great cheeses. How well the additional steps are employed determines whether or not one makes a great cheese.

Module 92: Ingredients used for cheese making**Ingredients****Milk:**

To make high-quality cheese, producers must start with high-quality milk. Cheese quality will never be better than the starting materials. Cheddar and most Cheddar-like cheeses can be made from raw, heat-treated, heat-shocked, or pasteurized milk, nonfat milk or cream, alone or in combination.

Extended storage of milk prior to pasteurization and cheesemaking not only enables growth of psychrotrophic bacteria, but also encourages solubilization of colloidal calcium phosphate and a shift in caseins from the micellar to soluble state. Although soluble caseins constitute less than 15% of the total casein in normal milk directly from the udder, the proportion increases to up to 42% of total casein during storage at 48C. Soluble calcium phosphate and casein are lost during whey drainage, which reduces cheese yield.

Because approximately 90% of both fat and protein from cheese milk are captured in the cheese), and these components make up 91% of the solids in cheese, detrimental effects on

either component will be realized in the cheese yield and quality. For consistency in yield and product composition, milk for Cheddar cheese is commonly standardized to a casein-to-fat ratio between 0.67 and 0.72..

Raw milk naturally contains low levels of endogenous enzymes, including alkaline phosphatase, plasmin, and lipoprotein lipase. Alkaline phosphatase (ALP) is slightly more heat stable than the most heat-resistant pathogenic microorganisms in milk. Thus, ALP is a convenient indicator of pasteurization.

Calcium Chloride:

Calcium chloride may be added to the cheese milk as a coagulation aid, in an amount not more than 0.02% (calculated as anhydrous calcium chloride) of the weight of the dairy ingredients. Addition of calcium chloride reduces the coagulation time and increases curd firmness (Lenoir and others 2000b). Calcium added to milk is solubilized during acidification and thus lost in whey, so it does not contribute to total calcium in the final cheese.

Starter Cultures:

Starter bacteria can be defined as isolates that produce sufficient acid to reduce the pH of milk to 5.3 in 6 h at 30 – 37°C, and aid in curd digestion and flavor development. Cultures selected for Cheddar cheese made throughout the world are typically “O” cultures, which are designated as cultures that produce lactic acid from lactose. The “O” type cultures are composed of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* (Harrits 1997; Strauss 1997). *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* can be differentiated by their ability to grow at 40°C and in the presence of salt. Whereas *L. lactis* subsp. *lactis* will grow at 40°C and in the presence of 4% salt, *L. lactis* subsp. *cremoris* will not grow at 40°C and will grow in the presence of salt up to a 2% concentration.

The “L” type cultures consist of “O” type cultures plus the citrate-fermenting culture *Leuconostoc mesenteroides* subsp. *cremoris*, which produces flavor compounds (e.g., diacetyl) plus small amounts of carbon dioxide. “L” type cultures may also be used in the production of Colby, because an open structure is allowed in Colby (Harrits 1997). Ideally, acid should be formed quickly and at a steady rate during curd formation.

Adjunct Cultures:

An adjunct culture is a one that is added, along with starter culture, for the desirable characteristics it may impart upon the cheese other than acid. Adjunct cultures are select nonstarter lactic acid bacteria (NSLAB), because not all NSLAB are desirable. Although the specific ripening mechanisms of NSLAB that contribute positively to Cheddar flavor have not been fully determined, to be successful, NSLAB adjuncts require two important features. First, strains must provide a balance of beneficial ripening reactions in cheese.

Secondly, strains need to be competitive against adventitious (not intentionally added) NSLAB and remain the dominant NSLAB during the ripening period (Crow and others 2001).

Color:

The recognizable yellow to orange color of Cheddar and Colby cheeses is derived from annatto, an extract from seeds of the “Lipstick tree,” *Bixa orellana*. Annatto was first used to make cheeses appear more fat-rich, when made during seasons of the year when the milk of cows produced less colorful cheese because the cows were fed diets lower in beta-carotene. Sheep and goat milk cheeses are naturally whiter than cow milk cheeses because the beta-carotene is efficiently hydrolyzed to vitamin A in the digestive tracts of these species.

Enzymes:

The Code of Federal Regulations permits the use of rennet/ chymosin and/or other clotting enzymes of animal, plant, or microbial origin, as well as enzymes of animal, plant, or microbial origin, used in curing or flavor development for Cheddar cheese. Enzymes of starter, adjunct NSLAB, and adventitious NSLAB naturally contribute to flavor and body/texture development in cheeses. It is possible to accelerate Cheddar cheese proteolysis and lipolysis using various liposome-encapsulated enzymatic cocktails. A neutral bacterial protease, acid fungal protease, and lipase were individually entrapped or mixed as cocktails and entrapped in liposomes then added to cheese milk prior to renneting. Certain enzyme treatments resulted in cheeses with more mature texture and higher flavor intensity or Cheddar flavor in a shorter time compared with control cheeses.

Salt:

Cheddar cheese typically contains 1.6 – 1.8% salt, in the form of NaCl. Salt enhances flavor, encourages syneresis, and slows or stops growth of salt-sensitive bacteria. Food-grade salt is essential to the production of safe, high-quality Cheddar cheese, and consistent salt grain size contributes to uniformity of salt concentration throughout the cheese matrix.

Investigators have demonstrated that reduced-salt, or low-salt Cheddar cheese can be made by replacing sodium chloride (NaCl) with potassium chloride (KCl) or mixtures of the two salts. Use of KCl to replace some of the NaCl for salting cheese has no detectable effect on the kinds of lactic acid bacteria, aerobic micro-organisms, aerobic spores, coliforms, and yeasts and molds in cheeses when compared with control cheeses.

Other Optional Ingredients:

The Code of Federal Regulations allows the use of antimycotic agents, applied to the surface of slices or cuts in consumer-sized packages. Some of the antimycotic substances allowed by the FDA are calcium propionate, methylparaben (methyl p-hydroxybenzoate), propylparaben (propyl p-hydroxybenzoate), sodium benzoate, sodium propionate, and sorbic acid.

Hydrogen peroxide is allowed, if followed by a quantity of catalase preparation sufficient to eliminate the hydrogen peroxide. The weight of the hydrogen peroxide shall not exceed 0.05% of the weight of the milk and the weight of the catalase shall not exceed 20 ppm of the weight of the treated milk.

Module 93: Selection of Cheese culture:

Bacteriophage (phage) resistance, salt sensitivity, and protease activity (desirable flavor development) are additional selection criteria. Homofermentative starter cultures are added deliberately to initiate Cheddar cheese manufacture. The starter bacteria produce L(p)-lactate from lactose and they grow, typically attaining cell densities of 10^8 cfu/g within hours of the beginning of manufacture. L(p) refers to an optically active substance that rotates the plane of polarized light counterclockwise (also called levorotatory). The optical isomer of L(p)-lactate is D(2)-lactate. The optical isomers are mirror images of each other and result from the tetrahedral geometry around the chiral carbon center.

Production of homogeneous, high-quality Cheddar cheese requires uniform lactose fermentation, lipolysis, and proteolysis, each of which varies among bacterial strains. A

relationship that exists between the extent of starter cell autolysis and the level of lipolysis during Cheddar cheese ripening. The rate and extent of both fermentation and proteolysis depend upon temperature and salt concentration. One of the main roles of starter bacteria is to provide a suitable environment for enzyme activity from rennet/chymosin (an acid protease) and favorable growth of secondary microflora with respect to redox potential, pH, and moisture content in cheese. Redox potential is a measure of the tendency of a system to donate or accept electrons, and indicates aerobic or anaerobic conditions. Typically, the environment inside cheese is anaerobic and reducing. Depending upon the type of culture preparation, usage rates vary between 0.75 and 1.25% for traditional bulk starter and 0.5% and 0.6% for pH controlled starter or DVS starter, respectively.

Module 94: Pre-treatment of milk for cheese making

Cheese Milk Pretreatments:

Centrifugal clarifier-separators are used to separate the cream and skim fractions, as well as remove solid impurities from milk prior to standardization. Cheese milk fat and protein content are commonly standardized for consistency of yield and composition. Fat may be increased with the addition of cream, and protein, particularly casein, may be increased with nonfat dry milk, skim milk, or condensed skim milk. A typical casein-to-fat ratio of between 0.67 and 0.72 may be used. Although cheese moisture is influenced by numerous factors during cheesemaking, higher fat levels in cheese milk are typically associated with lower moisture cheeses. As a general rule, an increase of 0.05 in the casein-to-fat ratio in milk generally results in a decrease of about 1.4% in the fat on a dry basis and an increase of about 0.8% in moisture in Cheddar cheese.

Mostly whole milk is preheated to 55 – 65°C in the regeneration section of the high-temperature short time (HTST) pasteurizer prior to separation. Following separation, the cream is standardized to a preset fat level and the fraction intended for standardization of milk is routed and remixed with the proper amount of skim milk to attain the desired fat and protein content. The surplus cream is directed to a separate cream pasteurizer, and the standardized milk flows through the pasteurizer.

Use of ultrafiltered milk for conversion into such cheeses as Cheddar, cottage, havarti, feta, brick, Colby, and Domiati is recommended because of an increase in yield of product. Ultrafiltration results in the concentration of milk proteins, with reduction in lactose and

mono- and divalent cations. Additional benefits claimed for use of ultrafiltered milk in cheesemaking include reduction in costs of energy, equipment, and labor, improved consistency of cheese flavor, and the potential production of new byproducts.

Homogenization:

Although homogenization of cheese milk is typically not employed in the production of Cheddar cheese, research has shown that homogenization of cream may have applications to Cheddar cheese. Homogenization is most efficient when fat globules are in the liquid state, so milk is preheated in the plate heat exchanger in the regeneration section of the HTST pasteurizer, where the temperature is raised to at least 60°C prior to homogenization. In a two-stage homogenizer, pressures typically range from 10 to 25 MPa in the first stage and 5 MPa in the second stage.

The only step in the dairy processing system that guarantees the killing of pathogenic microorganisms is pasteurization. Thus, pasteurization may be considered the most critical segment of the cheese processing line. An added side-benefit of pasteurization is that it also kills many spoilage microorganisms and inactivates enzymes that may contribute to quality defects in cheese. Pasteurization contributes to consistency in product quality. Of course, strict sanitation is critical up to and beyond pasteurization to assure the safety and quality of dairy products. In HTST pasteurization, milk must be held at a temperature of at least 72°C for a minimum of 15 s to be legally pasteurized. In batch or low-temperature long time (LTLT) systems (uncommon in large-scale operations), milk is continuously agitated in a single tank, at a set temperature (legally at least 62.8°C) for a given time (legally at least 30 min if at 62.8°C) to guarantee inactivation of pathogens. Any lower temperature or shorter time than legal pasteurization means the cheese must be treated as if made from raw milk, which means products must be aged for at least 60 days at 1.668°C or higher.

Regardless of pasteurization method, cheese milk is then cooled, either to incubation temperature for selected starter cultures, or to refrigeration temperature for future applications. Although all pathogens and most spoilage microorganisms are killed by pasteurization, potentially beneficial or flavor-producing microorganisms are also killed. And so, although cheeses made from pasteurized milk are safe, they also have less flavor than raw milk cheeses.

In addition to modifying milk microflora, pasteurization acts upon milk protein chemistry to influence cheese quality. Specifically, pasteurizing cheese milk influences the extent and characteristics of proteolysis during Cheddar cheese aging. Pasteurization causes heat-induced precipitation of whey proteins upon casein micelles that result in retention of additional whey protein in the cheese beyond that which is soluble in the aqueous phase of raw-milk cheese. The presence of heat-denatured whey protein in cheese may influence the accessibility of caseins to proteases during cheese aging, a consequence of which would be differences in proteolysis during aging. These differences may be another factor that contributes to differences in flavor development in Cheddar cheese made from pasteurized and raw milk. Temperatures higher than legal pasteurization (80 C) may be used to increase yield; however, gelling takes longer, the firming rate of the gel as well as its maximum firmness are reduced, and gel draining is more difficult and is incomplete. These factors are a consequence of denatured whey proteins, particularly β -lactoglobulin, which bind with caseins, particularly κ -casein. Indeed, a complex between β -lactoglobulin and κ -casein leads to modification in the conformation of the κ -casein chain at the chymosin cleavage site, detrimentally affecting coagulation properties. Denaturation of whey proteins is negligible at pasteurization temperatures, but reaches 10% after a treatment of 75 C for 15 s and 20% after 85C for 30s. Heat also decreases soluble calcium, ionized calcium, and soluble inorganic phosphorus.

Module 95: Cheese making process

In large automated plants, cheesemaking is typically held to a well-timed schedule. Culture, CaCl_2 , and color are typically added as cheese milk enters the cheese vat, after pasteurization and cooling. Chymosin is commonly added after the vat is completely filled with pasteurized milk. In pilot or small-scale operations, culture, CaCl_2 , and color are commonly added when an entire vat of cheese milk reaches target temperature. Chymosin is added after a ripening period of 15 – 30 min. Calcium Chloride Addition. If CaCl_2 is to be added to the cheese milk, typically 0.2% of cheese milk weight is adequate to improve coagulation properties.

Color Addition:

Annatto may be added to cheese milk at a rate of approximately 66 mL per 1000 kg of milk, adjusted to desired product color. Annatto binds with protein to form a straw to orange color in the final cheese, upon a concentration that occurs with whey expulsion (syneresis).

Culture Addition:

Prior to culture addition, raw or pasteurized milk must be tempered to the appropriate temperature for starter culture multiplication, approximately 26 – 30 °C for Cheddar and related cheeses. Inoculum level is defined by culture manufacturers, based upon whether the culture is DVS or bulk culture, typically from 0.5 to 5%. Cheese manufacturers may increase or decrease the amount of culture based on seasonal variation in milk composition. Optional addition of adjunct culture typically varies from 0.1 to 1%.

Ripening:

The titratable acidity (TA) of fresh milk is approximately 0.14 – 0.18, depending on composition, and pH is about 6.6 – 6.8. When starter culture is added, cultures need time to equilibrate to their environment (lag phase), so only a small rise in TA is noted during the 30-min ripening period. Little lactose is converted to lactic acid during the lag phase of the cultures, but TA rises steadily during the log phase of growth, during which time culture numbers increase exponentially. Even after lactic acid formation begins, little change in pH is noted because of milk's high buffering capacity, owing to the presence of proteins, citrate, and phosphate in milk.

Enzyme Addition:

In fresh fluid milk, charges on the k-casein “hairs” are negative (2), so casein micelles repel each other. With the production of acid, the charges on some k-casein hairs begin to change to positive (p). When pH declines to near 5.2, calcium and phosphorus are solubilized and the micelle structure changes. At pH close to 4.6, coagulation occurs, as repulsive charges are neutralized and micelles come into contact with one another and coalesce. Some cheeses are made exclusively with acid coagulation (e.g., cottage cheese). Because acid development is slow, cheese making procedures that rely entirely on acid coagulation are in the order of 10 – 18 h in length. The cheesemaking process is accelerated by the use of coagulating enzymes.

Chymosin, originally derived from the abomasum of milk-fed calves, but now microbially or fungally derived, is the most common coagulating enzyme used in the manufacture of Cheddar and related varieties. Chymosin is an acid protease, which means that it is more active at an acid pH than neutral or basic pH. Highest activity is observed at pH 5.5 and 42°C. Specifically, chymosin cleaves the peptide bond Phe₁₀₅ – Met₁₀₆, which leads to the

formation of k-para-casein (1-105) and glyco-macropeptide or caseinomacropeptide (CMP, 106 – 169). CMP is soluble in whey. When chymosin is added to milk, coagulation occurs in three steps:

1. k-Casein hydrolysis,
2. Aggregation of destabilized micelles, and
3. Reorganization of calcium phosphate, or reticulation.

The coagulation process is shortened because rennet/chymosin cleaves the negatively-charged k-casein hairs off the micelles, enabling approach and coagulation of micelles. During the coagulation process, calcium phosphate bridges form between micelles, and tighten as whey is expelled, forming a tight network of casein, which entraps some fat, water, and water-soluble components. As fermentation proceeds, Ca^{2+} are replaced by H^+ and the casein network continues to tighten. Approximately 5 – 50 mL of single-strength liquid chymosin should be used to coagulate 100 L milk. Chymosin should always be diluted (approximately 1 part to 40 parts water) prior to addition to the cheese vat to prevent localized coagulation. Dilution should always be done with cool (or room temperature) water immediately before adding to milk. Chymosin begins to lose its strength and activity immediately upon dilution, which is why dilution should not be done in advance. Also, chymosin is degraded by high temperatures and chlorine. Chymosin must not be over-mixed into the milk because cleavage of k-casein from casein micelle proteins begins immediately. In small operations, chymosin should only be mixed into milk for about 1 min to maximize yield. During the incubation period, the cheese vat must not be agitated or disturbed in any way, or a soft or weak curd will result and yield will be affected.

Chymosin is allowed to set the cheese for 20 – 30 min prior to curd testing. In large automated plants, the curd is typically not checked, and cutting begins at a set time. To check the curd, a spatula or knife may be used. A spatula works best for checking curd set because of its rounded shape. The blade is cut through the curd in a 5-cm vertical orientation and removed. The blade is then inserted at the bottom of the vertical cut, in a horizontal orientation, to form a T. The blade is pushed forward and lifted, to encourage the curd to split open. The curd is ready for cutting when the curd is firm, it breaks cleanly, and fills with clear yellow (not cloudy) whey.

Cutting. Cutting of the curd is an extremely important step in the cheesemaking process because it influences whey drainage and cheese yield. Cutting to a consistent size, with



sharp knives, is critical to minimize small curd particles (fines) that may get lost during whey drainage. Manually, the coagulated mass of cheese is cut with harps: knives constructed of stainless steel hardware and wire spaced at regular intervals. The wires on one harp are horizontally oriented, and the other harp wires are vertically oriented. The cutting progresses in such a way that first horizontal and vertical sheets of curd are cut with the harp knives. The sheets are then cut into cubes by perpendicular cuts with the vertical harp knives.

Large dairy plants have automated cheese vats that vary in size from 2000 to 25,000 L capacities. These cheese vats are equipped with a shaft to which agitators are attached. These agitators are designed in such a way that they cut the cheese when the shaft rotates in one direction and agitate the cubes gently when the shaft rotates in the other direction. Cheese vats are automated and allow the cheese curds to heal and cook before pumping the curds and whey onto a perforated conveyor belt where cheese curd is separated from the whey.

Cutting the coagulum increases the surface area of the curd and enhances syneresis. Upon cutting, curd particles immediately begin to expel whey and shrink, and the TA that had been rising in the cheese milk immediately drops in the whey, because the whey has lower apparent acidity, due to lower protein, citrate, and phosphate. The TA of whey will gradually increase as lactic acid is formed in the curds and released with whey during syneresis.

Healing:

Freshly cut curd is fragile and shatters easily, so curds are allowed to “heal” for 5 – 10 min prior to agitating and cooking. A healing period is particularly important when goat Cheddar cheeses are made, because the curds are naturally more fragile than curd obtained from cow milk. During healing, a tender skin is formed around each freshly cut curd. As the skin firms, the curd becomes more resistant to shattering and yield losses.

Cooking:

The cooking process is essentially a controlled increase in curd – whey temperature. Heating allows individual curd cubes to shrink, release whey, and firm. Cooking also increases reaction rates, specifically bacteria growth and metabolism, and enzyme activity. Temperature-sensitive bacteria strains are slowed down as temperature is raised. Prior to

raising the temperature of the curd – whey mixture, curds should be gently eased from the edges of the cheese vat, where they have matted. Curd cooking should begin slowly, with continual stirring of the curds. Hot water or steam may be used to increase the jacket temperature. The curd – whey mixture temperature should be raised slowly, about 28C every 5 min until 38C is reached (35 – 45 min). Stirring speed may be increased as the curds firm, but stirring too fast will shatter curds and reduce yield. For a drier cheese, temperature should be held at 38C for an additional 45 min, with stirring. In small or start-up facilities, whey TA should be recorded every 15 min. Regardless of plant size, good records should be kept of the entire cheesemaking procedure and final cheese quality. Failure to keep such records will reduce consistency.

For a short period after the curd is cut, lactose and lactic acid concentrations are at equilibrium in curd and whey. With time, the concentration of lactose drops faster in the curd than in the whey because starter bacteria concentrated in the curd deplete lactose in the curd. As the lactose is fermented within the curd, replacement lactose diffuses into the curd from the whey. As a neutral molecule, lactose diffuses easily through the matrix. Positively charged hydrogen ions exit the negatively charged curd much more slowly than lactose. As fermentation progresses, hydrogen ions are neutralized by the negatively charged proteins and phosphates. As the buffering capacity of the caseins and colloidal calcium phosphate (CCP) becomes saturated, the pH of the curd steadily drops. As pH drops, CCP is solubilized and lost into the whey.

Draining: Whey may be drained entirely, in the case of Cheddar, or partially, with washing, as in the case of Colby or Monterey. In large plants, a cleaned and sanitized finishing table/vat may be aligned with the exit port of the cheese vat. The drain of the vat is opened and curds and whey are allowed to flow onto the finishing table. Alternatively, in small plants, the cheese vat may double as a finishing table. A screen is installed ahead of the finishing table drain port to prevent curd loss as the whey is drained. Whey is commonly collected in a separate reservoir.

Cheese curds should be allowed to settle into the vat or finishing table, at an even depth throughout the length, and permitted to mat for 15 min. Whey TA will rise more quickly during this interval and should be recorded every 15 min from this point forward, throughout the cheddaring process. In the largest plants, curds are delivered to a perforated conveyor belt for drainage and cheddaring, which allows formation of a sheet of curd and

continuous whey drainage. The conveyer is enclosed in a tunnel. Upon drainage, in the absence of the whey bath, the curd pH will drop at a faster rate and the curds will continue to shrink and tighten. Much of the calcium is lost at drainage, particularly at low pH.

Washing. Washing, or curd rinsing, removes lactic acid and residual lactose and lactic acid from the curd and the result is a higher pH in the final cheese. Washing is rarely included during Cheddar cheesemaking, but when it is, the duration of such rinsing is so limited that only the whey on the surface of the curds is removed.

In Colby and Monterey production, whey is drained off until the curd on the bottom of the vat is visible, then sufficient cold water is introduced to reduce the temperature of the curd – whey mixture to 27 °C. Rate of syneresis is slowed if cool water used, resulting in higher cheese moisture content. Long wash time removes more lactose, resulting in higher final pH of cheese. Temperature-sensitive strains may be revived if cool water used.

Cheddaring. The step known as “cheddaring” was standardized into commercial practice by Joseph Harding in 1857. During manual cheddaring, curds are flipped and stacked at regular intervals, naturally pressed under their own weight, which enhances syneresis, yet still maintains a controllable level of moisture retention. The main purpose of cheddaring is to allow time for the acidity to increase and whey to be released. Curd particles fuse into a solid mass, syneresis continues as acid builds, rennet/chymosin continues to act, and these forces cooperate to tighten the casein network. As lactic acid continues to build, curds begin to flow or stretch under the weight of piled slabs. Cheddar gains its characteristic body through the process of knitting, stretching, and orientation of the casein network during cheddaring, which requires a pH below 5.8.

Cheddaring begins with flipping of slabs, one by one. The bottom becomes the top and the end toward the trench becomes the end toward the vat wall. Slabs should be allowed to settle 15 min before each subsequent step. The next step of cheddaring involves the flipping of one slab, followed by placing of an adjacent slab on top of the flipped slab (without flipping). This step is called “flip – stay.” The process continues for every pair of slabs. After 15 min, the top slab is placed (not flipped) into an empty spot in the vat. The previous bottom slab is then flipped and placed atop the new bottom slab. This step is called “stay – flip.” Cheddaring continues with flipping and stacking of slabs, alternating between “flip – stay” and “stay – flip” steps, until a whey TA of 0.35% as lactic acid is measured in a fresh sample of whey. In large plants, the process of cheddaring is auto-mated. As the perforated

conveyor mentioned previously transects a number of parallel planes during the approximately 90-min cheddaring process, the matted curds are flipped and stretched continuously in a tumbling motion.

Milling:

Milling is the process of cutting the slabs into cubes about 5 cm in size, which enables more uniform salt distribution, encourages syneresis and makes hooping more convenient. When curds are milled, more whey is expelled because milling greatly increases curd surface area and opens pores for syneresis. Salt distribution will be most uniform in cheese if curds are milled to a uniform size. In large plants, as the mat of curd arrives at the discharge point of the perforated conveyer, it is cut to desired size in a reciprocal dice-type mill or rotary curd mill.

Salting:

Milled curds of Cheddar and related hard cheeses are dry-salted rather than brine-salted. Cheese is salted because it:

Encourages further syneresis,

Inhibits further growth and metabolism of most microorganisms (thus arresting lactic acid production), and provides flavor.

Approximately 2.5 kg salt for every 100 kg of cheese curd is used. The salt is added in three equal applications and mixed for 5 min between applications. Adding salt too quickly will cause a “skin” to form on the curds, inhibiting salt absorption and syneresis. In large plants the milled cheese quantity is determined continuously by weight prior to entering the salting machine. The salter automatically calculates the salt and sifts it over the milled cheese. The pH and TA will only change slightly beyond the point of salt addition.

Salt, more specifically salt in moisture (S/M), directly influences the final pH of the cheese, growth of microorganisms and overall flavor, body, and texture of cheese

Pressing and Packaging:

Pressing gives cheese its final shape, reduces openings between curd particles, promotes fusion, and releases more free whey. In small plants, Cheddar curds are pressed overnight using a batch method. Pressure, approximately 1.4 atm, is applied to molds for 8 – 12 h at

room temperature. After one or two hours of pressing, cheeses may be flipped in the molds and lined with cheesecloth, which provides an attractive surface pattern. Large plants have a continuous “block-former” system. Curds are fed into a tower under a partial vacuum, whey is siphoned off, and for a short period, mechanical pressure is applied at the base of the tower prior to packaging. A block former cuts 20-kg blocks from the stack at regular intervals and the blocks are transferred to a vacuum packaging system prior to aging.

Aging. Aging enables flavor and texture development of hard cheeses. Nearly all residual lactose should be fermented within about 48 h. With cold storage, between 5 and 12°C, acid production slows down, but continues until limiting conditions occur (Banks and Williams 2004). Starter bacteria lyse (burst) and release proteolytic enzymes into the matrix. Residual plasmin and coagulant also contribute to proteolysis during aging. Caseins are broken down into peptides and amino acids, which yield flavor and modify cheese body/texture. Secondary fermentations can occur if NSLAB are still active, which results in further changes in flavor and body/texture. Cheeses with low S/M have a higher rate of proteolysis, resulting in a softer texture, than cheeses with high S/M.

Module 96: Quality Control of Cheese making

Shelf-life

The shelf-life of Cheddar and related cheeses is limited by quality, not safety. The quality of good Cheddar cheese improves with storage. Cheddar cheese may be removed from shelves due to flavor, body, or appearance defects. The most common flavor defects are high acid, bitter, unclean, and fermented/fruity. Common body defects are weak or crumbly body, gas holes, surface discoloration, and appearance of crystals on surfaces.

Evaluation

High-quality Cheddar cheese has a full, balanced nutty, sharp, but not bitter flavor. The ideal texture should be closed (no gas holes or mechanical openings), and the body should be firm, smooth and waxy (responds to moderate pressure). Colby and Monterey/Jack cheeses are similar to Cheddar, but are milder in flavor and possess a softer body. Colby and Monterey/Jack are prone to the same defects as Cheddar. However, due to higher moisture content, lower acid and salt, and higher microbial and enzymatic activity, some sensory defects may reach greater intensity and frequency in Colby and Monterey/Jack cheeses than Cheddar, particularly with extended aging.

Gas liquid chromatography (GLC) analysis of Cheddar cheese has shown that there are as many as 200 different compounds that may contribute to cheese flavor. However, flavor chemists believe that as few as 20 volatile compounds are pertinent to determination of the eventual flavor of Cheddar cheese. Cow diet, milk handling and sanitation

Lecture 11

FOOD ENZYME**Module 97: Food Enzymes**

Enzymes, produced by living things, are compounds that catalyze chemical reactions. Reactions involving enzymes may be said to proceed in two steps. In step 1, $E + S \rightarrow ES$ (where E = enzyme, S = substrate, and ES = unstable intermediate complex that temporarily involves the enzyme). In step 2, $ES + R \rightarrow P + E$ (where R = a substance in the substrate that reacts with the complex, P = the final product of the reaction, and E = enzyme liberated from the complex). Enzymes are critical to life because they have the ability to catalyze the chemical reactions that are important to life. Chemical reactions take place when the necessary reactants are present, but usually an energy input (activation energy) is required to start a particular reaction. The analogy usually given to illustrate this concept is that of a boulder located at the top of a hill. The boulder has the potential energy for rolling down the hill, but must first be pushed over the edge. The potential energy of the boulder could be great, depending on its mass and altitude. It could start a rock slide or landslide involving a great amount of energy. Although the energy required to push the boulder over the edge is insignificant compared to the total energy involved in the rolling of the boulder down the hill, that initial energy (called the activation energy) is nevertheless important, for without it there would be no landslide.

It is well known that the rate at which reactions take place is dependent on temperature. Because reactions can proceed more rapidly at higher temperatures but quite often have to accelerate within a system of constant temperature, such as within our bodies, only by the action of enzymes can they occur. Enzymes are produced by living organisms from the lowest single-celled members to the highest, most complex members of the plant or animal kingdoms, including humans. All life depends on enzymes to convert foods or nutrients to a form in which they can be utilized, and to carry out cellular functions.

In composition, enzymes always contain a protein. They may also contain or require complex chemical compounds in order to become functional. These compounds are known as prosthetic groups (also called coenzymes) and are usually vitamins, especially those belonging to the B group. Some enzymes also require trace amounts of a metal, such

as copper, to function. Enzymes therefore consist of either pure proteins, proteins with a prosthetic group, or proteins with a prosthetic group plus a metal cation.

Through their action, enzymes convert foods into less complex chemical substances that can be utilized for energy and for the building of cellular protoplasm. Proteins, fats, and carbohydrates are thus broken down to less complex compounds by enzymes in order that they may be utilized. More complex carbohydrates are broken down to glucose, a source of ready energy that can be absorbed and eventually converted to carbon dioxide and water or built up into fats that can be stored as a source of reserve energy. When reserve energy is needed, fats are first hydrolyzed (water is inserted to split the molecule), breaking down into glycerine and fatty acids. The fatty acids are then converted to acetates that can be utilized for energy. When the acetates are not completely used, those remaining may be reformed into fats and deposited as an energy reserve.

Proteins are broken down to their primary units (amino acids), in which form they may be incorporated into cellular protoplasm as proteins used for cell repair or growth. In some instances, the nitrogen portion of the amino acid may be removed and the remaining compound oxidized to provide energy.

As previously stated, enzymes always contain a protein (chain of amino acids) in which the amino acids are combined in a particular sequence, the protein itself having a particular shape or configuration.

There are approximately 20 to 22 known amino acids. Proteins consist of a large number of amino acids combined in a particular sequence. Also, chains of amino acids are crosslinked one to another and the different proteins form special configurations and shapes. Proteins are also sometimes combined with carbohydrates, lipids, or phospholipids (fatlike compounds containing phosphoric acid as part of the molecule). These are called conjugated proteins. The number and sequence of amino acids in the chain, the shape or relationship of one protein chain with another, and conjugation with carbohydrate or lipid all affect the functional properties and determine the manner in which a protein will react to physical and chemical energy.

Enzymes cause chemical reactions to occur at their fastest rates when the temperature is at an optimum level. For most enzymes, this is in the range of 60 to 150°F (15.6 to 65.5°C), but some action may occur at temperatures above or below the optimum

range. Thus, some enzymes are able to react slowly at temperatures well below that of the freezing point of water and others at temperatures above 160°F (71.1°C).

Because proteins are changed chemically and physically or are coagulated by high temperatures, especially when moisture is present, enzymes are usually inactivated at temperatures between 160 and 200°F (71.1 and 93.3°C). There are some exceptions to this, however, and at least one enzyme, which splits off fatty acids from fish phospholipids, is known to remain active even after steaming at 212°F (100°C) for 20 min.

Enzymes have an optimum pH at which they cause reactions to occur at the fastest rate. Water solutions having a pH value less than 7 are said to be acidic; those having a pH value greater than 7 are said to be alkaline; and those having a pH value of exactly 7 are said to be neutral. As in the case of temperatures, some action will occur at pHs above or below the optimum, although there are low and high limits beyond which a particular enzyme action cannot take place.

Module 98: Nature of Enzymes

Enzymes occur naturally in foods, and their presence may be either beneficial or detrimental, depending on the particular enzyme. When the presence of enzymes is undesirable, steps are taken to inactivate them. When their presence is desirable, either the enzymes or sources of the enzymes are intentionally added to foods. For example, the enzyme papain (from the papaya fruit) is added to steak to tenderize it. Many of the useful enzymes used in food processing are produced by microbes; consequently those microbes producing the desired enzyme may be added intentionally to food. For example, specific yeasts are intentionally added in the production of bread, beer, or cheese.

The use of enzymes as food additives presents no problem from the standpoint of safety, because enzymes occur naturally, are nontoxic, and are easily inactivated when desired reactions are completed. Enzymes called amylases are used together with acids to hydrolyze starch in the production of syrups, sugars, and other products.

Module 99: Proteolytic Enzymes

Enzymes involved in the breakdown or splitting of proteins are called proteolytic enzymes or, more simply, proteases. Proteases comprise two general classes: proteinases and peptidases. Proteinases split the protein molecules into smaller fragments called

proteases and peptones, then into polypeptides and peptides. Peptidases split polypeptides and peptides into amino acids. Because most amino acids in foods are water-soluble, food proteins are essentially liquefied by proteinases and peptidases.

In meat, such as beef, pork, or poultry, held in the eviscerated state (intestines and organs removed), the proteases present in the tissue are called cathepsins. The temperatures and times under which these products are held prior to utilization are not such that extensive proteolysis can occur. Therefore, while there may be some tenderization of the tissues during holding, which may, in fact, be due to proteolysis, there is not extensive breakdown of the tissues.

In fish, proteolytic enzymes are much more active than in meats. Even when fish is held in the eviscerated state in ice or under refrigeration, there may be sufficient proteolysis to cause softening of the tissues over a period of days. In fish held in the round (uneviscerated), proteolysis is accelerated owing to a concentrated source of enzymes present in blind tubules (the pyloric ceca) attached to the intestines. Thus, even though fish in the round are refrigerated, within a few days, sufficient proteolysis may occur to dissolve the tissues of the abdominal wall, exposing the entrails. Members of the herring and mackerel families handled in the uneviscerated state are quite subject to this type of enzyme deterioration, especially if they have been feeding when caught. Other fish such as flounders and ocean perch, handled in the uneviscerated state, seem not to be especially subject to this type of deterioration.

Lobsters provide an especially good example of a deteriorative change that may take place through the action of proteolytic enzymes. As long as the lobster is alive, autolytic proteolysis does not occur. However, if the lobster dies and then is held for some hours, even under refrigeration, but especially at high temperatures, proteolysis takes place to such an extent that the lower abdominal portion will be partially liquefied. When such a lobster is cooked, the flesh will be soft and crumbly (short-meated) and part of the tail portion will have dissolved, leaving only a part of this section intact. For this reason, lobsters should never be held long after death prior to cooking. The present accepted practice is to cook lobsters from the live state. Other crustaceans (shrimp and crabs) are also subject to enzyme proteolysis, although with shrimp, this usually is not extensive, especially if the head portion (cephalothorax) is removed shortly after the shrimp are caught. The relatively high activity of enzymes in marine species is attributed to the low-temperature conditions in the marine

environment. That is, in order to make reactions proceed at low temperatures, the activation energy system must be more efficient.

Plants also contain proteolytic enzymes, but these enzymes usually contribute little to deterioration, especially as long as the tissues of fruits and vegetables are not cut or damaged. Some plants provide an excellent source of proteolytic enzymes. Bromelain is found in unpasteurized pineapple juice and is so active that people who handle cut

pineapple that has not been heated must wear rubber gloves; otherwise the skin of the fingers will become eroded, and could result in the exudation of some blood. Papain is a proteolytic enzyme obtained from the latex (milky liquid) of the green papaya fruit. Ficin is a proteolytic enzyme obtained from the latex of certain fig trees. Proteolytic enzymes from plants may be extracted and purified and these enzymes may be employed, for instance, to tenderize meats.

Module 100: Oxidizing Enzymes

Oxidation has been defined as a loss of electrons in an atom or simply a chemical combination with oxygen. An example of complete oxidation is a wick of a candle burning, where the cellulose wick is converted to carbon dioxide and water. There are a number of oxidizing enzymes that bring about changes in foods that result in deterioration due to oxidation. In plants, peroxidases, ascorbic acid oxidase, tyrosinase, and polyphenolases may cause undesirable chemical reactions to occur. Peroxidases may oxidize certain phenollike compounds in root vegetables, such as horseradish, causing the prepared product to become darker in color. This does not happen while the tissues are intact but only when the vegetable has been cut up or comminuted. Ascorbic acid oxidase, present in certain vegetables, oxidizes ascorbic acid (vitamin C) to a form that is readily further oxidized by atmospheric oxygen. The resulting oxidation product is not utilized by humans as a vitamin. Therefore, the action of this enzyme may cause loss of the vitamin C content of foods. Peroxidases may also, indirectly, cause a loss of vitamin C in vegetables. In this case, the compounds formed by the action of peroxidase react with vitamin C. Phenolases are present in some fruits and vegetables. These enzymes oxidize some phenollike compounds, also present in plant product, causing brown or dark-colored compounds to be formed when the tissues are cut.

Tyrosinase oxidizes the amino acid tyrosine to form dark-colored compounds. The molecule rearranges and further oxidizes to form a red compound. Polymerization (combination of these compounds) results in the formation of dark-colored melanin compounds.

The enzyme tyrosinase, which is present in many fruits and vegetables, may cause discoloration of the cut tissue and will also oxidize compounds related to tyrosine. This enzyme is also present in shrimp and some spiny lobsters and may cause a discoloration called black spot. In shrimp, this often occurs as a black stripe on the flesh along the edges of the segments of the tail or as a pronounced band where the shell segments overlap. It is not generally recognized, but tyrosinases are also present in clams. Hence, shucked (deshelled) clams will darken at the surface if oxygen is present and if the enzymes have not been inactivated by heat.

These reactions occur only after the shrimp or clams die. In general, oxidizing enzymes do not cause deteriorative changes in tissues that are intact. In fruits and vegetables, the tissues must be cut or bruised or there must be a breakdown of cells by their enzymes before the action of oxidizing enzymes results in discoloration.

Module 101: Fat-Splitting Enzymes

Fats are composed of glycerine (glycerol) and fatty acids. Glycerine is a polyhydric alcohol (three alcohol groups), and fatty acids are short or long chains of carbon atoms to which hydrogen is attached, either to the fullest possible extent (saturated) or to a lesser extent (unsaturated), the latter resulting in reactive groups in the chain. At one end of the fatty acid chain there is an acid group.

In the formation of fats, each one of three fatty acids combines with one of the three alcohol groups of glycerine, splitting off water in each case. On the other hand, in the breakdown of fats, water and the enzyme lipase are present, and fats are split into their original component parts, glycerine and fatty acids.

The fatty acids in most fats that are found in nature consist of a chain of more than 10 carbon atoms, and these fatty acids have no particular flavor or odor. Hence, when lipase acts on most natural fats, no bad odors are generated. However, if fats or oils high in free fatty acid content (indicating deterioration) are used for deep-fat frying, the oil may smoke during heating, which is undesirable.

There are some fats that contain short-chain fatty acids, especially those fats present in the milk of cows or goats. These fats contain butyric (four carbons), caproic (six carbons), caprylic (eight carbons), and capric (ten carbons) acids. All these fatty acids have an odor and flavor. Butyric acid, especially, is pungent and considered to be distasteful. When lipase acts on butter, therefore, it splits off butyric acid, which gives the butter a strong, undesirable rancid taste. Actually, butter is an emulsion of water in oil and contains about 16% water, the water being present as fine droplets. Butter becomes rancid by the action of lipase produced by bacteria that grow in the water droplets. The lipase acts on the fat surrounding the water droplets. Lipase rancidity in butter, therefore, is really a type of deterioration caused by bacteria.

Phospholipases, enzymes similar to lipase, are split phospholipids. Most phospholipids are similar to fats in that they contain glycerine, two alcohol groups of which are combined with fatty acids. The third alcohol group in this case is combined with a molecule containing phosphoric acid, a short chain of carbons, and a nitrogen group with carbons attached to it. Lecithin is a typical phospholipid. Phospholipase splits off fatty acids from phospholipids. Such action may cause deterioration in foods in that it results in a destabilization of proteins that causes a toughening of the tissues and a loss of succulence (juiciness).

Module 102: Enzyme that decompose carbohydrates

Fruits contain pectin which supports the particular structure of the product. In processed fruit juices (for instance, tomato or orange juice), if the pectin is broken down, the solids tend to settle to the bottom, leaving a clear serum on top. Pectin consists of a long chain of galacturonic acid molecules with the carboxyl groups partially esterified with methyl alcohol. It has a high water-holding capacity. There are pectic enzymes that will either break down the pectin molecule to smaller units or completely decompose the molecule to its primary unit, galacturonic acid. The emulsifying properties of pectin may be lost, causing settling in fruit juices and softening in fruit. When the pectin in whole fruit breaks down, it may result in deterioration of the fruit as a result of the action of other enzymes or invasion of the tissues by microorganisms.

In the sugar cane plant, there is an enzyme, invertase, that breaks down cane sugar (sucrose), which has 12 carbon atoms, into glucose and fructose, each having six carbon atoms. Before sugar cane is harvested, therefore, apart of the plant must be removed to

eliminate the source of the enzyme. Were this not done, there would be a loss of sucrose during the processing of the cane. Many other carbohydrases, which break down cellulose or starch or break down more complex sugars to smaller units, exist.

Module 103: Glucose oxidase

Glucose oxidase is an enzyme that specifically catalyzes the oxidation of glucose to gluconic acid. This reaction is important in preventing nonenzymatic browning, because glucose is a reactant in the undesirable browning reaction. The most important application of this enzyme is in the treatment of egg products, especially egg whites, prior to drying. Eggs treated with this enzyme before they are dried do not undergo nonenzymatic browning during storage, because the sugar has been removed. In some cases, the enzyme is added to remove traces of oxygen to prevent oxidative degradation of quality. Examples of this type of application are mayonnaise and bottled and canned beverages (especially beer and citrus drinks).

Module 104: Catalases

Catalases are used to break down hydrogen peroxide (H_2O_2) to water and oxygen. Therefore, catalases are used when the presence of hydrogen peroxide is undesirable or when hydrogen peroxide is used for specific purposes, such as in bleaching, but then must be removed from the system. Examples of the latter case are the uses of hydrogen peroxide for preserving milk in areas where heat pasteurization and refrigeration are unavailable and in the manufacture of cheese from unpasteurized milk. Hydrogen peroxide is produced during the spray-drying process. Catalase is used to convert the unwanted H_2O_2 to water and oxygen.

Module 105: Application of Enzymes

Whereas enzymes may cause a deterioration of foods, they may also be used in the processing of foods to produce particular products or to modify the characteristics of particular products. Proteolytic enzymes obtained from plants may be used for tenderizing meat either by injecting animals with a solution of the enzyme prior to slaughter or by sprinkling the powdered enzyme on meat surfaces and allowing it to react, prior to cooking. In the manufacture of certain kinds of milk powder (e.g., to be used in chocolate), the lipases may be allowed to act on the milk fat prior to drying to obtain particular flavor in the finished product. Proteases may also be used to chill-proof beers, a technique used to remove

proteins that would cause clouding during cool storage. Protein hydrolysates can be produced from the use of proteases from both plant and animal sources. They have many uses, for example, as flavorings, nutrients, and stabilizers to improve texture.

The characteristic flavor of certain cheeses is due to the action of lipases on the milk fat contained therein. In order to obtain the particular flavors of Roquefort, Gorgonzola, or blue cheese, the milk fat must first be broken down to fatty acids that can then be oxidized. The lipases that decompose the fats to provide fatty acids are produced by the molds allowed to grow in these cheeses. Enzymes produced by molds then oxidize the specific fatty acids that ultimately produce the unique characteristic flavors. Lipases also can be added to dried egg whites to improve the whipping quality.

There are many applications of enzyme technology that involve the use of carbohydrate-splitting enzymes. In making malt, barley is germinated to obtain an enzyme that will convert starch to a sugar (maltose), which can be converted by yeasts to ethyl alcohol and carbon dioxide. By this means, various grains can be used as the source of sugar for fermentation. Carbohydrate-splitting enzymes are also used to modify starches used in foods and to modify starches used in sizing and laundering clothes. Others are used to form corn syrup from corn starch and then isomerize glucose to fructose in the formation of high-fructose corn syrup which is used extensively in the manufacture of soft drinks. Invertase (sucrase) is used in the production of chocolate-covered cherries. The cherries are rolled in a mixture of crystalline sucrose and the enzyme before they are covered in chocolate. During a short holding period, the enzyme splits the sucrose into a mixture of glucose and fructose, which has a higher water solubility and results in the sweet taste and creamy texture that is characteristic of the product. The presence of pectins in fruit juices may cause clouding. Pectinases can be added to remove the pectin and clarify the juice.

There are many other applications of enzyme technology in the food and other industries and it is expected that the number of applications for enzymes will continue to increase. One of the factors that will serve to widen the use of enzyme technology is the development of immobilized enzymes. It has been found that enzymes can be fixed chemically to the surface of inert substances, such as glass beads. In this form, they can be packed into a column through which a solution or suspension of the material to be acted on (called the substrate) is allowed to pass. In this manner, the enzyme responsible for the conversion or change in the substrate is not lost or washed out with the substrate. Thus, the

enzyme can be used for a number of substrate conversions. Moreover, in this form (immobilized enzyme), the active agent is much less subject to inactivation such as, for instance, by high temperature.

Restoration and enrichment of flavor in some processed foods can be accomplished by addition of enzymes. Examples of this include the conversion of allin in garlic into garlic oil by allinase and the addition of an enzyme preparation from mustard seeds to dehydrated cabbage which restores flavor by converting flavor precursors into the volatile sulfur-containing compounds responsible for the familiar flavors.

They may also prevent the formation of large ice crystals in frozen desserts. A significant potential for the use of gums lies in the production of certain low-calorie foods. For example, the oil in salad dressing can be replaced with gums to result in a product with the normal appearance, texture, and taste but without the calories normally associated with the product.

Lecture 12

FOOD ADDITIVES**Module 106: Introduction to Food Additives**

In September of 1958, the FD&C Act was amended to prohibit the use of food additives that had not been adequately tested to establish their safety. The term food additive was defined as follows:

“any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including any substance intended for use in producing, preparing, treating, packaging, transporting or holding food; and including any source of radiation intended for any such use), if such substance is not generally recognized among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or in the case of a substance used in food prior to January 1, 1958, through either scientific procedures, or experience based on common use in food) to be safe under the conditions of its intended use ...”

An additive may be reactive or inactive; it may be nutritive or nonnutritive; it should be neither toxic nor hazardous. Some substances, such as pesticides, are added to foods unintentionally, and these are, of course, undesirable, and may be hazardous to health. Because of their toxicity, their presence is closely regulated by strict government tolerance guidelines.

Module 107: Philosophy of Food Additives

Foods are made entirely of substances that, in the pure form, can be described as chemicals or chemical compounds. It is important to note that our knowledge of the composition of foods, because of its complexity, is by no means complete. For instance, it is reported that one of the most important of our natural foods, human milk, contains several hundred chemical compounds.

Unfortunately, the interpretation of the word *chemical* is too often inaccurate. Thus, some consumers are apprehensive about purchasing a food that is preserved by treating it with a chemical with which they are unfamiliar. However, a number of foods may be preserved with table salt, which is a chemical. Consumers are not apprehensive about

using salt as a preservative, because they are familiar with it, at least for adding taste and sometimes for bringing out the flavor in foods, yet table salt is definitely a chemical, with the name sodium chloride, and the formula NaCl. Refined sugar, vine-gar, spices, and other substances that are routinely added to foods are also chemicals or mixtures of chemicals, and the use of these is not questioned. The characteristics of chemicals that we use with confidence are familiarity and frequent use. The characteristics of chemicals that arouse skepticism in consumers are that they are uncommon and unfamiliar.

A large number of chemical additives are unfamiliar, and there is a need for regulatory agencies to question their use from the standpoint of safety. Obviously then, we should not fear the use of chemicals, but we do need to screen them for safety when their effects on human health are not known. Some lessons have been learned along these lines. For example, indiscreet use of certain additives for coloring candy and popcorn was reported to have caused diarrhea in children, resulting in the removal of these dyes from the FDA approved list of additives. There are a number of related concepts that must be remembered when dealing with food additives:

All foods are composed of chemical compounds, many of which can be extracted and added to other foods, in which case they are classified as additives.

Any additive or chemical compound can be injurious to health when particularly high levels of that compound are added to foods.

Any additive or chemical compound can be safe to use when particularly low levels of that compound are added to foods.

It is necessary to evaluate each additive for usefulness and toxicity in a sensible, scientific way, regardless of how safe its proponents say it is and how toxic its opponents say it is.

The use of radiation for preserving foods has been declared an additive, and whether or not it should be approved by the FDA makes it the prime example of extreme opposition and extreme favor. Quite often, the tendency to take a strong position for the use of an additive might make a proponent rationalize or overlook undesirable investigative scientific data concerning the additive. On the other hand, opponents tend to make irrational demands of investigators to prove the safety of an additive; for example, opponents of the

use of radiation for preserving foods have suggested that radiation should not be approved for preserving foods until all possible chemical effects of the process have been identified. This, without going into detail, is an impossible task. It would be just as impossible to identify all the chemical effects of frying foods and of baking food.

Given present capabilities, our most reasonable evaluation of an additive for safety can be made through conventional animal feeding studies. The overall physiological effects that an additive may have on animals of two or three different species over a specified number of generations is the most comprehensive, as well as the most reliable, way to evaluate the safety of a food additive.

It should be remembered that chemical materials cannot be added to foods unless their use, in the quantities added, has been approved by the FDA. Moreover, additives are tested for toxicity in concentrations much greater than those allowed in foods. It should also be remembered that most food additives are components of natural foods and that without these additives the quality of many foods would be greatly inferior to that to which we have become accustomed. The shelf-life or availability of many foods would also be greatly limited were all additives to be eliminated from foods. Food additives are difficult to classify mainly because they overlap each other in numerous combinations of effects. It should be remembered, therefore, that the following classifications are not precise.

Module 108: Antioxidants in Foods

Antioxidants are food additives used to stabilize foods that by their composition would otherwise undergo significant loss in quality in the presence of oxygen. Oxidative quality changes in foods include: (1) the development of rancidity from the oxidation of unsaturated fats resulting in off-odors and off-flavors and (2) discoloration from oxidation of pigments or other components of the food.

There is a large number of antioxidants, and although they may function in different ways, the purpose of each is to prevent, delay, or minimize the oxidation of the food to which they are added. One of the ways by which some antioxidants function involves their combination with oxygen. Others prevent oxygen from reacting with components of the food. When only a limited amount of oxygen is present, as in a hermetically sealed container, it is possible for some antioxidants to use up all of the available free oxygen, because they have a relatively great affinity for it. Some antioxidants lose their effectiveness when they combine with

oxygen; therefore, there is no advantage to using this type of antioxidant unless the food is enclosed in a system from which oxygen or air can be excluded. With the use of antioxidants, it should be noted that other precautions are necessary to minimize oxidation,



because heat, light, and metals are prooxidants, that is, their presence favors oxidative reactions. Many of the antioxidants used commercially occur naturally in foods (e.g., vitamin C, vitamin E, citric acid, amines, and certain phenolic compounds). However, the amines and the phenolic compounds can be toxic to humans in low concentrations; therefore their use and that of synthetic antioxidants require strict regulation. It should be pointed out that the potency of the naturally occurring antioxidants is not as great as that of the commonly used synthetic antioxidants. The antioxidants that are considered to be the most effective and therefore are most widely used are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propylgallate. These are generally used in formulations that contain combinations of two or all three of them, and often in combination with a fourth component, frequently citric acid. The main purpose of adding citric acid is that it serves as a chelator or sequestrant (a chelator ties up metals, thereby preventing metal catalysis of oxidative reactions).

Fats and shortenings, especially those used in bakery goods and fried foods, are subject to oxidation and the development of rancidity after cooking. To prevent this, chemical antioxidants in concentrations up to 0.02% of the weight of the fat component may be added.

Module 109: Nutrient Additives

The need for a balanced and ample nutrient intake by the human body is well known. Although nutrients are available in foods, losses of fractional amounts of some of them through processing and increasing frequency of improper dieting have led to the practice of adding minimum daily requirements or sizable fractions of minimum daily requirements of a number of nutrients to popular foods, such as breakfast cereals, baked goods, pasta products, and low-calorie breakfast drinks. Nutrient additives include mainly vitamins, proteins, and minerals.

Vitamin D is an exceptional example of the value of the food additive concept. The major source of vitamin D for humans is a precursor compound called 7-dehydrocholesterol which is produced in the liver. It circulates to an area just under the skin and is converted to previtamin D₃ by the ultraviolet rays of sunlight. Previtamin D₃ then goes through a number of steps and is converted to vitamin D₃ and finally to active vitamin D. However, in

many cases, exposure to the sun is sporadic and insufficient, especially in areas where there is normally insufficient sunshine or in cases where sunlight exposure is of insufficient duration. Thus, vitamin D is added to nearly all commercial milk in a ration of 10 micrograms of vitamin D (as cholecalciferol). This is equivalent to the old 400 IU per quart (0.95 liter). Vitamins A and C and some of the B vitamins are also added to some foods.

The addition of protein concentrate (produced from fish or soybeans) to components of the diet of inhabitants of underdeveloped countries has been used successfully to remedy the high incidence of protein malnutrition. It should be noted that soybean protein is incomplete and requires the addition of some amino acids in which it is deficient. Children, especially, succumb in large numbers to the disease, kwashiorkor, that results from insufficient protein intake.

Module 110: Flavorings and Flavor Enhancers

Flavorings are compounds, natural or synthetic, that are added to foods to produce flavors or to modify existing flavors. In the early days of human existence, salt, sugar, vinegar, herbs, spices, smoke, honey, and berries were added to foods to improve their taste or to produce a special, desirable taste. The variety of natural and synthetic flavorings available to the modern food technologist is very large. Essential oils provide a major source of flavorings. Essential oils are odorous components of plants and plant materials that give the characteristic odors of the materials from which they are extracted.

Because of the large production of orange juice, quantities of essential oil of orange are produced as byproducts. For this reason, there is little need for the production of synthetic orange flavoring.

Fruit extracts have been used as flavorings, but these are relatively weak when compared to essential oils and oleoresins. An oleoresin is a solvent extract of spices from which the solvent, usually a hydrocarbon, has been removed by distillation. Because of their weak effects, fruit extracts may be intensified by combining them with other flavorings.

Synthetic flavorings are usually less expensive and more plentiful than natural flavorings. On the other hand, natural flavorings are often more acceptable, but they are quite complex and difficult to reproduce synthetically. In fact, one of the problems with natural flavorings is that they may vary according to season and other uncontrollable factors. Synthetic flavorings, however, can be reproduced quite accurately. Many artificial flavors, such as

amyl acetate (artificial banana flavor), benzaldehyde (artificial cherry flavor), and ethyl caproate (artificial pineapple flavor), are added to confectioneries, baked products, soft drinks, and ice cream. These flavorings are added in very small amounts, often 0.03% or less.

Flavor Enhancers:

Flavorings either impart a particular flavor to food or modify flavors already present. Flavor enhancers, on the other hand, intensify flavors already present, especially when the desirable flavors are relatively weak. Monosodium glutamate (MSG) is one of the best known and most widely used flavor enhancers. This compound occurs naturally in many foods and in a certain seaweed that was used for centuries as a flavor enhancer in soups and other foods. It is only within the last hundred years that the reason for the effectiveness of the seaweed was discovered to be MSG. While it is effective at relatively low levels (parts per thousand), there are other compounds called flavor potentiators that also enhance flavors but are extremely powerful, effective in parts per million and even per billion. These compounds have been identified as nucleotides, and their effect is attributed to their synergistic properties (properties that intensify the effect of natural flavor components). Two in this group are disodium inosinate and disodium GMP.

Several theories attempt to explain how MSG and other flavor enhancers and potentiators work. One theory is that they increase the sensitivity of the taste buds, thus increasing flavor. A second suggests that an increase in salivation as a result of the flavor enhancers will increase flavor perception. A third theory of intensified flavor perception is based on the observation that flavor enhancers produce certain physical sensations in the mouth such as coolness and heat.

Module 111: Acidulants in foods

From the root word, *acid*, in acidulants, one can conclude that this class of compounds tends to lower the pH of any food into which the compounds are incorporated. Acidulants also enhance desirable flavors, and in many cases, such as in pickled products, are the major taste component. Vinegar (acetic acid, CH_3COOH) is added to relishes, chili sauce, ketchup, and condiments as a flavor component and to aid in the preservation of these products. Because the microbial spoilage of food is inhibited as the pH is lowered, acidulants are used for that purpose in many cases. Many acidulants occur naturally in foods

Ce.g., citric acid in citrus fruits, malic acid in apples, acetic acid in vinegars; all three are contained in figs). flavor. Citric acid is widely used in carbonated soft drinks. Phosphoric acid is one of the very few inorganic acids used as an acidulant in foods. It is widely used, comprising 25% of all the acidulants in foods. Citric acid accounts for 60% of all acidulants used in foods.

In addition to their preservative and flavor enhancing effects, acidulants are used to improve gelling properties and texture. Acidulants are also used as cleaners of dairy equipment.

Acidulants may be used in the manufacture of processed cheese and cheese spreads for the purpose of emulsification as well as to provide a desirable tartness.

Acid salts may be added to soft drinks to provide a buffering action (buffers tend to prevent changes in pH) which will prevent excess tartness. In some cases, acid salts are used to inhibit mold growth Ce.g., calcium propionate added to bread).

Lactose

Lactose ($C_{12}H_{22}O_{11}$), the sugar component of mammalian milk, is less sweet and less water-soluble than sucrose. Although most babies and young children generally are able to metabolize this sugar, some are unable to do so. The ability to metabolize the sugar appears to decrease with age. When a person is unable to metabolize lactose, the ingestion of milk may cause intestinal discomfort, cramps, and diarrhea. The major source of lactose is whey, a cheese byproduct. Because lactose is not as sweet as sucrose, larger amounts can be used in those foods in which the texture benefits from a high solids content.

Maltose

Maltose ($C_{12}H_{22}O_{11}$), or malt sugar, is produced during the malting process in brewing (enzyme conversion of starch). It is converted to alcohol by the action of yeasts through an intermediate conversion to dextrose. This sugar is much less sweet than sucrose, and it is used mainly in the manufacture of baked goods and infant foods.

Xylitol

Xylitol is a polyhydric alcohol having the formula $(C_5H_{10}O)_5$. At present it is used as a sweetener in chewing gum, mainly because of its noncariogenic property (it has not

been found to cause tooth decay). It occurs naturally as a constituent of many fruits and vegetables, and is an normal intermediary product of carbohydrate metabolism in humans and in animals. Commercially, it is produced by the hydrolysis of xylan (which is present in many plants) to xylose, which is then hydrogenated to produce xylitol. The xylitol is then purified and crystallized. Xylitol imparts a sweet taste, which also appears to have a cooling effect. As it is not metabolized by many microorganisms, it is quite stable.

Sorbitol

As has been pointed out, all microorganisms have a pH at which they grow best, and a range of pH above or below which they will not grow. Generally, it is not possible to preserve all foods by adding acid to the point where microorganisms will not grow. Most foods would be too acid to be palatable. The amount of acid may be enough to inhibit the growth of microorganisms provided that such treatment is combined with some other method of preservation. Certain dairy products, such as sour cream, and fermented vegetables, such as sauerkraut, are preserved with lactic acid produced by the growth of bacteria. Addition of the acid, along with holding at refrigerator temperatures above freezing, in combination will prevent growth of pathogenic and spoilage organisms. When sauerkraut is canned, it is given a heat process sufficient to destroy all spoilage and disease microorganisms.

Pickles are preserved by the addition of some salt, some acid, and a heat process sufficient to raise the temperature of all parts of the food to or near 212°F (100°C).

Pickled herring are preserved by the addition of some salt, some acetic acid (vinegar), and holding at refrigerator temperatures above freezing. In this case, the nonacid part of the acetic acid molecule has an inhibiting effect on the growth of microorganisms.

Module 112: Alkaline Compounds in Foods

Alkaline compounds, such as sodium hydroxide or potassium hydroxide, may be used to neutralize excess acid that can develop in natural or cultured fermented foods. Thus, the acid in cream may be partially neutralized prior to churning in the manufacture of butter. If this were not done, the excess acid would result in the development of undesirable flavors. Sodium carbonate and sodium bicarbonate are used to refine rendered fats. Alkaline compounds are also added to chlorinated drinking water to adjust the pH to high enough levels to control the corrosive effects of chlorine on pipes and equipment. Sodium carbonate is also used in conjunction with other compounds to reduce the amount of hardness in

drinking water. Sodium hydroxide is used to modify starches and in the production of caramel. Sodium bicarbonate is used as an ingredient of baking powder, which is used in baked products. (It is also a common household item used in a variety of cooking recipes.). Alkaline compounds are used in the production of chocolate and to adjust the acidity level in grape juice and other fruit juices that are to be fermented in the production of wine.

It is important to note that some alkaline compounds, such as sodium bicarbonate, are relatively mild and safe to use, while others, such as sodium hydroxide and potassium hydroxide, are relatively powerful reagents and should not be handled by inexperienced people.

Module 113: Sweeteners

Sweetening agents are added to a large number of foods and beverages. Table sugar (sucrose), the most commonly used sweetener in the country, and corn syrup, are covered in and therefore are not described in any detail here. Sweeteners include other sugars, as well as an abundance of natural and synthetic agents of varying strengths and caloric values.

Many sweeteners are classified as nonnutritive sweeteners. Although this classification might imply a lack of nutritional value, the implication is correct only in a relative sense. That is, the caloric value of a nonnutritive sweetener, such as aspartame, is about 4 cal/g, the same as that for sugar. However, because it takes only 1 g of aspartame to provide the same sweetness level as about 180 g of sugar (sucrose), it can be seen that the caloric contribution of aspartame is only about 0.5% that of sucrose. It is on this basis that a nonnutritive sweetener is classified as such.

Fructose

Of the natural sugars other than sucrose used by humans, fructose (also known as levulose), a monosaccharide ($C_6H_{12}O_6$), is the sweetest (nearly twice as sweet as table sugar, sucrose) and it is the most water-soluble. It is hygroscopic, making it an excellent humectant when used in baked goods. The value of a humectant in baked goods is that it retards dehydration. Solutions of fructose have a low viscosity that results in lower "body" feel than sucrose but have greater flexibility of use over a wide range of temperatures. Because of its greater solubility and more effective sweetness than sucrose, fructose is a better choice than sucrose when very sweet solutions are required, as fructose will not crystallize out of solution, whereas sucrose will. Fructose has sometimes been called fruit

sugar, since it occurs in many fruits and berries. It also occurs as a major component in honey, corn syrup, cane sugar, and beet sugar. **In** fact, sucrose, a disaccharide, is composed of glucose and fructose. Of these two components, the glucose moiety, or portion, cannot be metabolized by people with diabetes, and it is for this reason that the ingestion of sucrose cannot be tolerated by them. Fructose, on the other hand, does not require insulin for its metabolism and can, therefore, be used by diabetic individuals. Its use also appears to reduce the incidence of dental caries. When used with saccharin, it tends to mask the bitter aftertaste of saccharin. As it apparently accelerates the metabolism of alcohol, it has been used to treat those suffering from overdoses of alcohol. It has been recommended as a rapid source of energy for athletes and, in combination with gluconate and saccharin, as an economic, effective, safe, low-calorie sweetener for beverages.

Molasses

Molasses can be considered a byproduct of sugar production. The use of molasses as a sweetener in human foods is largely in baked goods that include bread, cookies, and cakes. **In** addition to sweetening, molasses adds flavor and acts as a humectant. It is also used in baked beans and in the production of rum and molasses alcohol. (The greatest use of molasses, however, is in the production of animal feed). Molasses comprises about 60% sucrose, but the sucrose content can be lower, depending on the grade of the molasses and on the raw material from which it was produced. Thus, the sucrose content of cane blackstrap (the final fraction of cane molasses) is only about one-half that of beet blackstrap (the final fraction of beet molasses). The fractions produced before the blackstrap are of higher grades and are those usually used for human consumption. Blackstrap generally is used for industrial purposes.

Honey

Honey, a natural viscous syrup, comprises mainly invert sugar. It is produced from the nectar of flowers, which is mainly sucrose, by the action of an invertase enzyme that is secreted by the honey bee. Honey is used as a direct sweetener, as an additive in a number of products, including baked goods, as well as in other ways. It is relatively expensive.

Maple Sugar

Maple sugar is produced from the sap of the sugar maple tree. It is comprised mainly of sucrose and small amounts of other sugars, including invert sugar. Maple sugar is

used in the manufacture of candies, fudge, baked goods, and toppings. It is among the most expensive of sweeteners.

Sorbitol is a polyhydric alcohol ($C_6H_5(OH)_5$) that is found in red seaweed and in fruits (apples, cherries, peaches, pears, and prunes). It was first isolated from the sorb berries of the mountain ash, hence its name. It is used as an additive because of its humectant property as well as its sweetening effect. It is used in cough syrup, mouthwashes, and toothpastes. Another of its desirable properties is that it is not easily fermented by microorganisms. Because sorbitol is largely transformed to fructose by liver enzymes in the body, it is tolerated by diabetic individuals, as fructose is not dependent on the availability of insulin for its metabolism. Sorbitol can be produced industrially by the electrochemical reduction or catalytic hydrogenation of glucose.

Mannitol

Mannitol is a polyhydric alcohol having the formula ($C_6H_5(OH)_6$). It is used in chewing gum, pharmaceuticals, and in some foods. It is a naturally occurring sweetener in many plants, algae, and mold. It occurs in the sap of the manna tree, an ash native to southern Italy, and can also be made by the reduction of either of the monosaccharides mannose or galactose. Industrially, it is produced by electrochemical reduction or catalytic hydrogenation methods. Although it is similar to sorbitol in many respects, it is less soluble than sorbitol.

Aspartame

Aspartame is the common name for aspartyl-phenylalanine. It is a combination of the two amino acids from which its name is derived. First produced in 1969, it is reputed to be about 180 times sweeter than sucrose. Like cyclamate, it was approved and later banned by the FDA. Exhaustive evidence of its safety has been presented by animal testing and by definition of its metabolic fate in animals and humans. It was subsequently reinstated as safe for use by the FDA.

Saccharin

Saccharin, the imide of o-benzosulfonic acid, is used as a sodium or calcium salt. It is about 300 times sweeter than sucrose (table sugar). It may leave a bitter aftertaste, and its safety

has been questioned as a result of some animal feeding tests. As an intense sweetener it is useful for diabetic individuals, and it reduces the incidence of dental caries.

Module 114: Starches

Although starches differ from each other somewhat, depending on the plant from which they are extracted, they are sufficiently similar chemically to be often classified together as starch. The two basic starch polymers are amylose and amylopectin. Starch is used as a source of carbohydrate, and because it is relatively inexpensive, is often used as an extender. Its properties also make it useful as a thickening agent. The major source of starch is corn, but some starch is also produced from sorghum, potatoes, and wheat.

Module 115: Gums

Gums, a class of complex polysaccharides, are defined as materials that are dispersible in water and capable of making the water viscous. Many gums occur naturally in certain land and sea plants. Examples are gum arabic and agar. Many gums, such as the cellulose derivatives, are modified or semisynthetic, and some gums, such as the vinyl polymers, are synthetic. Gums are used to stabilize ice cream and desserts, thicken certain beverages and preserves, stabilize foam in beer, emulsify salad dressings, and form protective coatings for meat, fish, and other products. Gums add "body" and prevent settling of suspended particles in chocolate milk, ice cream, and desserts.

They may also prevent the formation of large ice crystals in frozen desserts. A significant potential for the use of gums lies in the production of certain low-calorie foods. For example, the oil in salad dressing can be replaced with gums to result in a product with the normal appearance, texture, and taste but without the calories normally associated with the product.

Module 116: Polyhydric Alcohols

In addition to their use as sweeteners, many polyhydric alcohols (also called polyols) are used to improve texture and moisture retention because of their affinity for water. Many polyols are present in foods naturally, glycerine (glycerol) being the predominant one. However, only four of the many polyols are allowed as food additives. They are glycerine, sorbitol, mannitol, and propylene glycol. All but the last have a moderately sweet taste, although none are as sweet as sugar. Propylene glycol has a somewhat undesirable bitter

taste, but is acceptable in small amounts. Sorbitol imparts a cool sensation. Glycerine, on the other hand, imparts a hot sensation.

Polyols are used in the production of dietetic products including beverages, candy, gum, and ice cream to contribute to texture as well as to sweetness. These compounds have a less adverse effect on teeth than sugar, because they are not fermented as quickly as sugar and are usually washed away before they can be utilized by microorganisms.

Module 117: Surface Active Agents

Surface-active agents affect the physical force at the interface of surfaces. Commonly called surfactants, they are present in all natural foods, because by their nature they play a role in the growth process of plants and animals. They are defined as organic compounds that affect surface activities of certain materials. They act as wetting agents, lubricants, dispersing agents, detergents, emulsifiers, solubilizers, and so forth. One use for wetting agents is to reduce the surface tension of materials to permit absorption of water by the material. An example of their use is in powdered chocolate mixes used to prepare chocolate milk by addition of water.

Dispersions of materials depend on the reduction of interfacial energy, and this can be accomplished by certain surfactants. Surfactants are used in the production of foods to prevent sticking, such as in untreated peanut butter. Surfactants are also used in cleaning detergents used on food equipment, and they can stabilize or break down foams. Emulsifiers, such as lecithin, mono- and diglycerides, and wetting agents, such as

class of chemicals known as "tweens," may be added to bakery products (to improve volume and texture of the finished products and the working properties of the dough and to prevent staling of the crumb), cake mixes, ice cream, and frozen desserts (to improve whipping properties). Except for the tweens, the chemicals cited above are natural components of certain foods.

Module 118: Leavening Agents

Leavening agents are used to enhance the rising of dough in the manufacture of baked products. Inorganic salts, especially ammonium and phosphate salts, favor the growth of yeasts, which produce the carbon dioxide gas that causes dough to rise. Chemical

reagents that react to form carbon dioxide are also used in baked goods. When sodium bicarbonate, ammonium carbonate, or ammonium bicarbonate is reacted with potassium acid tartrate, sodium aluminum tartrate, sodium aluminum phosphate, or tartaric acid, carbon dioxide is produced. Baking powder is a common household leavening agent that contains a mixture of chemical compounds that react to form carbon dioxide, producing the leavening effect. Baking powder can be either single acting or double acting, giving the desired leavening effect in different products. The chemistry is shown below.

Single-Acting Baking Powder (Quick-Acting Baking Powder);

The CO₂ is liberated when sodium bicarbonate (the base) reacts with potassium acid tartrate (the potassium salt of tartaric acid).

Double-Acting Baking Powder:

The double-acting type has two acid-reacting ingredients (monocalcium phosphate monohydrate and sodium aluminum sulfate). The hydrated form of monocalcium phosphate reacts with sodium bicarbonate to release a portion of CO₂ during mixing a batter or dough. The remaining sodium bicarbonate will react with sulfuric acid which is produced from the sodium aluminum sulfate in hot water.

In this reaction, the major portion of the CO₂ is released after the product is heated in the oven.

Module 119: Chemical Preservatives

The practice of preserving food by the addition of chemical is quite old, ordinary table salt (sodium chloride) having been used as a preservative for centuries. It might be surprising to think of a naturally occurring substance as a chemical preservative, but many chemical substances used in the preservation of foods occur naturally. When they are used with the proper intent, they can be used to preserve foods that cannot be easily preserved by other means. They should not be used as a substitute for sanitation and proper handling procedures. Sometimes chemicals are used together with other processes, such as holding at refrigerator temperatures above freezing.

To preserve food, it is necessary either to destroy all of the spoilage microorganisms that contaminate it or to create and maintain conditions that prevent the microbes from carrying out their ordinary life processes. Although preservation is aimed

mainly at microbial spoilage, it must be remembered that there are other types of spoilage factors, such as oxidation.

Although foods can be sterilized (such as by heat processing) and contained in such a way as to prevent contamination by microbes during storage, it still is often necessary in some cases to forego sterilization, thus making it necessary to take other steps to prevent microbial degradation of the food. Foods can be protected against microbial attack for long periods (months to years) by holding them at temperatures below freezing. They can be preserved for shorter periods (several days) by holding them in ice or in a refrigerator at temperatures in the range 32 to 40°F (0 to 7.8°C). Foods can also be preserved by altering them to make them incapable of supporting microbial growth. Drying is an example of this type of preservation. Foods must also be preserved against color and texture changes.

Quite often it is either impossible or undesirable to employ conventional preservation methods, and a large variety of food additives is available for use, alone or in combination with other additives or with mild forms of conventional processes, to preserve foods. Usually, chemical preservatives are used in concentrations of 0.1% or less. Sodium diacetate and sodium or calcium propionate are used in breads to prevent mold growth and the development of bacteria that may produce a slimy material known as rope. Sorbic acid and its salts may be used in bakery products, cheeses, syrups, and pie fillings to prevent mold growth. Sulfur dioxide is used to prevent browning in certain dried fruits and to prevent wild yeast growth in wines used to make vinegar. Benzoic acid and sodium benzoate may be used to inhibit mold and bacterial growth in some fruit juices, oleomargarines, pickles, and condiments. It should also be noted that benzoic acid is a natural component of cranberries.

Salt is an excellent microbe inhibitor, mainly as a result of its suppression of the water activity of the material to which it is added. Its effectiveness is enhanced when the food is also dried or smoked or both. Smoking also imparts a partial preservative effect.

Weak acids, such as sorbic acid, or salts of weak acids, benzoates, propionates, nitrites, certain chelating agents (chemicals that tie up metals and prevent the catalytic action of metals), and other chemical additives are effective preservatives. Natural spices also have antimicrobial properties. Antibiotics have been used as food additives and are still used to preserve animal feeds and human foods in some countries. Their use in human foods is banned in the United States and in some other countries. Because many antimicrobial

agents are generally toxic to humans, their use must be regulated not to exceed established levels beyond which they are hazardous to human health.

Nitrites, proven inhibitors of *Cl. botulinum*, and nitrates are added to cured meats, not only to prevent botulism, but also to conserve the desirable color as well as add to the flavor of the products.

Module 120: Food Colorants

We are accustomed to specific colors in certain food, and colors often provide a clue to the quality of the foods. Color additives can be categorized into three major types: natural, nature-identical, or synthetic.

Many colorants (compounds that add colors to foods) are natural, and these include the yellow from the annatto seed; green from chlorophyll; orange from carotene; brown from burnt sugar; and red from beets, tomatoes, and the cochineal insect. Natural colors are simply pigments obtained from animal, vegetable, or mineral sources.

If synthetic counterparts of colors and pigments are derived from natural sources, the term "nature-identical" applies. These include the pure carotenoids such as can-thaxanthin (red), apocarotenal (orange-red), and beta-carotene (yellow-orange). These have all gone through toxicological studies and are approved by the FDA. Canthaxanthin and apocarotenal have maximum addition limits but beta-carotene can be added at the necessary level to accomplish its intended purpose.

Some colorants, however, are derived from synthetic dyes. The synthetic dyes in use have been approved and certified by the FDA. These certified color additives are divided into two groups: FD&C dyes and FD&C lakes. Dyes are water-soluble and are available in powders, granules, liquids, blends, and pastes. GMPs suggest that they not be used in amounts exceeding 300 ppm. The lakes are water-soluble FD&C certified dyes on a substratum of aluminum hydrate or aluminum hydroxide. The lakes must also be certified by the FDA. They are useful in foods that have very little water such as coloring oils. They are used in icings, fondant coatings, cake and doughnut mixes, hard candy, and gum products. They do not solubilize as do dyes but color by dispersion rather than solution. Some compounds are not color additives but are used to produce a white color. Thus, oxidizing agents including benzoyl peroxide, chlorine dioxide, nitrosyl chloride, and chlorine are used at the end of the production cycle to whiten wheat flour, which is pale yellow in color if untreated. Titanium

dioxide, on the other hand, is considered a color additive and may be added to some foods, such as artificial cream or coffee whiteners to add a white color.

Module 121: Determination of titratable acidity of fruit juices

Materials Required:

pH meter or phenolphthalein, burette, burette clamp and stand, gram scale, graduated cylinder, beakers, 0.1N NaOH solution. Optional: magnetic stirrer & stir bar, automatic titrator

Procedure

A. Obtain at least 50 mls of clear juice by one of the following methods:

1. Cut fruit, press with a hand press, and filter through cheesecloth, or
2. Cut fruit into a blender, homogenize, centrifuge slurry, and pour off clear liquid for analysis.

** Sugar levels often vary within the fruit, being higher at the stem-end and lower at the calyx-end. For this reason, it is important to use longitudinal slices of fruit (from end to end) when sampling.

B. Make sure samples are at room temperature before taking measurements.

C. Measure the pH of the samples with a pH meter and record the value.

D. For each sample, weigh out 6 grams of juice into a 100 ml beaker.

E. To each sample, add 50 mls of water.

F. Titrate each sample with 0.1 N NaOH to an end point of 8.2 (measured with the pH meter or phenolphthalein indicator) and record the milliliters (mls) of NaOH used.

G. Calculate the titratable acidity using the following formula:

% acid = [mls NaOH used] x [0.1 N NaOH] x [milliequivalent factor] x [100]grams of sample
Commodity Predominant acid

Milliequivalent Factor Stone fruit, apples, kiwifruit Malic Acid= 0.067 Citrus Citric Acid
0.064 Grapes Tartaric Acid

Lecture 13

PROBIOTICS**Module 122: Probiotics**

The expression “probiotic” was probably first defined by Kollath in 1953, when he suggested the term to denote all organic and inorganic food complexes as “probiotics,” in contrast to harmful antibiotics, for the purpose of upgrading such food complexes as supplements. In his publication “Anti- und Probiotika, Although numerous definitions have been proposed since then, none has been completely satisfactory because of the need for additional explanations, e.g., with regard to statements such as “beneficial balance,” “normal population,” or “stabilization of the gut flora.” A consensus and somewhat generalized definition as suggested by the Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinär-medizin (BgVV; now called BfR) states that probiotics are defined, live microorganisms, which when reaching the intestines in sufficient numbers (e.g., administered via food), will exert positive effects. The present-day concept refers to viable microorganisms that promote or support a beneficial balance of the autochthonous microbial population of the GIT. These microorganisms may not necessarily be constant inhabitants of the GIT, but their “...beneficial effect on the general and health status of man and animal” should be ascertained. This is also reflected in the suggestion of Havenaar et al., defining probiotics as “...mono- or mixed cultures of live microorganisms which, when applied to animal or man, beneficially affect the host by improving the properties of the indigenous microflora.” Probiotics are best known by the average consumer with relation to food where they are defined by the EU Expert Group on Functional Foods in Europe (FUFOSE) as “viable preparations in foods or dietary supplements to improve the health of humans and animals”.

Module 123: Administration and consumption of Probiotics

Viable strains of especially the *Lactobacillus acidophilus* “group” and *Bifidobacterium bifidum* were introduced into dairy products in Germany during the late 1960s because of their expected adaptation to the intestine and the sensory benefits for producing mildly acidified yogurts. Such products first became known in Germany as mild yogurts or “bio-yogurts”, while in the USA, acidophilus milk was better known.

Probiotics are available and may be administered in different forms, comprising foods, mainly in a fermented state, and pharmaceutical products, mainly as capsules or in microencapsulated form. By definition, probiotic strains may even be undefined organisms from fermented foods, which survive the gut passage and may exert positive effects in the GIT. If probiotic microorganisms constitute a defined part of a food, they are defined by FUFOS as “live constituents of a food which exert positive effects on health”. Probiotic foods comprise between 60 and 70% of the total functional food market. A continued increase is observed among the dairy-type probiotic foods, but even in the range of nondairy probiotic food products such as fermented meats and vegetable and fruit juices. Taking into account the wide range of potential (fermentable) substrates and the different conditions under which LAB strains may be challenged for “functional performance,” it can be expected that developments toward new food-based probiotics will proceed further in the future.

It is postulated that this positive effect is achieved when the proportion of lactobacilli and bifidobacteria in the intestinal population increases, either by increased intake of typical gut bacteria (e.g., as fermented foods or dehydrated preparations), or indirectly as a result of the stimulation of autochthonous gut bacteria belonging to these groups. The lactobacilli and bifidobacteria associated with the GIT are generally considered beneficial for such things as combating disturbances of the mucosa associated immune system and of the established gut population.

A particular feature of probiotic cultures is that they regulate the balance of the gut bacterial population, e.g., by competition for epithelium contact sites and nutrients and also by modulation of the pH value. Other features refer to the support of absorption of nutrients and the synthesis of vitamins such as riboflavin. Further stabilization of the gut microbiota is associated

Module 124: Microbiota of GI tract

Increasing microbial populations are found throughout the GIT, ranging from varying numbers of food-associated bacteria in the esophagus, to 10^1 - to 10^3 /ml (or g) in the stomach, 10^7 /ml in the jejunum (comprising mainly lactobacilli, Enterobacteriaceae and streptococci), up to 10^9 CFU/g in the terminal ileum, and ca. 5×10^{11} /g in the distal colon. Many microbes isolated from the duodenum and jejunum are considered to be typical transients, especially considering rapid chymus flow; indigenous colonization is, however, more likely to occur in

the lower parts of the ileum. The estimated total population of 10^{14} viable bacteria in the adult human GIT represents about 10 times more than all body tissue cells. The microbial population therefore represents an immense metabolic potential that not only supports the digestion processes but is also interactive in detoxification and toxification processes and, most importantly, comprises the major part of the human immune system. Bacteroides and the Gram-positive, anaerobic genera Eubacterium and Bifidobacterium predominate in the densely populated large intestine. Other groups such as the clostridia, peptostreptococci, “streptococci,” and lactobacilli also play an important role, e.g., in the maintenance of a stable gut mucosa and in the generation of SCFAs in a beneficial ratio. The role of the lactobacilli may be more important in the small intestines where they comprise a higher proportion of the total population. In healthy humans, lactobacilli are normally present in the oral cavity (10^3 to 10^4 CFU/g), the ileum (10^3 to 10^7 CFU/g), and colon (10^4 to 10^8 CFU/g) and are the dominant microorganism in the vagina.

Role and Functions of the Microorganisms of the Gut

A healthy intestinal epithelium, in association with an established and stable intestinal microbial population, presents a vital barrier against the invasion or uptake of pathogenic microorganisms, antigens, and harmful compounds from the gut lumen, while the intestinal mucosa also efficiently assimilates antigens. Specific immune responses are evoked by the specialized anti-gen transport mechanisms in the villus epithelium and Peyer's patches. The positive role of gut microorganisms in human health was largely overlooked for a long time, and the main focus was placed on enteric pathogens and factors leading to gastrointestinal disorders or “dysbiosis”. A stable barrier, typical of healthy individuals, ensures host protection and serves as support for normal intestinal function and immunological resistance. The gut-associated lymphoid tissues (GALT) are considered to be the largest “immune organ” in the human body, and its “barriers” serve for intrinsic protection against infective agents. Around 80% of all immunoglobulin-producing cells are found in the small bowel, while the gut microbial population is essential for mucosal immune stimulation and amplification of immunocompetent cells. Numerous physiological functions have been ascribed to the “normal” gut microbial population; some of the major functions are considered the following:

Maintenance and restoration of barrier function

Stimulation of the immune system

Maintenance of mucosa nutrition and circulation

Improvement of bioavailability of nutrients

Stimulation of bowel motility and reduction of constipation

Module 125: Probiotic Microorganisms

Probably the longest history of proven health benefits and “safe-use” of probiotic bacteria in food is documented for *L. casei* strain “Shirota” and some strains of the *L. acidophilus* group. Since at least 40 years in Japan and more than 30 years in Germany, LAB cultures of human origin are applied in the manufacture of fermented milk products. Viable strains of especially “*Lactobacillus acidophilus*” and *Bifidobacterium bifidum* were introduced in Germany during the late 1960s into dairy products because of their expected adaptation to the intestine and the sensory benefits for producing mildly acidified yogurts. In Germany, such products first became known as mild yogurts or “bio-yogurts”, whereas in the USA, *acidophilus* milk was developed. The functional properties and safety of particular strains of *L. casei/paracasei*, *L. rhamnosus*, *L. acidophilus*, and *L. johnsonii* have extensively been studied and are well documented.

Viable probiotic strains with beneficial functional properties are at present found among a wide and diverse number of microbial species and genera. They are supplied in the market either as fermented (mainly “yogurt”-type) food commodities or in lyophilized form, both as food supplements and as pharmaceutical preparations. Most strains currently in use as probiotics in food, nutrition, and in pharmaceutical preparations are members of the LAB. A number of “nonlactic” strains, e.g., *Bacillus cereus* (“toyoi”), *B. clausii*, *B. pumilis*, *Escherichia coli* (Nissle), *Propionibacterium freudenreichii*, *P. jensenii*, *P. acidopropionici*, *P. thoenii*, and *Saccharomyces cerevisiae* (“boulardii”), are also available in the market mainly as pharmaceutical preparations and some also as animal feed supplements.

With 65%, the probiotic milk products (mainly “yogurt”-like) represent the largest segment of the functional foods market in Europe, while in Japan they are estimated to comprise about 75% of the foods for specified health uses (FOSHU) market. Initiated by a national project team under the auspices of the Japan Ministry of Education and Science, specific regulatory measures on functional foods were first initiated in Japan in 1984

Module 126: Selection of Appropriate Strains

The prevalence of bifidobacteria in the feces of breast-fed infants may have been a major reason for selecting strains of this group for use as probiotics. Decisions on the use of Lactobacillus strains as probiotics have been determined by a number of favorable factors such as:

Their association with traditional fermented foods (earlier noted by Metchnikoff when he postulated benefits from the consumption of yogurt by the Caucasians), together with the high acceptability of lactic fermented foods. Their association with the human GIT, together with observations on their beneficial interactions in the gut ecosystem. The adaptation of many lactobacilli to milk and other food substrates and the relatively long history of technical application of LAB with the use of the first industrial strains dating back to 1890.

The selection of new strains presents a major challenge, both to science and industry. The primary objective is to select microbial strains with one or more proven functional properties. Even when probiotic microorganisms are suggested to promote health and well-being, the challenge remains to define particular end points or biomarkers by which such strains can be characterized and particular claims be sustained — either by in vivo or validated in vitro tests — even when all the mechanisms involved have not yet been fully elucidated. Approaches for selection of an “ideal” strain are therefore still difficult and indeed require considerable resources. Desirable technical features and factors related to health promotion or sustaining health serve as important criteria for strain selection. Five major aspects may be taken into account as key criteria for the selection of an appropriate functional strain.:

General aspects, e.g., origin, identity, and resistance to mutations

Technical aspects (growth properties in vitro and during processing, survival and viability during transport and storage)

General physiological aspects (resistance against environmental stress and to the antimicrobial factors prevailing in the upper GIT as encountered during the stomach-duodenum passage [pH 2.5, gastric juice, bile acid, pancreatic juice], adhesion potential to intestinal epithelium)

Functional aspects and beneficial features (adhesion, colonization potential of the mucosa, competitiveness, specific antimicrobial antagonism against pathogens, stimulation of immune response, selective stimulation of beneficial autochthonous bacteria, restoration of the “normal” population)

Safety aspects (no invasive potential, no transferable resistance against therapeutic antibiotics, no virulence factors)

Research during the past two decades focused mainly on functional features of strains selected for inclusion, e.g., in functional foods. Considering the worldwide increase in the consumption of dairy products containing probiotic strains of the bacterial genera *Bifidobacterium* and *Lactobacillus* during this period, relatively little attention has been given to technical and sensory properties of these strains and/or the resulting products. For the producer, technical properties related to growth, adaptation, and persistence of some probiotic strains, and also the sensory properties of the resulting products, are still major obstacles toward the large-scale production of functional foods containing probiotic strains. Information on particular production steps and modification of growth conditions are still well-protected industrial secrets for the technical production of some strains. Technical production of especially the bifidobacteria in milk substrate constituted a considerable technical challenge but was at least partly solved by some industries during the 1960s. Still, it is known that particularly strains of the “acidophilus” group and also bifidobacteria are not well adapted to the milk substrate and, in addition, do not influence the sensory properties of a product positively. Such strains therefore still constitute special technical challenges.

Module 127: Health Benefits of probiotics

Functional Properties

In spite of research progress in recent years, our understanding of the gut ecosystem is still fragmentary and consequently limits our comprehension of a normal or balanced microbial population. Thus, the impact of a functional strain on the composition and function of the intestinal population is still difficult to ascertain (39, 109). Numerous beneficial functions have been suggested for probiotic bacteria (36, 109), e.g.:

Nutritional benefits:

Vitamin production, availability of minerals and trace elements

Production of important digestive enzymes (e.g., β -galactosidase)

Production of β -galactosidase for alleviation of lactose intolerance

Barrier, restoration, antagonistic effects against:

Infectious diarrhea (traveller's diarrhea, children's acute viral diarrhea)

Antibiotic-associated diarrhea, irradiation-associated diarrhea Cholesterol-lowering effects by:

Cholesterol assimilation

Modification of bile salt hydrolase activities

Antioxidative effect

Stimulation and improvement of the immune system, e.g., by:

Strengthening of nonspecific defense against infection

Increasing phagocytic activity of white blood cells

Increasing IgA production

Regulating the Th1/Th2 balance; induction of cytokine synthesis

Enhancement of bowel motility, relief from constipation

Reduction of inflammatory or allergic reactions, by:

Restoration of the homeostasis of the immune system

Regulation of cytokine synthesis

Adherence and colonization resistance

Anticarcinogenic effects in the colon by:

Mutagen binding

Inactivation of carcinogens or procarcinogens, or prevention of their formation

Modulation of metabolic activities of colonic microbes

Immune response

Maintenance of mucosal integrity

Antioxidative activities

Effects such as lowering of the serum cholesterol level are not fully substantiated yet by placebo-controlled, double-blind, randomized clinical trials. On the other hand, strain-specific effects of probiotic lactic cultures on the human immune system and on diarrhea are well documented, e.g., for counteracting rotavirus or antibiotic-associated diarrhea, by application of strains such as the LGG strain of *L. rhamnosus* and the Shirota strain of *L. casei* (*L. paracasei*). Therapeutic use is also considered successful in cases of lactose intolerance, irritable bowel syndrome, colon cancer, and *Helicobacter pylori* infection. Complex underlying mechanisms, such as adhesive and immunomodulating properties of effective strains, are major challenges remaining to be solved by intensified research.

Module 128: Human Trials of Probiotics

Human trials (clinical or dietary intervention studies) are essential for proving health benefits of probiotic strains. Different designs have been applied such as pre- and postintervention designs and placebo-controlled designs, parallel and crossover designs, and case-control studies. The best evidence is probably coming from double-blind, randomized, placebo-controlled trials. In addition to the study design, the methods applied for analysis of various parameters are also of great importance. Epidemiologic evidence relating probiotics or probiotic-containing foods and disease incidence would be valuable but is hardly available. However, these studies would be difficult to control as fundamental parameters such as specific strain and dose would be unknown for most probiotic-containing food products. In human studies certain markers are usually applied in order to observe the effect of pre- and probiotics or functional foods in general on the human body. Markers can be classified into three categories:

markers that relate to the exposure to the food component under study, markers that relate to the target function or biological response, and [c] markers that relate to an appropriate intermediate endpoint. It was suggested that markers of type [b] might lead to enhanced function claims, whereas markers of type [c] might allow reduced risk of disease claims. In the case of probiotics, the detection of a certain probiotic strain in feces could be classified as a type [a] marker, an increase in natural killer cell activity and phagocytosis as type [b] (enhanced functioning of the immune system), and a reduced incidence of respiratory symptoms (e.g., cough, sore throat, runny nose) as type [c] (reduced risk of respiratory tract infections).

Module 129: Technological application of Probiotics in food products

Probiotic bacteria are applied in many different products worldwide. In addition to food products, probiotic cultures are also used in pharmaceuticals and animal feed. Most definitions of probiotics are based on live bacteria that confer a health benefit for the consumer. Thus, it is considered as important that probiotic products contain an effective dose of living cells during their whole shelf life. However, for some health benefits, viability of the microorganisms does not seem to be essential. Nonviable bacteria are, for example, applied in some pharmaceuticals and food supplements. The selective enumeration of probiotic species in a fermented product is sometimes impossible due to the product's background flora. Also, the choice of media may have a major impact on the viable cell counts.

Dairy Products

Probiotic bacteria have been applied in fermented dairy products for many years. In some cases fermented milk products are monocultures of probiotic bacteria, but usually support cultures are applied to speed up the acidification process and provide the desired texture and flavor. Many lactobacilli and bifidobacteria survive in fermented milk products for 4 to 8 weeks. There are several parameters that may influence the growth and survival of the probiotics, e.g., the starter culture, fermentation temperature, pH, sugar content, presence of oxygen, packaging material, fruit preparations, and other ingredients. Therefore, the survival of a probiotic culture should be reconfirmed in the final product formulation. Probiotics may also be applied to unfermented milk products such as milk-based sweet or acidified drinks and ice cream.

Other Food Products

The applicability of probiotics in food products depends in general on factors like water activity, processing and storage temperature, shelf life, oxygen content, pH, mechanical stress, salt content, and content of other harmful or essential ingredients. For many products, excess water activity is a critical parameter that increases the death rate of bacteria. Products with an unfavorable water activity are, for example, cereals, chocolate, marmalade, honey, and toffees. These products are too “dry” for applying live bacteria and too “wet” for the application of freeze-dried bacteria. Freeze-dried bacteria could be applied in these products if the bacteria could be protected from moisture, as small amounts of moisture can be very detrimental to the dried culture. In addition to dairy products, fruit juices have been shown to be suitable carriers for probiotics. The limiting factor for many of the probiotic strains is the

low pH of the juices. There is growing interest in applying probiotics to fermented meat products. Lactic acid bacteria have been used for the fermentation of meat products for many years, and today some strains are also utilized as protective cultures. Probiotics might be an instrument to change the perception of meat products toward a healthier image. This might, however, also be a hurdle in the marketing of probiotic sausages. Freeze-dried probiotic bacteria are applied to infant nutrition powders and powdered milk drinks. In these products, the water activity is very low, which is essential for the stability of freeze-dried bacteria. ☰

Food Supplements and Over-the-Counter Products

Most probiotic food supplements and over-the-counter (OTC) products are available as powders, tablets, and capsules. As these products also contain dried bacteria, the water activity in the final product must be very low. Another critical parameter for tablets is the pressure applied in tableting and the heat that is produced. An enteric coating can be applied on tablets and capsules in order to protect the bacteria from the acidic environment in the stomach and improve their survival rate.

Lecture 14

FOOD SAFETY

Module 130: Concept of Food Safety

Food safety is the assurance that food will not cause harm to the consumer when it is prepared and eaten according to its intended use. All requirements relating to the safety characteristics of a food must be met; there must be no unacceptable health risk associated with a food. The assurance that a food will not cause harm, injury, or illness is determined by: (1) whether all harmful substances present in the food have been eliminated, reduced to an established acceptable level, or prevented from exceeding the acceptable level; and (2) the food has been prepared, handled, and stored under controlled and sanitary conditions in conformance with practices prescribed by government regulations.

Systems and Programs of Food Safety:

For decades, the food industry has depended on the use of quality programs based on inspection and testing of food products for hazards, and on GMPs for addressing food safety. Since the late 1980s, there has been widespread use of the HACCP system specifically to achieve food safety; the system addresses food safety primarily on the basis of prevention or elimination of unacceptable hazard levels. The GMPs, which were used to address food safety requirements prior to the use of the HACCP system, have been incorporated into prerequisite programs for the HACCP system. A food company that does not operate with the HACCP system must continue to use the GMPs.

Module 131: Concept of Food Quality

Food quality, as distinct from food safety, is the extent to which all the established requirements relating to the characteristics of a food are met. Common examples of quality characteristics of food, excluding the food safety characteristics, are:

- 1 Identity of a food in relation to a standard (e.g., standardized food)
- 2 Declared gross or net quantity (e.g., weight or volume) of a unit of the food or net fill of a food container
- 3 Declared or claimed amount of one or more stated components of a food

- 4 Appearance (e.g., size, shape, color)
- 5 Flavor
- 6 Aroma
- 7 Texture
- 8 Viscosity
- 9 Shelf-life stability
- 10 Fitness for use as human food
- 11 Wholesomeness
- 12 Adulteration
- 13 Packaging
- 14 Labeling

Some of these quality characteristics are covered in food laws and regulations. For instance, failure of a food to meet regulatory requirements relating to a standard of identity, the declared quantity, declared ingredients, or label claims, can be considered as misrepresentation, misbranding, or fraud. The spoilage, deterioration, or decomposition of foods with the absence of any resulting harmful substance that can lead to illness or injury, can be considered as failure to meet food quality requirements based on fitness for human use or wholesomeness criteria. Unacceptable levels of foreign matter or extraneous materials that are not necessarily harmful to health or do not cause injury can also be considered as failure to meet food quality requirements; in the U.S., defect action levels have been established for naturally occurring, unavoidable, extraneous materials in many foods. The Codex Alimentarius defines the term food suitability (distinct from food safety) as the assurance that food is acceptable for human consumption according to its intended use; food suitability criteria include fitness for human use, wholesomeness, and extraneous matter.

Module 132: Responsibility of Food Safety

The overall responsibility for food quality and food safety is shared by all segments of the food system, including the various food industry sectors, government regulatory agencies,

and consumers in general. The food industry has both the legal and moral responsibility for providing customers and consumers with foods that meet all established quality and safety requirements. Within a food company, overall responsibility for the implementation and effective use of these programs and systems rests with senior management.

Governments worldwide have enacted food laws and regulations designed to ensure that foods are fit for human consumption. Such laws protect consumers from harm resulting from unsafe foods and from deception resulting from misrepresentation or fraud relating to certain established food quality characteristics. Governments have also established various agencies that enforce these food laws and regulations; this legal framework is intended to provide consumers with confidence in the safety and quality of foods.

Within the food supply chain, customers who purchase raw materials, ingredients and food contact packaging materials for manufacture of consumer foods, must ensure that these materials are safe and fit for use. When making purchases, customers need to be vigilant in their assessment of foods for safety and quality. In particular, customers and consumers must pay attention to the instructions for handling, storage, preparation, and use of foods.

Module 133: Factors of Food Quality and Systems

The food industry, like many other industries, has used basic quality control programs, and more complex quality assurance programs and quality management systems, in its efforts to achieve food quality; some food companies use the ISO 9000 Quality Management System Standard. These programs and systems can include components that are devoted specifically to food safety. For instance, GMPs and the HACCP system can be integrated into a food industry, quality management system, or inspection and monitoring of materials, products, and processes for food safety hazards can be part of a quality control program.

Module 134: Food Safety and Food Safety Systems

- For decades, the food industry has depended on the use of quality programs based on inspection and testing of food products for hazards, and on GMPs for addressing food safety.
- Since the late 1980s, there has been widespread use of the HACCP system.
- HACCP system is designed to achieve food safety: Addresses food safety primarily on the basis of prevention or elimination of unacceptable hazard levels.

- The GMPs, were used to address food safety requirements prior to the use of the HACCP system.
- GMP have also been incorporated into prerequisite programs for the HACCP system.
- A food company that does not operate with the HACCP system must continue to use the GMPs.

Module 135: Food Laws and regulations

The legal requirements for food safety and food quality have been established by many national governments, with the objective of protecting consumers and ensuring that foods are fit for human consumption. These requirements are contained in food laws and regulations, the scope of which varies from one country to another. In the U.S. and Canada, food laws and regulations govern all aspects of food safety and some aspects of food quality. The food laws and regulations of the U.S. are likely the most extensive of any country. It is essential that food industry professionals be familiar with the laws and regulations that govern their specific industry sectors in their countries. 

The legal framework of food laws and regulations of a particular country depends on the overall government regulatory system of that country. In the U.S. and Canada, the federal or national food laws are statements of government policies that cover both the general and specific aspects of adulteration and misbranding of foods, while the food regulations deal with the enforcement of government policies that are embodied in the food laws. These food laws and regulations are intended to ensure that foods do not cause harm, illness, or injury; are not adulterated or misbranded; and are wholesome and fit for human consumption. Food laws and regulations apply to all foods produced domestically, as well as all foods imported into a country; foods cannot be imported if they do not conform to the food laws and regulations of the importing country. Examples of food laws are the U.S. Federal Food, Drug, and Cosmetic Act (FDCA), which is the primary law governing the safety and quality of most foods in the U.S., and Canada's Food and Drugs Act, which is the primary food law in Canada. The U.S. Code of Federal Regulations (CFR) Title 21 and Canada's Food and Drug Regulations are examples of food regulations that address food safety and food quality.

Food laws protect consumers from illnesses and injury by prohibiting the presence of any poisonous or harmful substance in foods that are intended for human consumption. For example, in the U.S., adulterated food is regulated primarily under the FDCA, which covers

all aspects of food safety and certain aspects of food quality. In addition, food laws protect consumers from fraud and deception by prohibiting false or misleading information relating to foods. For example, in the U.S., misbranded food is prohibited under the FDCA.

Module 136: Food Standards

In addition to food laws and regulations, food standards also establish requirements for the safety and quality of foods; however, unless a food standard is part of food regulations (e.g., standard of identity in the U.S. CFR Title 21), it is not a legal requirement. The Codex Standards are the best examples of food standards. The Codex Alimentarius Commission has the mandate to implement the joint Food and Agricultural Organization (FAO)/ World Health Organization (WHO) Foods Standards Program. This has resulted in the Codex Alimentarius, a collection of standards for food quality, food suitability, and food safety. These food standards have been adopted by countries worldwide and are intended primarily to protect consumers and to facilitate international food trade. They include codes of practice such as The Codex General Principles Of Food Hygiene, standards for maximum residual levels (MRL) for pesticides and for veterinary drugs in foods, and standards for specifications for food additives.

Module 137: Food Safety hazards and health risks:

The safety of a food can be related directly to certain harmful substances that are present in the food; these substances are food safety hazards. Any substance that is reasonably likely to cause harm, injury or illness, when present above an established acceptable level, is a food safety hazard. An unacceptable level of a food safety hazard in a food presents a health risk to the consumer. Food-borne illnesses from food safety hazards occur frequently; each year a relatively large number of deaths attributed to these hazards occur among North American consumers.

There are three recognized categories of food safety hazards: biological hazards, chemical hazards, and physical hazards. The origin of these hazards in foods can be from naturally occurring substances or agents in foods, from deterioration or decomposition of foods, or from contamination of the foods with the hazard at various stages of their production, harvesting, storing, processing, distribution, preparation, and utilization. For many hazards, government regulatory agencies have established an acceptable level of the hazard in a food; the Codex Alimentarius has also established acceptable levels of certain hazards as part of its

Food Standards Programme. For some hazards, such as pathogenic bacteria (e.g., *Salmonella* spp.), there is zero tolerance; this means that the presence or the detection of the hazard in the food is unacceptable. The strategies used to address hazards in foods include the prevention or elimination of hazards, or the reduction of hazards to acceptable levels. These strategies are employed in the HACCP system.

Food safety hazards and health risk

For a known food safety hazard, the extent of the harmful effects of the hazard on the health of the consumer is established by risk analysis and by hazard analysis. Risk analysis is usually conducted by a national food or health regulatory agency and addresses a public health concern regarding a particular food safety hazard associated with a sector of the food industry. A risk analysis is comprised of risk assessment, risk management, and risk communication. A primary objective of risk analysis is to establish a national food safety objective for a hazard in a food. The food safety objective for a hazard is the maximum frequency and concentration of a hazard in a food at the time of consumption that provides the appropriate level of protection from the hazard. The food safety objective can be considered as the maximum acceptable level for the hazard in a food.

Module 138: Biological Safety Hazards

Biological hazards in foods

Pathogenic bacteria

Food-borne pathogenic bacteria are responsible for a large proportion of food poisoning incidents in North America. Therefore, the importance of this group of hazards must be emphasized. More than forty different pathogenic bacteria are known; however, a large proportion of the reported cases of food poisoning can be attributed to the following pathogenic bacteria: *Salmonella* spp., *Escherichia coli* 0157:H7, *Listeria monocytogenes*, *Clostridium perfringens*, *Clostridium botulinum*, *Staphylococcus aureus*, and *Campylobacter jejuni*. Food poisoning from these organisms occurs frequently, with symptoms that include headache, muscle pain, nausea, fatigue, chills or fever, stomach or abdominal pain, vomiting, and diarrhea. Numerous severe and fatal illnesses occur as a result of food poisoning from pathogenic bacteria; infants and the elderly are particularly vulnerable. The foods that are commonly involved in these food poisoning incidents include meat and poultry and their

products, seafood and seafood products, egg and egg products, milk and dairy products, fruits and vegetables and their products, low-acid canned foods, and water.

Viruses

Foods can be the medium for transmission of certain viruses. Examples of viruses that are known to be food safety hazards are the hepatitis A and E viruses, the Norwalk group of viruses, and rotavirus.

Parasites

Several human parasites can be transmitted by foods. The most common human parasites include parasitic protozoan species (e.g., *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*), and parasitic worms (*Ascaris lumbricoides*, *Taenia solium*, *Trichinella spiralis*).

Module 139: Chemical Safety Hazards

Permitted food additives

Government regulations permit numerous chemical and biochemical substances to be added to foods at specified maximum levels. These substances are intended to impart some improved nutritional effect (e.g., vitamin fortification) or some specific technical function (e.g., preservative action, sensory attribute, stabilizing effect, etc.). Permissible food additives with their established levels for use can be found listed in government food regulations (e.g., U.S. CFR Title 21, Canada's Food and Drug Regulations). In addition, the Codex Alimentarius contains specifications of permitted food additives. Although food additives are permitted by government regulations, many can be harmful if they are present in the food at levels above the maximum established, and are therefore, potential chemical hazards. In some instances, a permitted food additive present below the maximum allowable level in a food can be a health hazard for specific segments of the population. For example, sodium bisulfite is a permitted food additive in some foods; however, individuals who are asthmatic could be at risk from foods containing sodium bisulfite. The labels on the containers containing the foods must clearly indicate the presence of the additives for the benefit of individuals who may be at risk from these additives.

Naturally occurring harmful compounds

It is well known that many foods contain as their normal or inherent components naturally occurring substances that can be harmful if they are present in excess of certain levels; examples are oxalate in rhubarb, alkaloids in potatoes, toxins in mushrooms and in shellfish. In the U.S., the FDCA considers foods containing these naturally occurring substances to be adulterated only if the harmful substance is present in sufficient quantity that is likely to cause illness.

Unavoidable contaminants

Some foods can contain naturally occurring harmful substances that are not normal or inherent components of the foods. These substances are considered unavoidable contaminants in the food and cannot be removed through processing or manufacturing practices; examples are aflatoxins from molds in peanuts and in some cereals. If the normal level of a naturally occurring harmful substance in a food is increased to an unsafe level as a result of mishandling of the food or by any other action, then the harmful substance can be considered as an added harmful substance.

Agricultural residues

Agricultural residues are a group of residual chemical or biochemical substances found in foods and are directly attributable to certain substances that have been approved for use in the production of crops and livestock for food. They include residues of permitted pesticides, herbicides, fungicides, drugs, hormones, and antibiotics. Some of these residues are considered as added harmful substances attributable to human actions and are regulated by governments. In the U.S., these residues are regulated under several laws including the FDCA. The Codex Alimentarius establishes maximum residual levels (MRL) for various harmful pesticides and veterinary drugs.

Industrial contaminants

Several harmful chemicals that enter the environment as a result of industrial activity have been shown to be present in foods. These substances include heavy metals (lead, mercury, arsenic), organo-chlorinated compounds such as polychlorinated biphenyls (PCBs), and are considered as industrial or environmental contaminants. In the U.S., the CFR Title 21 considers PCBs as unavoidable environmental contaminants because of their widespread occurrence in the environment, and provides tolerances for PCB residues in several foods (e.g., milk, dairy products, poultry, eggs, etc.).

Chemical residues

In food processing operations, some chemical compounds that are not per-mitted substances in food are used during certain operations and care must be taken to prevent unintentional contamination. These substances include chemical compounds used for cleaning and sanitizing food contact surfaces of processing, handling, and storage equipment, and for lubricating certain parts of food processing equipment.

Prohibited chemicals

No chemical substance is permitted for use in a food unless it meets all of the requirements that are covered in the applicable food laws and regulations. In addition, in the U.S. CFR Title 21, some chemical substances are specifically prohibited from direct addition to food or from indirect addition to food through food contact surfaces.

Food allergens

Certain foods are known to contain inherent components that cause serious immunological, allergic responses in a relatively small proportion of food consumers. These foods are entirely safe for most consumers who are not sensitive to the allergens. The following foods and some of their products are generally considered to be the most common food allergens: peanuts, soybeans, milk, eggs, fish, crustacea, tree nuts, and wheat. Some other foods (e.g., sesame seeds) are also known to cause allergenicity occasionally.

In addition, sulfites (including bisulfites and metabisulfites) used as ingredients in certain foods can produce nonimmunological allergic reactions in certain sensitive individuals.

Module 140: Physical Safety Hazards

Physical hazards include organic or inorganic substances, commonly referred to as foreign objects, foreign matter, or extraneous materials. Hard and sharp physical hazards are of particular concern. Depending on their size and dimensions, hard and sharp physical hazards can cause injury to the mouth or teeth, or can cause serious injuries if swallowed. In addition, some physical hazards, depending on their size, shape, and texture, have the potential to cause choking if swallowed. Physical hazards in foods can be particularly harmful to infants.

Certain hard and sharp foreign objects that are natural components of food (e.g., prune, date or olive pits; fish bones nutshells) are not considered physical hazards since it is expected that

the consumer will be aware that these objects are natural components of the foods. However, if the food carries a label stating that the hard and sharp object has been removed(e.g., pitted prunes), the presence of the hard and sharp object in the food represents a hazard, since it is not expected by the consumer.

The common hazards considered as avoidable physical hazards in foods include broken glass, pieces of hard or soft plastic materials, stones, pieces of metal, pieces of wood, and personal articles.

Broken glass

In a food plant, the common potential sources of broken glass include light bulbs, glass containers, and gauges with glass covers. Every effort must be taken to protect or eliminate these sources of broken glass, and to protect food from contamination with this hazard. In addition, many foods are packaged, distributed and sold in glass containers. For these foods, the glass packaging itself can be a source of broken glass.

Plastic

Both hard and soft plastic foreign objects are sometimes found in foods. In some food plants, some utensils and tools used for cleaning of equipment are made from hard plastic material; this type of plastic can become brittle from use over an extended period of time, and pieces can adulterate foods. The common sources of soft plastic foreign objects in food are plastic material used for packaging food and gloves used by employees who handle food.

Metal pieces

The most common sources of metal pieces in a food plant are food processing equipment, metallic cleaning tools, and equipment maintenance activities. In many food plants, magnets are used to eliminate some metals from foods, and metal detectors are used to detect the presence of metals in foods.

Wood pieces

The most common sources of wood pieces in a food plant are wood structures and wood pallets. The presence of these sources should be avoided whenever possible in food processing and production.

Stones

Many plant foods and particularly field crops such as peas and beans can contain small stones that become incorporated with the foods during harvesting. In addition, in food processing plants, a common source of stones is concrete structures, particularly concrete floors.

Personal articles

A variety of personal articles can become foreign objects in foods, resulting from unintentional adulteration by employees during preparation, handling, processing, and packaging. Personal articles that have been found in foods include jewelry, pens or pencils or their parts, Band-Aids, and ear plugs.

Module 141: Good Manufacturing Practices (GMP)

Food quality, food safety, and good manufacturing practices (GMPs)

Government regulatory agencies have established minimum requirements relating to the sanitary practices and controlled conditions for processing, handling, and storage of foods (e.g., current good manufacturing regulations in U.S. CFR Title 21, Part 110). These requirements are commonly referred to as GMPs, and are some of the basic food quality and food safety activities in food companies. If a food is prepared, handled, or stored under conditions that are unsanitary, or if certain required practices or operations are not followed, the food can be considered to be potentially unsafe, unfit, or unsuitable for consumption. Food companies that operate with the HACCP system, incorporate the GMPs within the HACCP prerequisite programs.

Module 142: HACCP

At the level of production, processing, handling, or storage, a food company performs hazard analysis as part of the development of an HACCP plan for the food. Hazard analysis is the first of the seven HACCP principles, and is performed to determine the health risk associated with a hazard present in a food when it is produced, processed, handled, or stored, according to an established sequence of steps at a particular location. Once a food safety objective for a hazard has been established by risk analysis, it must be considered during the hazard analysis step of HACCP plan development.

Seven Principles of HACCP

1) Conduct a hazard Analysis

2) Identify Critical Control Points

3) Establish Critical Limits

4) Monitor Critical Limits

5) Establish Corrective Actions

6) Verification

7) Record Keeping

Lecture 15

FOOD ENGINEERING

Module 143 Food engineering principles

Converting raw food into desired products involves equipment & processes. To design equipment, an engineer must consider numerous criteria that are inherent to a process. For example, in designing a heat exchanger, one must consider the heat transfer, fluid flow, and various physical, chemical, and biological aspects of food.

Overall engineering process involve energy balance and material balance. Overall process can be broken down into series of operations. These are called unit operations. Efforts are directed towards maximizing efficiency and reducing losses in every unit operations.

The essential concept is therefore to divide physical food processes into basic unit operations, each of which stands alone and depends on coherent physical principles. e.g. heat transfer is a unit operation and the fundamental physical principle underlying heat transfer principles.

Because of the dependence of the unit operation on a physical principle, or a small group of associated principles, quantitative relationships in the form of mathematical equations can be built to describe them. The equations can be used to follow what is happening in the process, and to control and modify the process if required.

Important unit operations include fluid flow, heat transfer, drying, evaporation, contact equilibrium processes (which include distillation, extraction, gas absorption, crystallization, and membrane processes), mechanical separations (which include filtration, centrifugation, sedimentation and sieving), size reduction and mixing.

The law of conservation of mass and energy is applied. Hence losses are calculated and discouraged in both materials and energy for best optimization of process.

Module 144: Importance of of Hygienic design and layout

Hygienic design of equipment is essential in a modern food processing plant. A major concern in processing foods is to prevent microbial contamination that may be facilitated in poorly designed equipment. Such equipment is difficult to clean or may require longer cleaning times and more use of chemicals. The key principles of hygienic design are

Use materials of construction that are suitable for hygienic processing of food.

Product contact surfaces must be easily accessible for inspection and cleaning.

Incorporate design features that prevent harboring microbial accumulation and their growth.

Module 145: Food Process Equipment Design

To ensure clean food processing equipment, the product contact surface has an important role. If the surface is rough and/or porous it will most likely allow food particles to build up and it will be more difficult to clean when compared with a smooth, polished surface. The contact surface must not chemically interact with the food and its ingredients. It should be free of corrosion and it must be inert to the chemicals used during cleaning.

If the contact surface is hidden during visual inspection, it would be impossible to know whether it is properly cleaned. Therefore, all contact surfaces must be accessible during inspection and cleaning. In some cases, it may require complete disassembly; in other circumstances ports for inspection may be strategically located. Often, access doors are provided to inspect the contact surface. The door fasteners should be easy to open without the use of tools; quick release type fasteners are preferred. Many of the small equipment such as pumps should be installed 15 cm or more from the floor, whereas larger pieces of equipment should be elevated 30 cm from the floor so that the floor areas under them are easily cleaned. Sealing process equipment to the floor should be avoided as sealants (such as caulking) crack over time.

The noncontact surfaces should be designed to prevent accumulation of any solid materials. These surfaces should prevent any absorption of liquids or water.

Motors used to power equipment should be placed where any lubricant used in the motor does not contaminate the product. Direct drive systems are generally preferable. While drip pans may be used, they should be avoided as much as possible by using a direct drive system. Another source of contamination in drive systems is bearings. Use of food grade material such as nylon, sealed or self-lubricating bearings are preferable. Additionally, seals should be nontoxic and nonabsorbent. It should be easy to remove seals for inspection and sanitation purposes. Hoods used to exhaust steam or dust collection should be easy to clean. For processing liquid foods, kettles should

Module 146: cleaning and sanitizing in food Premises

Cleaning and sanitizing in a food processing plant involves a number of steps. The first step is to remove any gross soil. Then a chemical agent is used to remove any visible soil residues. Next, a rinse of a cleaning agent is used. The rinse is followed by the use of a sanitizer that assists in killing, removal, or inhibiting any microorganisms. If necessary, a final rinse cycle may be used to remove the sanitizer.

Cleaning is influenced by temperature, time of cleaning, concentration of chemicals, and the mechanical action used in cleaning. Use of higher temperatures during cleaning of fat and grease is beneficial; however, it should not be excessively high to cause protein adhesion to a surface.

Many properties of soil are important in determining the ease or difficulty in removing it, for example, particle size, viscosity, surface tension, wettability, solubility of a liquid soil in a solid soil, the chemical reactivity with the substrate, the attachment of soil to a surface or entrapped in voids, and any forces such as cohesion, wetting, or chemical bonds that influence the attachment.

The selection of a cleaning compound depends upon:

1. the type of soil on the surface;
2. the type of surface to be cleaned; the amount of soil on the surface;
3. the method of cleaning (such as soaking, use of a foam, or clean-in-place);
4. the type of cleaning agent – liquid or powder; the quality of the water;
5. the amount of time available for the cleaning cycle; the cost of the compound.

Cleaning involves first the separation of the soil from the surface, followed by dispersion of the soil in the detergent medium. It is important that the soil must not redeposit on the surface.

The effectiveness of a detergent is evaluated based on its:

1. penetration and wetting ability; control of water hardness;
2. efficient removal of soil; ease of rinsing;
3. noncorrosiveness to the surface

A sanitizer used in the food industry must produce a 99.999% (or 5 log) reduction in populations of 75– 125 million *Escherichia coli* and *Staphylococcus aureus* within 30 s at 20°C (70°F). The purpose of using a sanitizer is to destroy pathogens or other organisms on a

clean surface. Furthermore, the sanitizer should not adversely affect the equipment or the health of the consumer.

Module 147: process control

Process control may be defined as manipulation of process variables so that the desired product attributes are obtained. The variables employed in a process have a marked influence on the final attributes of the product. Therefore, an appropriate control of these variables is an important objective in food processing operations.

Advances made in computer technology since the 1970s have made it possible to automate process operations. By controlling process variables, the desired consistency of operation is achieved along with reduced costs of production and improvements in safety. When equipment begins to deviate from what it is designed for, automatic controls provide a higher level of consistency than if human intervention is allowed that often leads to higher levels of variability. The production productivity is enhanced as less out-of-specification product is produced. Automatic controls provide a higher level of screening of unsafe conditions to improve overall safety of the equipment.

A feedback process model:

Consider a manual temperature control installed on juice being pumped through a steam heat exchanger. The temperature of the juice is the control parameter. The measurement device is a thermometer used to measure temperature. An operator decides whether the temperature is too hot or too cold. A steam valve is used to make adjustments. If the juice temperature is too high, then the operator adjusts the valve towards the closed position. This is an example of a negative feedback control, because a positive error requires a negative response from the operator. Initially the operator adjustment may be too large. When the desired set point is approached, the operator is able to make finer adjustments.

The process variable is measured and compared with a set point, and this generates an error signal. For the given error signal, an algorithm is used to determine the type of control response. The control response manipulates the control element. Thus the control variable is modified and the loop is repeated. With decreasing error, the control response becomes smaller

Transducer: A transducer is a sensing element that detects the process variable. It converts the signal into some measurable quantity. Often the measurable quantity is an electrical signal. For example, a thermocouple receives information about temperature and converts it into a millivolt signal.

The output signal of a transducer may or may not be convenient to transmit for long distance. The modern control systems can accommodate a variety of signals, such as millivolt, frequency, and variation in current. The output signal may or may not be linear with respect to the measured quantity.

Transmitter: Transmitters help in converting the measured variable into a standardized signal. Often the variable is linearized to the measured signal. Typical output of a transmitter is 4–20 mA. Often a power source of around 24 V is used as a direct current source, and any other voltage generated due to electrical noise is usually not a problem as only the change in the current is measured. Within limits of the power source, devices may be driven by including them within the 4- to 20-mA loop.

Controller: A controller reads the transmitted signal and relates it to the set point. The controllers are able to handle a variety of electrical signals such as current, voltage, or frequency. In special situations where electrical circuits may cause hazardous conditions such as explosion, pneumatic controllers are used.

Digital controllers are used to convert analog to digital signals. The digital signal is read by a digital computer that processes the data and calculates the deviation of the transmitted signal from the set point. The digital controller then converts the digital signal received from the computer into an analog signal in the form of 4–20 mA; however, other output signals are also possible. The output signal is used to adjust the control element. In case of a current to pneumatic converter, the 4- to 20-mA signal is converted into a 3- to 15-psig output signal. In addition to controlling valves, other food processing devices may also be controlled such as a variable speed motor used to drive a pump.

Sensors: There are a wide variety of sensors used in the industry. Several considerations are necessary when seeking a sensor for food processing application, including type of material contacting with food, range, accuracy, and cost.

Module 148: handling solid foods in processing plant

The design and layout of the plant and how materials are handled between various processing equipment have a major impact on the production efficiency. In the case of transporting solid foods in a food processing plant, it is typical to consider the movement of product in any direction, horizontal or vertical. A variety of conveyors and elevators are used for this purpose. These include belt, chain, screw, gravity, and pneumatic conveyors, and bucket elevators. In some cases fork-lifts and cranes are employed. Some salient features of these different conveyors are described below.

Belt conveyors: An endless belt operating between two or more pulleys is one of the most ubiquitous conveyors used in transporting solid foods in a processing plant. Between the pulleys, idlers are used to support the weight of the belt. Some key advantages and disadvantages of belt conveyors are as follows:

1. High mechanical efficiency as load is carried on antifriction bearings.
2. Minimal damage to product, as no relative motion between the product and the belt.
3. High carrying capacity.
4. Ability to convey long distances. Long service life.
5. High initial cost.
6. Requires significant floor area.

Module 149: Food Transport: Screw Conveyors

Screw conveyors consist of a helix turning inside a circular or U-shaped trough. Screw conveyors are suited for handling powders, sticky and viscous products such as peanut butter, and granular materials. They are also useful for mixing in batch or continuous mode. Screw conveyors are useful to empty silos of flour and powder materials. Often they are used as metering devices.

The flights of screw conveyor are made of a variety of materials including stainless steel. Their operating power requirements are high, and they are used for distances less than 25 m. In a standard screw conveyor, the pitch of a screw is the same as the diameter. Screw conveyors are suitable for horizontal as well as incline up to 20 degrees. For horizontal screw conveyors an oval trough is used, whereas for a steep incline a cylindrical trough is necessary.

The power requirement of a screw conveyor depends upon a number of factors including:

1. the length of the conveyor;
2. elevation; pitch;
3. speed;
4. type of flights and hanger brackets;
5. weight and properties of the material being conveyed;
6. the coefficient of friction between the product and the material of the flights and housing.

The startup power requirement of a screw conveyor is generally higher than for continuous operation.

Module 150: Food Transport: Elevators

Bucket elevators: The bucket elevator consists of an endless belt with buckets attached to it. The belt operates on two wheels; the top wheel is referred to as the head and the bottom is called the foot. Bucket elevators are highly efficient as there is absence of frictional loss between the product and the housing material. The bucket elevators are enclosed in a single housing referred to as a leg; in certain cases, the return is housed in a second leg. A chain or a belt is used to carry the buckets that are shaped with either rounded or sharp bottoms. The belt or chain operates between two wheels – the head and foot. For longer lengths, idlers are installed to prevent belt whip.

The product carried by the buckets is discharged at the top when the bucket turns around the head wheel, and the product is thrown out by the centrifugal force. The speed of the bucket as it goes around the head wheel must be maintained within limits to ensure that the product is discharged in a desired region.

The conveying capacity of a bucket elevator depends on the product density, belt speed, bucket size and spacing of buckets on the belt. Typical applications of bucket elevators are for handling cereal grain, animal feed, and meal. The energy requirements of bucket conveyors for cereal grains range from 0.1 to 0.2 kWh/m³.

Module 151: Storage of Fruits and Vegetables

Many fruits and vegetables are highly perishable. Therefore in post harvest management, proper techniques of handling and storage of fruits and vegetables are essential to minimize losses. The range of post harvest losses varies anywhere from 5 to 50% or even higher. In



developing countries, post harvest losses are enormous often due to lack of adequate infrastructure and poor handling practices. As a result, the growers and those engaged in the food handling chain suffer major financial losses. Moreover, the shelf life of these products is severely reduced and poor quality product is delivered to the consumer. Unfortunately any gains made in increasing the production yields of fruits and vegetables are compromised by increased post harvest losses due to inadequate practices.

In industrialized countries, major progress has been made in developing proper systems for the handling of fruits and vegetables. Post harvest losses are reduced in a significant manner and the product is delivered to the consumer with minimal quality loss

The respiration process:

Fruits and vegetables continue to undergo physiological changes after harvest. These changes are largely the result of the respiration process. The metabolic pathways active in a respiration process are complex. As a result of the respiration process, the starch and sugars present in the plant tissue are converted into carbon dioxide and water. Oxygen plays an important role in the respiration process. The oxygen concentration within a product is very similar to that of the normal atmosphere. When sufficient amount of oxygen is available, the respiration process is called aerobic. If the surrounding atmosphere becomes deficient in oxygen then anaerobic respiration occurs. Anaerobic respiration results in the production of ketones, aldehydes, and alcohol. These products are often toxic to the plant tissue and hasten its death and decay. Therefore anaerobic respiration must be prevented. Furthermore, to extend the shelf life of fruits and vegetables, the oxygen concentration must be controlled in such a manner as to allow aerobic respiration at a reduced rate. Control of the respiration process has become one of the most important methods of storage in commercial practice. 

An important physiological change during fruit storage is the production of ethylene gas. Based on their ethylene production, fruits are classified as either climacteric or nonclimacteric. Climacteric fruits exhibit a high production of ethylene and carbon dioxide at the ripening stage.

Module 152: Transport of Fruits and Vegetables

One of the most common ways to transport perishable foods such as fruits and vegetables is by using a refrigerated trailer that may be either pulled via a truck on a highway or placed in a ship for trans-ocean shipment. Any perishable product stored in the trailer must be kept

refrigerated for the entire duration. A typical refrigerated trailer includes a refrigeration system to cool air and an air handling system to distribute air within the trailer. It is vital that there is uniform air distribution within the trailer; otherwise regions with no air circulation can lead to product heating and spoilage.

Temperature control of the air circulating inside the trailer is vital to the shipment of perishable foods. Modern trailers are equipped with temperature sensors and controllers that automatically control the re-refrigeration unit based on the temperature of air exiting the refrigeration unit. The control of air temperature at the exit of the refrigeration unit is important in protecting fresh produce that is sensitive to chilling injury or freeze damage. The temperature of the thermostat in these systems is set within 0.5° C of the long-term storage temperature. In older trailers used for chilling/freezing-sensitive produce, the temperature control is typically based on the return air to the refrigeration unit, and the controllers should be set at least 1.5–2.5° C above the long-term storage temperature of the produce. For frozen products, the temperature is controlled based on the return air temperature. The trailer used for frozen products should be set at –18° C or colder. The frozen food industry generally requires that at the time frozen food is loaded into a trailer, the temperature of the product should be less than –12° C.

Module 153: Water Quality and Waste water in Processing Plant

Water is a ubiquitous resource on our planet, yet the availability of a clean and reliable supply of water is becoming increasingly scarce. The food processing industry relies heavily on access to clean water. Water use in food processing has increased with the widespread mechanization of harvesting operations; raw agricultural products arriving at a food processing plant require large quantities of water for cleaning. Inside the plant, water is used in a variety of processing and handling operations such as product conveying, peeling, blanching, cooling, generating steam, and washing equipment and floors.

The quality of water used in food processing depends on the function of water in the manufacturing process. For example, water quality for initial cleaning of raw produce has a different requirement than water required for the formulation of carbonated beverages, beer, and bottled drinking water. Water obtained from ground wells or surface areas (such as lakes, rivers, and springs) may require certain treatment before its use in the food processing plant. Water has high levels of hardness due to the presence of dissolved solids, then processes such as precipitation, ion exchange, distillation, or reverse osmosis may be used often

pretreated using coagulation, flocculation, sedimentation, or filtration. The presence of dissolved organics in water causes off-flavor, odor, or color that is often removed by activated carbon adsorption.

Lecture 16

MICROBIAL PRODUCTION OF FOOD INGREDIENTS

Module 154: Main techniques used for production of food ingredients by microbes

Microorganisms play an important role in the generation of natural compounds, particularly in the field of food flavors. For a long time, plants were the sole sources of flavor compounds and most of them were isolated from essential oils. However, active compounds are present in low concentrations, which makes their isolation difficult. Another disadvantage of plants as a source of flavors is the dependence on factors that are difficult to control such as the weather and the risk of plant diseases. The production of flavor compounds by biotechnological methods has been an interesting alternative due to consumers' preference for natural ingredients. Microbial processes seem to be the most promising methods for the production of natural flavors.

Many microorganisms are capable of synthesizing flavor compounds when growing on a culture media. They have the ability to perform conversions that would require multiple chemicals steps. Microorganisms are used to catalyze specific steps. They are also an economical source of enzymes, which can be utilized to enhance or alter the flavors of many food products. In this way, biotechnological processes involved in the production of flavor compounds can be divided into two groups: microbiological and enzymatic. Microbiological methods are subdivided into biosynthesis and biotransformation. The first is the production of chemical compounds by cells (fermentation or secondary metabolism). The second refers to the use of microbial cells in the specific modification of chemical structures.

In fermentation, the production of flavors starts from cheap and simple sources such as sugars and amino acids. The product is generated by the complex metabolism of the microorganism. When microorganisms are used in order to catalyze specific conversions of precursors and intermediates, the process is called biotransformation. Although fermentation requires C and N sources, a specific substrate is necessary for microbial transformation. The enzymatic catalysis precedes a simple and specific transformation of the substrate molecule. The substrate does not have to be “natural”; according to Schreier (1989) “non-natural” substrates can also be biotransformed.

It is important to distinguish research with the purpose of obtaining complex products with natural characteristics from those that try to obtain isolated molecules. The first consists in

the experience of nature imitation and in developing a process with one or more microorganisms and enzymes. The second tries to obtain a higher yield of the characteristic components. The choice between them determines the methodology, which will be employed in vivo or in vitro, through biosynthesis or bioconversions (Delest 1995).

Module 155: Selection of Microorganisms and development

For centuries microorganisms have been employed for the production of fermented food products (i.e., cheese, soy sauce, sauerkraut, wine, and bread). The consumption of some live microbial cultures (probiotics) has proven to provide a health benefit to humans and animals. Some foods containing live cultures are yogurt, buttermilk, and acidophilus milk. These microbial fermented food products also have an extended shelf-life compared to the perishable starting raw material. Thus, microorganisms not only provide a nutritional benefit to humans but act to extend the shelf-life of the food supply.

Microorganisms employed by the food industry include bacteria, yeasts, and molds. These microorganisms have several morphological and physiological differences. Morphologically bacteria are small and difficult to remove, yeasts are larger and will sometime settle out of solution, whereas molds are filamentous and are typically removed by filtration. Physiologically they differ in pH preferences (yeasts and molds prefer a lower pH than bacteria), nutrient requirements (different concentrations and types of nitrogen and other trace elements), growth rates (bacteria grow much faster than yeasts and molds), and more. Thus, different culture media, fermentation methods, and product recovery methods are required depending on the microbial system being cultured.

Microorganisms are the biocatalysts that produce and maintain a host of enzymatic pathways that are used to produce the food component of interest. The characteristics of a good industrial microorganism for the production of food ingredients are (1) it must be effective in producing large quantities of a single product, (2) it can be efficiently isolated and purified, (3) it is easy to maintain and cultivate, (4) it is genetically stable, (5) it grows best in an inexpensive culture medium, and (6) it is safe for human consumption. The first step is to isolate the hardiest starter culture possible, then to begin strain improvements via classical mutagenesis or genetic engineering.

Module 156: Culture Media and Upstream compounds

The ideal culture medium will use inexpensive components to supply their complex nutrient

requirements. Miller and Churchill (13) provide an excellent summary of inexpensive media components and their makeup. These ingredients are crop, animal, marine or yeast based components. The culture medium alone can represent 30 to 70% of the fermentation production costs. Slight changes in medium micronutrients can have a major impact on the fermentation. Thus, the food industry demands a consistent product from suppliers of these complex components. Failure to provide a consistent product will eliminate the commercial use. What decides the culture medium makeup? Essentially, it is the nutritional requirements of the microorganism of choice and its ability to biosynthesize essential elements such as amino acids, vitamins, lipids, and carbohydrates. For example, bacteria and yeast are high in protein (40–50%), whereas molds are not (10–25%). Yeast can generally grow in a minimal medium, whereas lactic acid bacteria, essential for the fermented dairy products, require a host of micronutrients to grow.

Each microorganism has different micronutrient requirements. Typically the exact micronutrient which benefits the fermentation that is being supplied by the complex nutrient employed is unknown. Generally it involves specific amino acids, vitamins, trace elements, and lipids. Furthermore, the concentration ratio of carbohydrate to nitrogen and phosphorous has a dramatic impact on microbial growth. Carbon–nitrogen imbalances can result in the production of other byproducts such as extracellular polysaccharide, and fermentation end products such as ethanol. In the case of ethanol production in yeast, excess glucose in the presence of oxygen will direct yeast to produce ethanol. This is called the Crabtree effect. Yeast cells typically consist of 48% carbon and 8% nitrogen on a dry weight basis.

When the C:N ratio is 10:1, yeasts grow aerobically consuming little substrate while producing maximum cell mass, CO₂ and H₂O, but when the C:N ratio is 50:1 yeasts grow anaerobically, consuming much substrate while producing little cell mass, and much CO₂ and ethanol. This difference in yeast growth is also called the Pasteur effect. Why the increase in industrial microbiology fermentation processes over the past 20 years? It is the result of the corn syrup sweetener industry and computer technology. Corn is 70% starch, and when dried to 19%, it can be stored for more than two years. Annually 10 billion bushels of corn are produced in the United States of America. Thus, the liquefaction of corn starch to glucose syrups for the production of high fructose corn sweeteners represents a consistent, low cost supply of substrate for most industrial microbiology fermentations. Not only is glucose produced, but customized substrates can be also produced. For example, corn syrups containing 19% dextrose, 14% maltose, 12% maltotriose, and 55% higher saccharides are

used to control microbial growth rates and biological heat production in many fermentations. Thus, glucose is the platform chemical used for the microbial production of organic acids, amino acids, vitamins, and more. In fact some food grade fermentation facilities have located adjacent to a corn sweetener facility to permit glucose syrup to be piped directly to their fermentors. Glucose corn syrup is also shipped via truck or rail cars as liquid or dried product. reliable and easy to operate dissolved oxygen, pH, foam, temperature, and sterilization controls of the process. Sirakaya et al. (19) described fermentation software to monitor and control the utilization fermentation process.

Module 157: Bioreactor monitoring systems and design

The stirred tank bioreactor design is the most common fermentor and consists of agitator, baffles, aeration sparger for aerobic culture growth, sterilizable monitoring probes for pH, dissolved oxygen, temperature, and antifoam, filling and draining ports, and often culture medium sterilization capabilities in the reactor tank. Reactor agitation is essential for temperature control, pH adjustments, oxygen absorption into the liquid medium, overall culture health, and mixing of any required additions of substrate, and nutrients. Typical commercial reactor working volumes for food grade ingredients are 5,000 to 40,000 gallons.

Fermentation health requires real time monitoring system. Microbial growth can be monitored via several methods. The most common method is indirect measurement of biomass by absorbance of the fermentation broth at 620 nm by using a spectrophotometer. The measured absorbance values can then be used to estimate biomass concentrations by using a standard curve. Standard curves are developed by collecting fresh log phase cells via centrifugation, washing cells with water or 0.1 M ammonium acetate pH 7.0 buffers, then serially diluting the pellet. Absorbance for each dilution is then determined spectrophotometrically at 620 nm. The actual dry weight biomass (g/l) is determined for each dilution via direct biomass measurement after oven drying of each dilution in preweighed boats at 70°C for 24–36 hr. This needs to be performed in at least replicates of three. By washing biomass with water, any influence of culture medium on dry weights can be eliminated or minimized. Finally, a standard curve can be constructed by plotting absorbance versus actual dry biomass weight (g/l). This method allows for quick, reliable, and easy conversion of absorbance to dry biomass (g/L).

Substrate consumption and product formation rates can be monitored by high pressure liquid chromatography (HPLC) or by membrane bound enzymes biosensors, which requires 20 and

1 min to run, respectively. HPLC analysis is time consuming, but the concentration of multiple metabolites can be monitored simultaneously. HPLC does not provide real time feedback on the health of the fermentation, and it has long sample preparation and run times.

In contrast, membrane bound enzyme biosensors such as YSI 2700 select analyzer (Yellow Springs Instruments, Yellow Springs, OH) can analyze a sample in 1 min. However, these units are restricted by the availability of substrate specific oxidases which generate H_2O_2 , the measurable product by their electrode. Some compounds currently measurable are glucose, ethanol, maltose, lactic acid, and lysine. Sample preparation is simply filtration (0.45 μ m) and dilution with water if the value falls outside the instrument window.

Organic acid production can be continuously monitored via alkali addition rates for pH control. Alkali consumption can be easily monitored by feeding alkali solution from a sterile burette. Microbial respiration for aerobic cultures can be continuously monitored via dissolved oxygen probe or the concentration of CO_2 in the exit gas, which can be monitored via off gas analyzers or simply via alkali (4 N NaOH) traps followed by pH titration. However, more technologies are needed to acquire real time measurements of microbial growth, ensuring optimal fermentation time, and product formation in the shortest time possible.

Module 158: Fermentation Types Employed: The Actual Production Process

The work horse of the industry is batch and fed-batch fermentation. Batch fermentations are closed fermentations. The fermentation sequence starts with medium sterilization, reactor inoculation [1 to 2% (v/v) typically], incubation for complete microbial growth cycle with lag, log, and stationary phases, fermentation termination, draining the reactor for product recovery down stream, cleanup of the reactor, and starting over. Percentage yield is calculated by taking the concentration of the product formed (g/l) divided by the concentration of substrate consumed (g/l) times 100%, whereas productivity is a measure of the product formation rates. It is calculated by dividing the product concentration (g/l) by the fermentation time (hours); thus, it is presented in g/l/hr.

In fed-batch fermentation, additional carbohydrate is supplied to the batch fermentation during the run. High carbohydrate concentrations in the initial culture medium are toxic to many microorganisms. Thus, an optimal carbohydrate concentration is employed initially, which permits maximum culture growth to late log phase. When the carbohydrate

concentration is reduced to almost zero, additional sterile carbohydrate is injected into the bioreactor to bring the carbohydrate concentration back to the starting level. When this is consumed, the process is repeated until end product inhibition forces the whole bioconversion to stop. Ideally, at the end of the fermentation you will have a product concentration which is three to four times greater than single batch fermentation with no residual carbohydrate.

This will generate the highest yield possible. Also, due to increased end product accumulation with each carbohydrate addition, culture production rates will decrease. Thus, the decision as to how many fed-batch fermentations to perform before harvest is based on the desired final product concentration and the optimal fermentor use time. For example, in

lactic acid fermentations a final product concentration ~ 120 g/l is desired to enhance product recovery. For *Lactobacillus casei* this concentration can only be achieved via fed batch fermentation for a total fermentor run time of eight days.

Continuous fermentations are open fermentations, whereby fresh medium is continually added to the bioreactor, while spent culture medium, cells, and product are continually leaving. This fermentation is desired by the industry, because the reactor volume is 10 to 100 times smaller than batch fermentations, a steady stream of fermentation product is produced which will optimize downstream processes, bioconversion rates are always at maximum, operation costs are less, and the system can be fully automated and computer controlled to the point where only two operators are needed to manage the fermentation each day. However, it requires a continuous supply of sterilized or pasteurized culture medium, dilution rates are linked to microbial growth rates and the operational speed of downstream recovery process. Startup is slow, so any facility shut downs have an impact on production, and you are constantly fighting contamination. Thus, not all fermentations can be operated this way. The best example is ethanol production for gasohol, which is commonly a continuous fermentation with a four bioreactor train with increasing working volumes in each bioreactor. Thus, the dilution rate is decreasing in each bioreactor over the course of the fermentation. This dilution gradient in the train is critical, because as ethanol builds up in the culture medium, the yeast growth rate slows.

Specific growth rate equals dilution rate. Finally a holding tank at the end is used to ensure complete bioconversion of any residual sugars to ethanol. The CO₂ is collected and concentrated, then sold as another valuable byproduct. Fermented beverages (i.e., wine and beer) are still performed in batch. For some continuous fermentations, an increased

concentration of biomass in the reactor is required. This can be achieved by cell recycling, immobilized cell, and biofilm fermentations. Cell recycling reactors employ a filtration unit that allows for the constant bleeding of culture supernatant while retaining biomass (26). However, filtration unit fouling is a problem and must be constantly monitored. This type of operation has found use in the treatment of food processing from some starchy food waste streams.

Module 159: Bioreactors Design

One of the most common forms of immobilized cell bioreactor is entrapment, where high concentrations of cells are trapped in a polymer matrix such as alginate and carrageenin. Thus, a high cell density is continuously retained in the fermentor while substrate is continuously converted to product. This higher concentration of biocatalysts in the reactor results in higher productivities and yields. The disadvantages of this method are migration of substrate through the matrix to the cell and the migration of product out, potentially high concentrations of product around the cells causing end product inhibition, cell leakage from the polymer matrix due to cell growth, and bead swelling and disintegration over time causing the whole fermentation to be stopped, cleaned, and restarted. Biofilms are a natural form of cell immobilization in which microorganisms are attached to a solid surface. In this bioreactor, cells are continually growing, and sloughing off. Thus, the reactor is a mixture of immobilized and suspended cells. Continuous biofilm fermentations are truly open immobilized cell bioreactors. Their operation is equivalent to a suspended cell continuous fermentation with the added advantage of increase biomass concentrations in the bioreactor. Biofilms are typically resistant to harsh conditions, and can tolerate changes in the fermentation feed and conditions.

However, not all microorganisms form biofilms. Filamentous microorganisms such as fungi and actinomycetes are natural biofilm formers. For nonfilamentous bacteria to form a biofilm, an extracellular polysaccharide needs to be generated by the bacterium. Some bacteria will form biofilms on any surface such as metal, plastic, and glass. However, certain bacteria, such as lactobacilli, require something to stimulate this biofilm development. Plastic composite support (PCS) developed at Iowa State University has proven to stimulate biofilm development of *Lactobacillus case*, *Zymomonas mobilis*, *Saccharomyces cerevisiae*, and *Actinobacillus succinogenes*. PCS is a high temperature extruded material consisting of at

least 50% polypropylene, plus ground soybean hulls, bovine albumin and various culture micronutrients.

Soybean hulls keep the extruded product porous due to the release of steam as the PCS leaves the extruder die. Bovine albumin performs as a natural plastizer which protects the temperature sensitive micronutrients. Micronutrients are selected based on the specific cultural requirements for amino acids, vitamins, and lipids. Monosaccharides are avoided due to poor PCS production. For example, the PCS blend for lactobacilli contains 50% (w/w) polypropylene, 35% (w/w) ground dried soybean hulls, 5% (w/w) bovine albumin, 5% (w/w) yeast extract, 5% (w/w) soybean flour, and mineral salts. PCS have been evaluated in batch, fed-batch, and continuous lactic acid fermentations. In every application the percentage yields and productivity rates were significantly higher than suspended cell lactic acid fermentations. Furthermore, repeat batch fermentations have operated for more than 1.5 years with virtually no change in percentage yields and productivities. This longevity is attributed to the fact that once a biofilm has established on these customized materials, it will continue to grow as a biofilm. This is supported by the fact that a PCS biofilm reactor washed with concentrated ammonium hydroxide, rinsed with mineral salts solution, and then reinoculated with a fresh culture and medium will reestablish itself overnight. Commercially, the quick vinegar process is the most common biofilm process in current operation which uses wood chips for supports and *Acetobacter aceti* for production.

Solid substrate fermentation is when a substrate such as soybeans is ground, inoculated with *Aspergillus oryzae*, then incubated for three days for the production of soy sauce. It is a simple fermentation process and commonly used for aerobic fermentation due to its large surface area. Thus, oxygen concentration is high without using any mechanical forced air systems. Solid substrate fermentations require large areas or incubation space.

A temperature controlled environment, intermittent monitoring for contamination and quality of starting material is essential for success. 

Module 160: Future Research in food Fermentation:

Research is still needed for isolation of new microbial strains with improved production efficiencies and higher yields. More real time measurements are needed for culture conditions and metabolite formation. Recovery will continue to be the key factor associated with final product purity and cost. As an industry we cannot rely solely on genetic engineering as our

method of improving current fermentations. As we have illustrated, there are many other techniques which can be employed to improve productivity and yield, including new inexpensive medium ingredients, more continuous fermentation processes, and new exotic microbial reservoirs in nature and in the food industry.

Lecture 17

GENETIC MODIFICATION OF PLANT STARCHES

Module 161: Genetic modification of plant starches for food applications

Starch is a unique natural material, valued for its uses in food, feed, and industry. It is found in higher plants, mosses, ferns, and some microorganisms where it serves as an important store of energy. In higher plants, starch is deposited as transitory starch in leaves and as storage starch in specialized storage organs such as seeds or tubers. Starch is also an important component of many fruit crops such as apple, pear, melon, banana, and tomato. Storage starch is one of the main components of cereal grain (seeds) harvested from crops like wheat, maize, oats, barley, sorghum, and rice as well as of tubers harvested from crops like cassava, yam, and potato. Whether in its native state in grain or tubers, or in isolated granular form, starch is a convenient stable material, cheap to produce, suitable for long term storage without spoilage, convenient for high volume transport, and an important source of calories, retaining functional properties for use in many potential product applications. Grain and tubers are often used directly for animal feed or human food, with little or no processing, such as cooked whole cereal grain or potatoes. Cereal grain is also ground or milled to make flour or meal, which is subsequently mixed with other ingredients and cooked to make breads and pastries. Alternatively, starch may be extracted from the storage organs and the purified starch used as a key functional ingredient added to foodstuffs such as pie fillings, puddings, soups, sauces, gravies, coatings, candies, confectionary products, yogurts, and other dairy products. Extracted starch also has many nonfood industrial uses, such as paper sizing aids, textile sizing aids, molded plastics, ceramics, dye carriers, or suspension aids. Globally, starch is an essential commodity providing most (80%) of the world's calories. This vital commodity supply comes from just six different plant species: three cereal crops (rice, maize, and wheat) and three tuber crops (potato, yam, and cassava).

As a result of advances in genetics and biochemistry, we have discovered much about how starch is synthesized in crop plants. Furthermore we have also unraveled the biochemical and genetic basis of some useful natural genetic variations that affect starch synthesis and consequently starch structure and functionality. Some of these variants are already commercially exploited. Examples include variants that accumulate less starch and more sugar (e.g., sweet peas, sweet corn, sweet potato) and others that cook to form clear sols rather than opaque gels (e.g., waxy corn, waxy rice, waxy wheat) and yet others that are

useful industrially (e.g., amylose extender corn), and finally others valued for imparting stickiness when cooked (e.g., indica vs. japonica rice). Further progress in this area depends upon improvements in our understanding of the relationship between starch synthetic genes and enzymes, starch structure and functionality. Thus, by linking these findings with further advances in our understanding of the genes required for starch synthesis, an opportunity has appeared for us to make starches with increased usefulness and value.

STRUCTURE

Physically, after extraction and drying, normal starch is a white powder consisting of a mixture of amylose and amylopectin in semicrystalline granules. Starch granules are microscopic structures approximately 0.5 to 100 μm in diameter. In shape, they are spherical, elliptical, or polyhedral. The size and morphology of starch granules is characteristic of the organ and species in which they are produced. Starch granules appear rather similar in size and morphology with and without amylose. Under most environmental conditions, starch granules can be considered moderately inert with little capacity to hold water. These characteristics of starch granules make them ideal vessels for storage and shipping, whether in grain or tubers or from processed isolated starch.

Chemically, starch is classified as a complex carbohydrate and is a mixture of two polymers of glucose: amylose and amylopectin. Amylose is a generally linear -1,4 glucan which is sometimes lightly branched with -1,6-glycosidic linkages. Amylopectin is normally higher in molecular weight than amylose. It is also an -1,4 glucan, but is highly branched with -1,6-glycosidic linkages. The proportions on a dry weight basis of amylose and amylopectin in starches isolated from storage tissues like potato tubers or cereal grain is normally between 20 to 30 percent amylose and 70 to 80 percent amylopectin. In addition to amylose and amylopectin, granules contain small quantities of protein and lipid. Between species there is variation in the structure of amylopectin, the size and structure of amylose, and the nature and amounts of proteins and lipids. Because starch physical behavior is dependent on all of these components there are specific uses of starches from different species. In addition, within a given species, rare examples have been found of grains, tubers, or roots producing starches that deviate from the typical amylose to amylopectin ratio or have altered amylopectin structure. These plants have been selected because of their unique cooking behavior due to their unusual starch composition that confers unique properties to the crop storage organ. Some of these natural variants are now cultivated on a commercial scale.

In starch granules, some of the chains of amylopectin are believed to be associated with one another through hydrogen bonding, forming double helices. The double helices either form higher ordered crystalline structures or may exist independently of crystalline order. The double helices are oriented radially within the granule, with the reducing ends of the chains oriented toward the center or hilum of each granule. Within the granule, crystalline regions, often referred to as growth rings, are separated in a radial fashion from each other by amorphous regions. The crystalline regions are further subdivided into amorphous and crystalline lamellae, which have a periodicity between clusters of approximately 9 nm. The branch points in amylopectin are believed to be the primary component of the amorphous lamellae, with the ordered amylopectin side chain double helices clustered in the crystalline lamellae. Differences in the internal chain lengths of amylopectin affect starch crystallinity.. Important new insights into how amylopectin chain architecture may affect packing have been advocated based on small angle x-ray scattering studies and analogies with liquid crystals. Using these models it is possible to discuss the mechanisms and kinetics of interchain associations in the context of visualizing starch as a liquid crystalline polymer having different degrees of crystalline order depending on physical conditions. Amylose contributes to the overall crystallinity of normal starch through the formation of crystalline complexes of amylose with lipids and, it is believed, through participation

Module 162: Modification of starches

Most of the maize starch mutants affecting starch structure were discovered before the genes and enzymes responsible for starch synthesis were known. Their names are therefore based upon the phenotypic changes observed in that storage organ or on the properties of the starch, rather than that of the gene or enzyme affected by the mutation. For example, numerous mutant phenotypes have been reported for maize and several phenotypes (e.g., waxy, amylose extender, dull, shrunken, sugary-2, and sugary) have been described extensively with regard to their effects on carbohydrate composition and response to genetic background, allelic dosage, or interaction with other mutations. Examination of maize kernels with differing starch phenotypes has been instrumental in determining which enzymes are required for starch synthesis in this storage organ. Mutations that are responsible for most of the abnormal starch phenotypes have been located in genes encoding starch synthetic enzymes. Related isoforms, for which there are as yet no mutants available, have also been identified and characterized. Thus, the presence of a functional GBSSI enzyme from a single locus pair is sufficient to produce a starch with an amylose content of at least 16%. Others have shown

that low amylose wheat starches having amylose content between 14.1 and 16.7% can be created through ethyl methanesulphonate (EMS) mutagenesis of the seeds. Amylose free wheat starches were created using triple null combinations of GBSSI mutants and using mutagenesis of a double null wheat known as Ike to generate a nonnull wheat which stained red when stained with iodine.

Two functional Wx alleles of rice exist: Wxa, which produces a large amount of amylose, and Wxb, which produces a smaller amount of amylose. Studies of the effects of the two alleles on the gene expression at the waxy locus in rice showed that the Wxb allele resulted in an ineffective production of GBSSI enzyme and amylose in japonica rice, while the Wxa allele produced larger quantities of GBSSI enzyme and amylose in indica rice. On a specific activity basis, other authors have shown that the Wxa allele was less effective in the production of amylose than the Wxb allele based on analysis of 40 rice varieties. It was observed that for two wild-type rice alleles, Wxa and Wxb, Wxb had a GBSS expression tenfold lower than Wxa at the protein and mRNA levels. The decrease in the expression of Wxb compared to Wxa was the result of a point mutation within the genetic sequence for the normal rice enzyme (Wxa allele). The Wxb allele resulted in the synthesis of a 3.4 kilobase pair mRNA transcript compared to a 2.3 kilobase pair mRNA transcript for Wxa as a result of the inclusion of an intron into the mRNA sequence as a result of the point mutation. Amylose produced from rice plants was related to the ability of the plant to excise the intron 1 from the mRNA sequence. Plants expressing high levels of mature mRNA (without intron 1) and no pre-mRNA (containing intron 1) produced the highest levels of GBSSI protein and the highest levels of amylose (20.0 to 27.8% amylose). These were all indica species. With more balanced expression of mature and pre-mRNA, lower levels of GBSSI protein and amylose were observed (6.7 to 16.0% amylose). Both indica and japonica species were within this group. When all of the mRNA contained intron 1 and no mature mRNA was observed, no GBSSI protein was observed and no amylose was detected. This pattern relating amylose content to mature mRNA with properly excised intron 1 could be applied across 31 different rice cultivars. Thus, based on extensive studies, low amylose rice appears to be the result of a decrease in the amount of normal GBSSI through a mutation which results in problems with mRNA processing rather than due to a mutation in the mature mRNA sequence. However, some differences in the behavior of Wxa and Wxb may be present in different rice species.

Modification of SS Activity

Modifications in starch synthase activity result in changes in amylopectin content or amylopectin structure. Starch synthase may have either a subtle or profound impact on the starch, depending on the activity or inactivity of a specific isoform on the structure and composition of the starch. Although SSI is a relatively minor isoform in potato, it is the predominant isoform of SS in the cereals. Of the starch synthases, the effects of SSIIa and SSIII on starch structure and composition are the best elucidated, especially in maize. Mutants lacking the putative SSIV isoform have not yet been reported. The first reported SSI mutant was in rice and no mutants have yet been reported in other plant species. In rice, the SSI mutant has only a minor effect on starch content and quality. In maize, SSIIa maps to a locus known as Sugary-2 which when mutated produces the sugary-2 also been found which, like maize, do not have increased amylose content and do not further increase amylose when combined with SBEIIb mutants. High amylose rice starches appear to have amylose contents between 30 and 50% of the weight of the starch. However, with the variability of amylose content of what may be considered normal rice varieties and the recent implication that other starch biosynthetic enzymes affect the amylose content of such normal rice starch with a few exceptions, it is difficult to know whether deficiencies in SBE are explicitly responsible for some of these high amylose rice starches. In such instances, the rice starch granules have the characteristic changes in starch properties, amylose properties, and amylopectin structure seen with high amylose maize starches. Similar to rice, high amylose barley starches with an amylose content between 30 and 45% of the weight of the starch clearly exist, although it is unknown whether these starches are a result of down regulation of SBE activity or are a result of changes in the expression and activity of other enzymes.

Reports of high amylose potato starch obtained through transgenic down regulation of multiple starch branching enzymes have been published recently. Work on development of a high amylose potato starch has been occurring for at least the past 10 years. Amylose contents as high as commercially available maize starches have been obtained in potato with a decrease in the overall molecular weight distribution.

Modification of DeBE Activity

Modifications in debranching enzyme activity can result in significant changes in starch granule structure. ISAI mutants accumulate starch in compound instead of simple starch granules (compound refers to amyloplasts containing many small granules, while simple refers to amyloplasts containing one major granule) and sometimes also accumulate phy-

toglycogen (a highly branched nongranular storage product). Mutants known in ISAI include the sugary1 locus in maize and sugary of rice and in barley by lines named Riso17 and Notch-2. Similar results were observed using antisense technology to reduce ISAI activity in rice. In potato where antisense constructs for ISAI and ISAI were combined, the tubers accumulated large numbers of small granules. Mutations in PUI have been identified, but effects on starch content are minimal. Modifications in the other isoamylase (ISAI and ISAI) have not yet been identified, although there is some evidence that they each play distinct roles in starch synthesis (90). The maize sugary mutants are important because they are one of the main sources of producing sweet corn.

Modification of Multiple Pathway Enzymes

By eliminating multiple starch biosynthesis enzymes, other alterations of the starch biosynthetic pathway can occur resulting in even more novel starches. Several patents exist of amylopectin gels occurs during days or weeks of storage. As a consequence of their different molecular weights and chain length profiles, the rates of retrogradation of amylose and amylopectin are not the same. The rapid setting of the structure of breads is believed to be due to rapid (within seconds or minutes) amylose retrogradation to form a network structure. Starch functionality is therefore a consequence of the degree of gelatinization and is influenced by retrogradation, time, temperature, concentration, and the presence of other food components or additives. In addition, modifying starch using chemical, enzymatic, or physical treatments alters and extends its functional properties.

Measuring starch functionality and applying it to food applications is problematic because the results are subject to extrapolation to systems and processes which are far more complex than laboratory testing is able to emulate. Analytical instruments (e.g., Differential Scanning Calorimetry, DSC) are frequently used to quantify the temperature range and amount of energy needed to melt crystalline starch. The amounts and molecular size of amylose and amylopectin may be measured using gel permeation or size exclusion chromatography. Granule size and shape are measured microscopically or by light diffraction techniques, and granule viscosity is measured using various rheometers. Rheological measurements may include various temperature and shear programs that attempt to mimic thermal treatments, pumping, and shearing forces that occur during food processing. Such measurements of texture provide information on adhesiveness, cohesiveness, yield stress, viscous flow, and rigidity of starch sols and gels.

Module 163: functionality of Starches

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Amylose free starches have useful functionality that has encouraged their commercial development. They are considered useful as water binders, viscosity builders, and texturizers in food as well as in industrial applications. However, these starches are less resistant to shear, acid, and high temperatures than are normal starches, and extended cooking results in stringy, cohesive pastes. Amylose free starches are generally recognized for their improved transparency after processing compared to normal starches and have better freeze and thaw stability compared to normal starches once cooked. They are also recognized for their improved long term storage capability as they require weeks to gel if they could be considered to gel at all. To correct for some of the negative paste attributes of amylose free starch, such as poor stability to temperature, shear and acid and undesirable paste quality, most amylose free starches are chemically modified by substitution, cross linking, or both.

Module 164: Application of Starches

In recent years there has been significant interest in developing amylose free starches in other crops so as to take further advantage of any species specific qualities of the starch

produced from that species. However, although mutants are readily found in some plant species (such as maize, rice, and barley) this is more difficult in other plant species where there are multiple copies of each gene (such as the polyploid species like potato, oats, and wheat). In the case of wheat, the advances have been made by screening for mutants (non-GM), while in potato the waxy types were made using biotechnology (GM). Applications of amylose free wheat and potato are at present still being developed. However, the most likely applications for amylose free potato include the paper, adhesive, textile, and packing industries. In the EU, certain GM varieties of modified amylose free (high amylopectin) potato have not been approved thusfar (http://europa.eu.int/comm/food/fs/sc/scp/out24_en.html), while others appear to be in the approval process (http://europa.eu.int/comm/food/fs/sc/scp/out129_gmo_en.pdf). Amylose free wheats are finding applications in foods such as noodles and baked goods including breads.

Low Amylose Starch

Although staining starch with iodine readily identifies amylose free starches, care must be taken when using this quantitatively to adequately account for the iodine binding capacity of the amylopectin. For example, amylopectin from an amylose free plant having an inactive GBSSI enzyme might appear to contain 5% amylose based on iodine binding, blue value, measurement. After carefully considering these potential problems certain low amylose starches have been identified. For example, in the early 1940s, a waxy maize mutant (*wxa*) was discovered in two exotic Argentinean small seeded flint varieties that contained a starch that had an amylose content of 2.4% and stained a pale violet color with iodine. Additionally, the amylose content of the starch increased from 0% (full waxy) to 0.65% to 1.3% to 2.4% (full *wxa*) with increasing dose of the *wxa* allele. With these same crosses, the viscosity of starch pastes decreased with increasing dose of the *wxa* allele. The *wxa* allele was described as resulting in a 95% reduction in the amount of GBSSI protein produced and a starch with a low amylose content.

Wheat starches have been produced with amylose contents of about 7.5% and 13.5% by crossing normal wheat with amylose free wheat. Peak viscosities of all starches differed by less than 20% of the peak viscosity of the amylose free wheat starch, with the low amylose starches having a higher peak viscosity than both normal and waxy wheat starch. The gelatinization temperatures and enthalpy were highest for waxy wheats and decreased in the order waxy . 13.5%, amylose wheat . 7.5%, and amylose wheat . normal wheat starch. The

retrogradation temperatures and enthalpy were insignificantly different for amylose free wheat, normal wheat, or any of the low amylose wheat starches. Starch granules extracted from a wheat strain derived from mutagenized Tanikei A6099 had an apparent amylose content of 1.6% and stained dark brown with dark cores compared to red-staining waxy wheat starch with 0.4% apparent amylose. The Tanikei A6099 mutant wheat starch had a higher initial pasting stability than an amylose free wheat starch (0.4% amylose). However, the viscosity of the low amylose starch paste decreased dramatically, to the same viscosity as the amylose free wheat, during continued cooking and remained at the same viscosity as amylose free wheat after cooking. The mutagenized Tanikei A6099 wheat is known to produce a mutant GBSSI enzyme.

By screening microtubers from plants exposed to x-ray radiation an amylose free mutant of potato was identified. Potato starches considered amylose free have been shown to have an amylose content varying from 0 to 7.9%. The amylose free potatoes were null for the GBSSI enzyme. Staining with iodine gave varied results: sometimes the starch stained red and other times reddish brown and blue. These results are indicative of a heterogeneous mixture of amylose free starch and amylose containing starch of unknown quality within the potato tuber. In further attempts to understand the link between function and activity of GBSSI in potato, antisense transgenic plants having amylose contents between 3.0 and 8% were produced. These tubers had both blue and red brown staining portions again indicative of heterogeneous mixtures of amylose free starch and amylose containing starch of unknown quality. Others observed additional heterogeneity at a granule level, with starch granules having blue cores surrounded by a red brown shell of starch. The size of the blue core appeared to be correlated with the amylose content of the starch. Starch extracted from plants produced from crosses between an amylose free potato and a normal potato had no linear correlation between GBSS activity and amylose content. Additionally it was observed that the swelling and rheological properties of the granules could not be clearly linked with amylose content.

The waxy barley starches have been shown to contain up to approximately 5% apparent amylose. However, this apparent amylose is due to a mixture of starch granules within the barley seeds. The amylose content of the granules typically ranges from an undetectable level up to approximately 10%, with the granules closest to the surface of the seed having the highest amylose content. Recent work with waxy barley starch (with amylose contents up to 6.44%) shows delayed peak viscosity development and viscosity varying during cooking

under shear. Additionally, all of the waxy barley starches began to develop viscosity at a similar time and temperature in the cooking process.

Low amylose rice starches have been shown to have amylose contents between 7 and 15%. Shimada et al. produced several antisense rice plants with starch having an amylose contents between 6 and 13%. The iodine staining qualities of these starch granules were not reported. Further, any cooking properties of the starches, the elastic properties and gelling abilities of pastes and the gel properties of gels produced from these low amylose rice starches produced by transgenic rice plants are unknown.

High Amylose Starch

Commercialized high amylose maize starch is a result of down regulation of the maize BEIIb enzyme, utilizing the amylose extender mutation. High amylose maize starches bring important differentiated properties for food applications, having amylose contents between 30 and 90% of the weight of the starch. The starch imparts gelling ability to the food system, improving adhesion to water impermeable surfaces and altering product texture. These starches also have improved film forming ability and improved fat impermeability compared to the normal starch counterparts. High amylose starches provide firmness, extend cooking times and increase the crispiness of coatings.

Because of their high resistance to processing and subsequent digestion, in addition to their rapid retrogradation if gelatinization occurs, high amylose maize starches are also being utilized as a source of resistant starch in a number of food products. The most widely available high amylose starches originate from maize using the amylose extender mutation that results in a starch having 40–50% amylose. As a result of extensive breeding and selection work higher amylose contents (up to 90%) have been achieved. High amylose starches are also available in barley and rice.

Amylopectin Chain Length

As already stated, care must be taken when using the iodine binding assay for quantifying amylose content. In a more extreme example of this problem, the combination of the absent GBSSI activity and the ae mutation produces an amylose free maize starch that stains blue or purple and appears to have an amylose content of 15–26%. This is because the ae mutation causes a decrease in starch branching enzyme activity, which results in the formation of long

chain amylopectin, which will itself stain blue with iodine. Other examples of this come from genetic modifications that affect amylopectin structure.

Loss of SSIIa activity in maize results in a starch with an amylose content near 40% of the total starch weight. This starch develops viscosity very slowly at temperatures above 90°C and forms stable gels that strengthen only very slowly compared to normal starch gels. The loss of SSIIa activity resulting in elevated amylose contents highlights the often observed disconnect between the viscosity development of the starch and the thermally detected gelatinization of the starch. Despite the resistance of the starch to develop viscosity, likely a consequence of the elevated amylose content, loss of SSIIa activity results in a decrease in the gelatinization temperature range of the starch from about 70°C to 80°C for normal starch to approximately 55°C to 65°C. Examination of the chain length distribution of the maize starch in combination with the absence of amylose, as a result of the inactivity of GBSSI, indicates that the starch has an elevated proportion of short component chains below a DP of 30 compared to normal starch and additionally an elevated proportion of very short component chains below a DP of 10 compared to normal starch. This high proportion of very short chains imparts a decreased tendency to retrograde compared to normal starch.

Recently, potatoes have been engineered to eliminate both SSIIa and GBSSI activity, resulting in an increase in short chains below a DP of 14 compared with normal starch (108). This had the benefit of a decrease in the tendency for the starch to retrograde after cooking which may have implications for improved freeze and thaw tolerance. Potatoes engineered to have reduced SSIII activity had decreased amounts of chains longer than DP 17.

The SSIIa enzyme of rice has been implicated as one of the major enzymes that affects whether the grain is of the indica-type vs. the japonica-type. Indica-type rices have been long known to have higher kernel integrity and higher granule stability than japonica-type rices. The properties of indica rice starches have been attributed to their higher proportion of longer chains than japonica-type rice starches.

It has been known for some time that the inactivation of SSIII in the dull mutant of maize results in an elevation in the apparent amylose content to 30 to 40%. Beyond this, a number of chromatographic studies on dull starches, including those additionally lacking GBSSI enzyme activity, indicate that the chain distribution of maize starch is only slightly different from normal starch, with some elevation in the chains with a DP less than 30 to 50. The beta-limit dextrins, starches with the exterior chains digested to stubs of maltose to maltotriose,

provide a more complete picture of the changes observed with elimination of SSIII in maize. In this case the lengths of the residual chains containing all of the branching are considerably shorter when SSIII is absent during synthesis. It has been suggested that this change has implications for the retrogradation of starch produced in the absence of SSIII. Thus, during gelatinization and then continued heating after gelatinization, the starch chains are less able to randomly orient themselves compared with starches having longer spacing between branch points (i.e., normal or waxy starch). Thus, the retrogradation rate of du wx starch is less concentration dependent than wx starch despite the similar chain length distributions of the two starches after debranching.

Module 165: novel Starches

Novel Starches

Recent developments in biotechnology are opening ways of making novel starches in plants. Thus starches obtained from different crops having varied functionalities due to differences in composition may now theoretically be readily transferred into crops from one or another different plant species. In recent years a number of patents have been filed covering novel genetic modifications of starch in plants. In general we can consider making changes in any aspect of amylose/amylopectin, phosphate, protein, phospholipids, crystallinity, gelatinization, and pasting characteristics, flavor and starch granule morphology. One merely has to understand the genetic basis of the differences.

One interesting example of a cross species difference that may be exploited is phosphate content in potato. Recent work has shown that starch phosphate content may be attributed to a novel glucan water dikinase enzyme. This discovery opens up the possibility of creating novel genetically modified starches having varied phosphate contents. Although it is too early to conclude whether there would be useful applications in food, possibilities include changing starch digestibility or starch viscosity after cooking. Another interesting character in one species that is not available in others is associated with the genes responsible for endosperm texture in some cereal crops. In this case the puroindoline genes, described as PinA and PinB are interesting candidates. Transforming the wheat genes into rice successfully created the softness trait in the seed. At this time it is still too early to say how useful this character will be in food applications.

Although starch granule morphology varies extensively across species and starch from different species is highly valued for certain applications, there are few specific reports that granule morphology is the attribute desired for valuable food applications. Furthermore, although there have been some reports of progress in understanding the mechanisms that control and influence starch granule size, for granule morphology there is little that can be defined as clear enough to arouse interest in a genetic approach. Similarly, flavor can be considered to vary extensively across species and a bland flavor of (e.g., tapioca starch) is valued over certain cereal starches that impart a mealy character. However, as with granule morphology, our understanding of the genetic components determining this character is rather poor, making flavor a difficult target for genetic modification at the present time. Thus in these cases it is difficult to see a way of using such differences in granule morphology and flavor to exploit that character in another species.

Another novel concept has come from bifunctional domains involving cellulose and starch, which may enable developments with novel biomaterials. Another possibility is to explore incorporation of inulins with starch. Even more novel possibilities to consider include adding new functionality to starch using gene constructs containing fusion proteins. The fusion proteins can be proteins, which might affect starch properties or enzymes, or other bioactive proteins

Lecture 18

PRODUCTION OF FOOD FLAVORS

Module 166: biotechnology of food flavors production

Microorganisms play an important role in the generation of natural compounds, particularly in the field of food flavors. For a long time, plants were the sole sources of flavor compounds and most of them were isolated from essential oils. However, active compounds are present in low concentrations, which makes their isolation difficult. Another disadvantage of plants as a source of flavors is the dependence on factors that are difficult to control such as the weather and the risk of plant diseases. The production of flavor compounds by biotechnological methods has been an interesting alternative due to consumers' preference for natural ingredients. Microbial processes seem to be the most promising methods for the production of natural flavors.

Many microorganisms are capable of synthesizing flavor compounds when growing on a culture media. They have the ability to perform conversions that would require multiple chemicals steps. Microorganisms are used to catalyze specific steps. They are also an economical source of enzymes, which can be utilized to enhance or alter the flavors of many food products. In this way, biotechnological processes involved in the production of flavor compounds can be divided into two groups: microbiological and enzymatic. Microbiological methods are subdivided into biosynthesis and biotransformation. The first is the production of chemical compounds by cells (fermentation or secondary metabolism). The second refers to the use of microbial cells in the specific modification of chemical structures.

In fermentation, the production of flavors starts from cheap and simple sources such as sugars and amino acids. The product is generated by the complex metabolism of the microorganism. When microorganisms are used in order to catalyze specific conversions of precursors and intermediates, the process is called biotransformation. Although fermentation requires C and N sources, a specific substrate is necessary for microbial transformation. The enzymatic catalysis precedes a simple and specific transformation of the substrate molecule. The substrate does not have to be “natural”; according to Schreier (1989) “non-natural” substrates can also be biotransformed.

How to obtain flavors

Flavor from fermented foods

The sensory properties of fermented foods are one of the key parameters in distinguishing these products from foods that have undergone undesirable spoilage. The organoleptic properties of fermented foods usually differ from those of the unfermented substrate and are dependant upon the biochemical activities of the associated microorganisms.

Fermentation has been practiced for the production of food since ancient times. It has become an effective technology for the production of organic acids, flavor compounds, and other biologically important chemicals. New aroma and flavors includes acids, alcohols, carbonyl compounds, esters, and pyrazines.

The use of microorganisms in the production of food has been practiced for a long time to improve the sensory quality of the food. Products such as beer, wine, distilled beverages, bakery, vinegar, fermented vegetables, milk, soybean, and meat are preserved, modified, and flavored using microorganisms. The flavor compounds of traditionally fermented foods originate from a complex microflora that acts in the chemical precursors of a food matrix.

Lactic acid and alcoholic fermentations are the two important processes responsible for fermented food flavors. However, in some cases, the flavor is formed by specific fermentations. The creation of new fermented products can result in the development of novel flavors and textures.

Dairy Products. Cheese flavors find application in snacks, sauces, baked goods, and several other products. Yogurt and buttermilk flavors are also useful. The cheese flavor results from the action of microorganisms and enzymes on milk's pro-teins, fats, and carbohydrates. Numerous breakdown products are formed, among them, short-chain fatty acids, acetic and lactic acids, alcohols, aldehydes, ketones, esters, sulfur and nitrogen compounds. It has been reported that the use of bacteria strains for cheese ripening with enhanced flavor production is promising. They also mentioned that the catabolism of amino acids is presumably the origin of some major flavor compounds.

The starter cultures used in dairy technology are mainly prokaryotes like *Lactococci*, *Lactobacilli*, *Leuconostocs*, *Bifidobacteria*, *Propionibacteria*, *Streptococci*, and *Brevibacterium linens*.

Yeasts, such as *Kluyveromyces*, *Debaromyces*, *Candida* or *Trichosporon* are present in many manufactured milk products. These microorganisms modify the sensory characteristics of the products by synthesizing or assimilating volatile nitrogen and sulfur compounds.

Several chemical reactions take place in the surface of ripened cheeses such as Camembert and Brie during maturation due to fungi growth. The Fungal mycelium of *Penicillium roqueforti* grows rapidly and the resulting products are used directly for flavoring foods with a blue cheese-type flavor.

Alcoholic Beverages. Flavor compounds are produced as byproducts of yeast metabolism during alcoholic fermentation. Many flavor compounds have been identified in alcoholic beverages.

During alcoholic fermentation, yeasts transform sugars (glucose, fructose, and sucrose) into ethanol and carbon dioxide by the Embden – Meyrhopf – Parnas pathway. This is the main bioreaction, but not the only one and, at the same time, several secondary byproducts are formed. Higher alcohols, organic acids, and esters are the main flavor compounds.

Higher alcohols, which contain more than two carbons, are also called fusel alcohols. They constitute the major portion of the secondary products of yeast metabolism. They include n-propanol, isobutyl alcohol, 2-methyl butanol, amyl alcohol, isoamyl alcohol, and 2-phenyl ethanol. Isoamyl alcohol accounts for more than 50% of the total concentration of higher alcohols.

Esters at appropriate concentrations impart flowery and fruity flavors. They are formed by esterification of fatty acyl-CoA or of organic acid by alcohols. Esters are present in very low amounts, near their threshold level. However, ethyl acetate has been found in wine in high concentrations.

Beer. Acetaldehyde, the most important aldehyde in beer, is formed as a metabolic branch point in the pathway from carbohydrate to ethanol. Its level varies during fermentation and ageing of beers, reaching 2 – 20 mg/L. At concentrations of 20 – 25 mg/L, acetaldehyde causes “green” or “vegetable” flavor.

Diacetyl and pentane-2,3-dione (vicinal diketones) have a characteristic flavor described as “buttery”, “honey” or “toffee-like”. They have a very high off-flavor potential, dependent on

the fermentation temperature. The threshold for diacetyl in lager-type beers is 0.10 – 0.14 mg/L. At levels above 1 mg/L, it becomes increasingly “cheese-like” and sharp.

Volatile acids are usually present in beer at concentrations of 20 – 150 mg/L. Butyric and iso-butyric acids in concentrations of 6 mg/L cause a “butyric” or “rancid” flavor. Valeric and iso-valeric acids cause an “old-hop” and “cheesy” flavor. Fatty acids with 6 to 12 carbon fatty acids give the characteristic flavor of “cheesy”, “goaty”, or “sweaty”.

Wine. The chemical composition of wine is determined by many factors, among them grape variety, geographical and viticultural conditions, microbial ecology of the grape, fermentation processes, and winemaking practices. Microorganisms affect the quality of the grape before harvest and during fermentation. They metabolize sugars and other components into ethanol, carbon dioxide, and hundreds of secondary products that contribute to the characteristic flavor of wine

Bakery Products: Although *Candida* yeast has occasionally been used for baking and some *Saccharomyces carlsbergensis* strains have been patented for use as baker’s yeast, pure strains of *Saccharomyces cerevisiae* are almost universally employed.

Mushroom Flavors. The commercially important mushrooms belong to the orders Ascomycetes and Basidiomycetes. Truffles (*Tuber* sp.) and morels (*Morchella* sp.) represent the Ascomycetes. Basidiomycetes are represented by *Agaricus bisporus*, *bitorquis*, *Lentinus edodes* (Shiitake), *Volvariella volvacea*, *Pleurotus* sp., and *Flammulina velutipes*. The main chemical compound responsible for the mushroom flavor is 1-octen-3-ol, although several others, including glutamic acid and 50-guanylic acid, can modify the flavor, giving each mushroom species its distinctive characteristic. There is interest in growing mushroom mycelium in submerged culture and then utilizing the dried mycelium as a flavor compound.

Module 167: Production of Esters and Aldehydes

Biochemical reactions as well as several nonenzymatic reactions involving sugars, fatty acids, and amino acids give rise to flavor during fermentation. Several reports and reviews have been published on the production of volatile compounds by microorganisms. Although several bacteria, yeasts, and fungi have been reported to produce flavor compounds, a few species of yeasts and fungi are often preferred. However, only a few of them find application in the food industries due to their GRAS (Generally Recognized As Safe) status.

Flavor compounds derived from microorganisms are often produced in low concentrations. These compounds have low thresholds and can be detected by chromatographic methods in parts per million (ppm, mL/L). The amount and type of compounds secreted by microorganisms depend on the strain, with its enzyme-specific action, chemical composition of the culture medium, pH and temperature control, age of inoculum, and water activity of the substrate.

Flavor compounds produced by *Trichoderma viride*, *Penicillium roqueforti*, and *Penicillium decumbens* have been detected during the phases of growth or sporulation, depending on the culture medium.

Esters. Esters are a very important class of flavor compounds of fresh fruits and fermented foods, which are found in concentrations between 1 and 100 ppm. The production of the ester ethyl acetate by the yeast *Candida utilis* from glucose is observed when the yeast grows on a medium containing a specific initial ethanol concentration.

Esters of low molecular weight are responsible for fruity odors and consist of acids and their derived compounds such as acetates, propionates, and butyrates. Some examples are ethyl butyrate and isoamyl acetate, which are found in strawberry and banana.

The presence of esters such as ethyl acetate and butyric acetate in the culture medium can eventually describe a detoxification mechanism by which the microorganism avoids the accumulation of toxic compounds. The production of acetates occurs in order to detoxify the medium by converting acetic acid and high alcohols.

Module 168: Production of Alcohol and Terpens

Two metabolic pathways can be followed in the formation of esters: alcoholysis of acyl-CoA compounds and the direct esterification of an organic acid. Yeasts follow predominantly the first pathway, and filamentous fungi and bacteria prefer the second.

Some ramified amino acids are important precursors of flavor compounds and are related to fruit maturation. The initial reaction is called the enzymatic Strecker degradation. Several microorganisms including yeasts and bacteria such as *Streptococcus lactis* can modify the majority of the amino acid structures. Even if alcohols are related to fruit maturation, esters have a dominant role. Ethyl acetate comprises, with other compounds, banana flavor. 2-Methyl-ethyl-butyrate has a great impact on characteristic apple flavor.

Janssens and others (1987) found and quantified the fruity banana flavor produced by the yeast *Hansenula mrakii* and by the fungus *Geotrichum penicilliatum* in submerged fermentation using a synthetic medium. In the study using the yeast it was concluded that the fruity aroma occurred due to the biosynthesis of esters and alcohols. Seventeen compounds were identified in concentrations greater than 50 mL/L, including ethanol, ethyl acetate, isobutanol, ethyl propionate, isobutyl acetate, and isopentyl acetate. Alcohols were formed in the exponential growth phase, but the esters were formed in the stationary phase. Ethyl acetate was the main product. In both studies, some precursors of fruity esters were added, such as vanillin, leucine, isoleucine, and phenylalanine. Thirty-three compounds were identified in concentrations greater than 50 mL/L: ethanol, ethyl acetate, ethyl propionate, and others. Ethyl acetate was produced in the highest concentrations (9924.1 mL/L).

Inoue and others (1994) reported the tolerance of *Hansenula mrakii* to ethyl acetate, which can be used as a sole carbon source. In this study, the esters formed during the production of sake by *Hansenula mrakii* and *Saccharomyces cerevisiae*, were compared. Ethyl acetate, isobutyl acetate, and isoamyl acetate were preferably formed, determining the beverage quality. The formation of these compounds was catalyzed by the enzyme acetyl transferase from isoamyllic acid and acetyl CoA.

Ethyl acetate, ethanol, acetic acid, and acetaldehyde were also produced by submerged fermentation and identified in the glucose metabolism of *Hansenula anomala*. In this case, the production of ethyl acetate was recognized as an aerobic process (Davies and others 1951).

Strains of *Ceratocystis* were also identified as ester producers. Lanza and others (1976) studied the production of acetates with different carbon (glucose, galactose, and glycerol) and nitrogen (urea and leucine) sources. They concluded that the type of flavor compounds produced depended on both sources (carbon and nitrogen), which is different for other microorganisms such as *Trichoderma viride*.

Collins and Morgan (1961) identified esters synthesized by different species of *Ceratocystis* (*C. moliniformis*, *C. major*, *C. coerulescens*, and *C. fimbriata*) during submerged fermentation in a dextrose potato medium. Ethyl acetate and ethanol were found in higher concentrations, except for *C. fimbriata*, which had isobutanol as the main compound. A strong banana flavor was detected when using dextrose and urea due to the presence of isoamyl acetate, which was also identified in leucine- or isoleucine-based media. For the combination galactose – urea, the main flavor was citric due to the formation of terpenes.

Aldehydes. Aliphatic, aromatic, and terpenoid aldehydes are important contributors to the flavor of fermented dairy products. They are synthesized by micro-organisms as intermediates in the formation of alcohols from keto acids. An example is the bioconversion of ethanol to acetaldehyde by *Candida utilis*.

Flavor production using immobilized lipase from the yeast *Candida cylindracea* in a nonaqueous system has been studied for producing a broad range of esters including ethyl butyrate, isoamyl acetate, and isobutyl acetate. Ethyl butyrate has a pineapple – banana flavor, which has a large market demand, and sells at a price of US\$150/kg upwards. This process has shown a great stability of the enzyme (more than a month) if kept hydrated intermittently.

Alcohols: Alcohols do not contribute as a flavor component unless present in high concentration. They are formed as a primary metabolite from microorganisms' activity or due to the reduction of a carbonyl. Fusel alcohols can be formed from either carbohydrate or amino-acid metabolism and are the predominant volatiles of all fermented beverages, in addition to ethanol.

Different alcohols can be found in the culture of yeasts such as ethanol, propanol, isobutanol, and phenyl ethyl alcohol. In filamentous fungi it is possible to find methyl-3-buthanol, butanol, isobutanol, pentanol, hexanol, octanol-3, and phenyl ethanol from the metabolism of amino acids such as leucine, valine, isoleucine, and phenylalanine.

Carbonyls: Among the ketones, odd-numbered 2-alkanones from five to eleven carbons, along with free fatty acids and 2-alkanols, determine the flavor of *Penicillium*-ripened cheese and have received much attention. Bacteria such as *Aureobasidium*, yeasts, and higher fungi produce 2-alkanones, but only *Penicillium* has been used industrially.

Terpenes. Terpenes are the most important natural components of essential oils to be used as flavors. Microorganisms are able not only to synthesize but also to degrade or transform terpenes. Fungi are the main microorganisms responsible for terpene production, but bacteria are capable of synthesizing a few volatile terpenoids, such as geosmin and cadin-4-ene-1-ol (Berger 1995).

The synthesis of monoterpenes by *Ceratocystis variispora* has been studied by Collins and Halim (1972). Numerous other microorganisms are able to synthesize monoterpenes, among

them *Ceratocystis moniliformis*, *Kluyveromyces lactis*, *Sporobolomyces odorus*, *Trametes odorata* and *Trichoderma viridae*.

Microbial bioconversion of terpenes has been studied by several authors. Monoterpenoid compounds like citronellal, citral, limonene, and menthol (acetates) can be biotransformed in citronellol, geranic acid, carveol and 1-menthol, respectively.

Valencene is a sesquiterpenoid available from orange oil and has little commercial use. Some bacteria are capable of transforming valencene to nootkatone, a main flavoring component of grapefruit.

From the economic point of view, the development of biotechnological processes for the production of terpenes is not viable due to the low yields obtained and the abundance of vegetable sources available. The real meaning of these studies is the understanding of the steps of the catabolism of terpenes.

Lactones. Lactones are associated with fruity, coconut, buttery, sweet, or nutty flavors. *Trichoderma viridae*, a soil fungus, generates a characteristic coconut flavor due to the production of 6-pentyl-2-pyrone. The main component of peach flavor, 4-decalactone, can be synthesized by *Sporobolomyces odorus*. *Aspergillus niger* can transform β -ionone into a complex mixture resembling tobacco flavor. Lactones make a significant contribution to the flavor of several fermented foods like dairy products and alcoholic beverages.

Some microorganisms such as *Ceratocystis moniliformis*, *Trichoderma viride*, *Sporobolomyces odorus*, and some species of *Candida* have been reported as lactone producers. However, the production is not very significant and has low yields (mg/L), except for the in situ production of lactones from dairy products.

Lee and Chou (1994) verified that the addition of 3% castor oil to the culture medium raised the production of lactones by *Sporobolomyces odorus*, with a yield of 8.62 mg/L. Among lactones, 6-pentyl- α -pyrone (6-PP) presents the most interesting flavor properties. It is a molecule with a strong coconut flavor and is also present in the aroma of peaches and nectarines. The production of 6-PP by *Trichoderma harzianum* with sugar cane bagasse by solid-state fermentation was studied by Sarhy-Bagnon (1999) as an alternative for the production by submerged fermentation, giving a six fold raise in concentration.

Pyrazines. Pyrazines are typical flavor components of heated foodstuffs. They give the roasted or nutty flavors characteristic of roasted nuts, coffee, and cocoa beans, and baked and meat products. Microwave foods need the addition of pyrazines because they do not develop a characteristic nonenzymatic browning flavor during cooking. *Bacillus subtilis* was the first organism found to produce pyrazine. Pyrazines were also identified in cultures of *Septoria nodorum* and *Aspergillus parasiticus*.

Module 169: biotechnology of food flavors production

Enzymatic Technology

Enzymatic processes that are used to obtain flavors can be described by the hydrolysis of some compounds without microbial growth. The majority of the enzymes used in food processing are hydrolases, such as amylases, proteases, pectinases, cellulases, pectonases, invertase, and lactase. They are used, for example, in cheesemaking (lipases, proteases), wine and juice production (pectinases), lactose reduction (lactase). Immobilization techniques, such as gel inclusion, microcapsules entrapment, and covalent or adsorptive binding onto solid supports has improved technical aspects such as handling, recycling, and long-term stability. Microbial enzymes have become an integrated part of processes in the food industry, So it is natural to see their use for the generation of flavor compounds.

Filamentous fungi are capable of producing enzymes that are used to hydrolyze plant cell wall and liberate its content. However, the enzymatic extraction needs a thermal treatment, which sometimes can destroy or change flavor compounds.

Pectinase, cellulase, and hemicellulase of *Aspergillus*, *Penicillium*, *Rhizopus*, and *Trichoderma* are enzymes more commonly used to increase extraction efficiency during fruit, vegetable, cereal, or juice processing.

Lipases often show complex patterns after isoelectric focusing, but this heterogeneity is due to the varying degrees and positions of glycosylation of the protein core. Pre-pro-lipase and pro-peptides are now studied in detail by genetic engineering.

Microbial hydrolases have been reported to improve the sensory quality of food by the synergistic action of mono-, oligo-, and poly-glucanases. Various carbohydrases have been purified and characterized, among them, β -glucosidase from *Aspergillus niger* and α -glucosidase, with maltase properties from banana pulp. Carbohydrases have contributed to

the assessment of the identity and origin of plant products, to the understanding of changes during processing and maturation, and to the selection of flavor-rich cultivars. Stability and selectivity data will be decisive for sensory changes in a food and thus for the future application of new enzymes in food processing. Smaller peptides and free amino acids, which are end products of various proteases, contribute to the non-volatile flavor fraction and act as precursors of volatiles.

Cheese treated with enzymes to enhance flavor, or a significant portion of the flavor profile, is considered to be enzyme-modified cheese (EMC). It provides the food manufacturer with a strong cheese note in a cost-effective, nutritious, and natural way (Moskowitz and Noelck 1987). Such EMCs are ideal in frozen cheese, because proteins from natural cheese tend to coagulate and produce a grainy texture, but the proteins in EMCs have been hydrolyzed to more soluble peptides and amino acids, overcoming these problems (Missel 1996).

EMC flavors available commercially include Cheddar, Muzzarela, Romano, Provolone, Feta, Parmesan, Blue, Gouda, Swiss, Emmental, Gruyere, Colby, and Brick. These cheese flavors have a wide range of applications in salad dressings, dips, soups, sauces, snacks, crisps, pasta products, cheese analogs, frozen foods, microwave meals, ready-made meals, canned foods, crackers, cake mixes, biscuits, quiches, gratins, cheese spreads, low-fat and no-fat cheese products, and cheese substitutes.

Microbial fermentation is a promising biotechnological technique for the production of natural flavors. Although many biotechnological processes have been reported, most have not yet been applied in industrial production. The major reason for this is the low yield. Microbial flavors are often present in low concentrations in fermentation broths

Module 169: Determination of specific gravity of sugar

Uses

Measure amount of air incorporated in products such as whipped cream, egg white foams, creamed shortening, and cake batters.

Procedure:

1. Weigh a dry container to the nearest gram.

2. Fill container with cooled, boiled deionized water at room temperature. Complete fill on balance; judge at eye level. Weigh to nearest gram.
3. Fill dry cup with test material. Do not pack. Remove excess with spatula. Wipe outside of container. Weigh to nearest gram.
4. Calculate the specific gravity as follows:

weight filled container ÷ weight container specific gravity = -----

volume container

where volume container = (weight container + water) – weight container. Since specific gravity is the density of a substance relative to water it has no units.

SPECTROPHOTOMETER

Measures the absorption of light at a particular wavelength by the sample.

Uses

Qualitative identification and quantitative determination of colored substances.

Procedure

- (i) Turn on instrument by rotating the left knob clockwise and allow to warm up for 15 to 30 min. Turn wavelength dial to appropriate setting.
- (ii) Zero instrument with cuvette chamber empty and lid closed by adjusting left knob until needle is at 0% transmittance. Insert cuvette containing reagent or tissue blank and adjust right knob until needle reads 100% transmittance. Each time the wavelength is changed, zero the instrument again.

Lecture 19

IMPROVING QUALITY OF FRUITS AND VEGETABLES**Module 171: Biotechnological approaches to improve nutritional quality and shelf life of fruits and vegetables**

Fruits and vegetables, which can be consumed as fresh and as processed products, are important ingredients of a healthy diet. They are valuable sources of vitamins, minerals, antioxidants, and fiber. The important quality factors of fruits and vegetables are their color, flavor, texture, and nutritive value. Consumers always prefer to buy fruits and vegetables of high quality. As used by the industry, quality is a concept involving measurable attributes: degree of purity, firmness, flavor, color, size, maturity, condition, or any other distinctive attributes or characteristics of the product (1). The qualities of the produce bought by the consumer are influenced by many factors, such as the cultivar, the environmental conditions affecting growth, cultural practices, exposure to pests and diseases, time of harvesting, and postharvest and storage conditions used. Today, with the advancement of technology in several areas, the only factor on which the grower has no control of is the environment of the field conditions. Heredity (the identity of the cultivar) plays a major role in determining the quality of fruits and vegetables, as evidenced by the various varietal differences in quality. Even though traditional crop breeding is still being used as one means of crop improvement, continuing advances in knowledge and technology have dramatically expanded the biotechnological tools available for genetic improvement and production of vegetables and fruits. The term biotechnology is broad, encompassing a wide range of disciplines in science.

Module 172: Factors effecting reducing sugars in potatoes**Potatoes**

Potatoes are ranked fourth in production of all agricultural commodities in the world and yield more dry matter and protein per hectare than the major cereal crops (2). They are consumed as fresh and processed products, and used as raw material for many industrial purposes such as starch extraction. Potato chips and french fries are two of the most popular processed potato products. The consumer preference for these products is influenced by the color and crispness of these products. The primary problem associated with potato processing is the nonenzymatic browning of the product that occurs under the high temperature conditions used during frying, when reducing sugar levels are high in the tissue, a phenom-

enon known as Maillard reaction (3). The reducing sugars, glucose and fructose, combine with the -amino groups of amino acids at the high temperatures used in frying operations, resulting in darker and more bitter flavored french fries and chips that are unacceptable to the consumer. The ideal content of reducing sugars is 0.1% of the tuber fresh weight; 0.33% is the upper acceptable limit (4). A four year study was conducted to determine the compositional differences during low temperature storage between low sugar accumulating and high sugar accumulating cultivars in relation to potato chip processing quality (5). Pearson analysis of the above data showed that chip color was most closely correlated with reducing sugar concentration. Multiple regression analysis revealed that the relative contribution of each of the parameters studied, such as sucrose, reducing sugars, nitrogen, protein, ascorbic acid and dry matter content, varied greatly among cultivars and selections evaluated and from season to season (5).

Factors Affecting Accumulation of Reducing Sugars in Potatoes

The factors that affect the sweetening or breakdown of starch into sucrose and its component reducing sugars glucose and fructose are drought, excess nitrogen during growth, high temperature at harvest, handling, aging, identity of the cultivar, anaerobic conditions, and low temperature during post harvest storage.

Low Temperature Sweetening in Potatoes

Low temperature sweetening (LTS) in potato tubers is a phenomenon that occurs when tubers are stored at temperatures below 10°C in order to minimize respiration and sprouting. LTS results in the accumulation of starch breakdown products, primarily sucrose and the reducing sugars glucose and fructose, which cause Maillard browning during potato chip frying operations. Fry color of Russet Burbank and Shepody potatoes has been shown to be more closely associated with glucose concentration than with fructose, total reducing sugars, sucrose, or total sugars. In order to avoid Maillard browning, processing potatoes are generally stored at temperatures around 10°C; but at this storage temperature potato tubers will sprout. To prevent sprouting during storage, the processing tubers are treated with chemical sprout inhibitors. However, due to health and environmental concerns, there is increasing pressure to reduce the use of chemical sprout inhibitors. The only solution to avoid this problem is using cultivars that are resistant to LTS.

Low temperature storage of potato tubers has many advantages, such as control of sprout growth and senescent sweetening, reduction of physiological weight loss due to decreased respiration and losses associated with bacterial and fungal pathogens, and extended marketability. Low temperature storage has several advantages, but the main drawback associated with it is the accumulation of reducing sugars and the resulting browning of processed products such as chips and fries.

The mechanism responsible for the initiation and subsequent regulation of LTS in potato tubers has not been fully elucidated. Many theories have been proposed to explain LTS based on starch metabolism, sucrose metabolism, glycolysis and the oxidative pentose phosphate pathway (PPP), and mitochondrial respiration, as well as membrane instability, lipid peroxidation, and electrolyte leakage. It has been suggested that in mature, cold stored potato tubers, the glycolytic or respiratory capacity plays a key role in the ability of potatoes to regulate their sugar concentration. Although LTS has not been elucidated at the molecular level, many factors may play a role in it. For example, chilling may influence compartmentation and membrane permeability by altering the phase transition of lipids in the bilayer, resulting in the leakage of key ions such as inorganic phosphates. This can alter the activity and synthesis of key enzymes involved in the metabolic pathways, ultimately resulting in LTS.

Many theories have been postulated and documented to explain LTS at the level of carbohydrate metabolism in stored potato tubers. The mechanism is complex and may involve the interaction of several pathways of carbohydrate metabolism and the genes that regulate these pathways. This discussion focuses on a theoretical model of the mechanisms involved in LTS based on information available about the roles of the major tuber carbohydrate metabolic pathways as well as changes in membrane stability.

Metabolism of Starch in Tuber

Starch is the major component in the main crop plants of the world, as well as an important raw material for many industrial processes. Potato tubers contain 60–80% starch, of which sugars represent only a small fraction (up to 3% on a dry weight basis). There is evidence that the principal event in LTS is the cold induced synthesis of sucrose. The carbon needed for the synthesis of sucrose and reducing sugars for LTS is generally, but not always, provided by a net breakdown of starch. An increase in potato tuber sugar content occurs early during cold storage; over two to three months at storage temperatures of 1–3°C, tubers can

convert as much as 30% of their starch content. In mature King Edward tubers stored at 2°C, the sugar content increased from 0.3 to 2.5% in three months, with the initial appearance of sucrose followed by reducing sugars. Coffin found that sucrose content increased within two days of 5°C storage for both mature and immature tubers of four cultivars, while fructose and glucose content increased more slowly. Pollock reported an increase in both sucrose and reducing sugar content within 5 days in tubers stored at 2°C, and after 20 days storage, the sugar content was approximately six times greater than at day 0. The sweetening response of tubers to low temperatures is fairly consistent, but is influenced by cultivar, locality, and conditions prior to cold storage. Isherwood related energy requirements to possible biosynthetic pathways and concluded that sucrose was formed from starch when potato tubers were moved from 10° to 2°C and that starch was reformed when tubers were moved back from 2° to 10°C, although different metabolic pathways were involved. Reconditioning of tubers is sometimes used to improve chipping quality by decreasing the level of reducing sugars. After cold storage, potatoes are reconditioned at 18°C where sugar content decreases and starch content increases as sugars are resynthesized to starch. However, the response to reconditioning is neither consistent nor completely restorative, and tends to be cultivar dependent.

Preconditioning treatment has been used to lessen chilling injury in chilling sensitive plants. Storage at 10°C prior to cold storage can acclimatize tubers and lessen the LTS effect. Katahdin tubers preconditioned at 15.5°C for one to four weeks before 0°C storage did not show a change in sugar accumulation patterns or respiration rates. The use of intermittent warming (15.5°C for one week following 0°C for three weeks) decreased sugar levels and respiration rates to levels lower than those of tubers stored continuously at 0, 1 and 4.5°C, although sugar levels were not low enough for desirable chipping potatoes

Module 173: Starch Synthesis

Starch is synthesized in plastids (amyloplasts) upon tuber initiation, and both the number of starch grains and the grain size increase during tuber growth. Starch consists of two types of glucose polymers, the highly branched amylopectin, and relatively unbranched amylose. Potato starch is comprised of 21–25% amylose and 75–79% amylopectin. Starch is synthesized from ADPglucose by the concerted action of ADPglucose pyrophosphorylase (ADPGPase), starch synthase, and the starch branching enzymes. Following the conversion of sucrose into hexose phosphates in the cytosol, glucose-6-phosphate is transported into the

amyloplast where it is converted into glucose-1-phosphate. A study involving antisense inhibition of plastidial phosphoglucomutase supported the theory that carbon from the cytosol was imported into potato tuber amyloplasts in the form of glucose-6-phosphate. Glucose-1-phosphate is subsequently converted to ADPglucose by ADPGPase. The starch synthases catalyze the polymerization of the glucose monomers into -1,4-glucans using ADPglucose as a substrate, while the starch branching enzyme catalyzes the formation of the -1,6-linkages of amylopectin.

ADPGPase is often referred to as a rate-limiting step in starch synthesis. It is subjected to allosteric activation by 3-P-glycerate and inhibition by inorganic phosphate. Strategies to alter the starch metabolism in tubers by genetic manipulation of ADPGPase may be helpful in reducing the accumulation of reducing sugars during LTS. It has been reported that transgenic tubers with an 80–90% reduction in ADPGPase activity have reduced starch content relative to wild type tubers. The reduction in ADPGPase activity resulted in a major reduction of carbon flux, with increased flux to sucrose and decreased flux to starch. Stark have reported that hexose accumulation was greatly reduced in cold stored tubers with overexpression of the mutated ADPGPase gene, *glgC16*, from *E. coli*. The *glgC16* gene produces a mutant form of ADPGPase that is less responsive to allosteric effectors. It has been suggested that the observed decrease in hexose concentration could be due to the higher starch biosynthetic capacity of the transgenic tubers. Lorberth developed transgenic potatoes with decreased levels of R1 protein, a starch granule bound protein capable of introducing phosphate into starch-like glucans. By reducing the activity of the R1 protein using anti-sense technology, the phosphate content of starch was reduced, resulting in a starch that was less susceptible to degradation at low temperatures relative to the starch of wild type tubers. It has been observed that after two months of storage at 4°C, the transgenic tubers contained up to ninefold lower concentrations of reducing sugars compared to the wild type. However, the commercial value of the modification of starch could not be assessed because the authors did not analyze the processing quality of transgenic tubers.

Module 174: Starch Degradation

The differential response of potato cultivars to LTS may be the result of starch properties that affect the ability of enzymes to degrade it. The various starch properties ascribed are:

Chemical modifications of glucose units by attachment of covalently bound phosphate. Phosphate esters are attached to C3 or C6 glucosyl residues of amylopectin in the larger

interbranch chains and are absent around the branching points. This affects the cleavage sites and degradation product patterns.

Surface property alteration caused by the negative surface charge from surface phosphate, lipid, or protein can affect the properties of enzymes and other soluble compounds.

Association with starch-metabolizing enzymes such as endoamylase activity in cotton leaves and starch synthase in potato tubers.

Physical characteristics of starch. Isolated starch grains from two cultivars differing in their sensitivity to LTS showed an increase in starch grain size over time with the disappearance of smaller starch grains while ND860-2, the resistant cultivar had a consistently smaller mean starch grain size. Higher levels of amylose in ND 860-2 were believed to be responsible for a more ordered crystallinity within the starch granule, decreased thermomechanical analysis swelling, increased resistance to gelatinization and decreased susceptibility to α -amylase hydrolysis.

In addition to the various properties of starch, which affect its degradation, other factors such as enzymes responsible for starch degradation during LTS have been studied. The pathway of starch breakdown during LTS is not well established. The degradation of starch is believed to occur in the amyloplast. The widespread distribution of α -glucan phosphorylase, α -amylase, β -amylase, and maltase suggests that starch breakdown could be phosphoryltic, hydrolytic, or both. However it is assumed that starch breakdown in cold stored potato tubers is mainly conducted by starch phosphorylase, because sucrose is the first sugar to accumulate upon transfer of tubers to chilling temperatures. Amylase activities are too low at such cold temperature to catalyze the required rate of starch degradation, and no increase in either maltose or polymers of glucose larger than maltose, the common products of amylolytic starch degradation, have been observed during LTS.

Two types of potato phosphorylases are recognized based on glucan specificity, monomer size, and intracellular location. They are noninterconvertible proteins with different primary structures and different immunological properties. Type 1 isozyme, also known as type H, is localized in the cytoplasm, has a low affinity for maltodextrins, has a high affinity for branched polyglucans, and cross reacts with type H phosphorylase from potato leaves. Type 2 isozyme, also known as type L, is located in the amyloplast, has a high affinity for maltodextrins, has a low affinity for branched polyglucans, and cross reacts with type L

enzyme from the leaf. Type L and type H isozymes do not cross react immunologically. The function of these isozymes in starch degradation and LTS is unknown. There are reports which suggest that starch breakdown during LTS is phosphorylitic. Kumar et al. have demonstrated that although the activities of cytosolic and plastidic isozymes of starch phosphorylase were reduced by up to 70% in transgenic potatoes expressing antisense cDNA constructs of starch phosphorylase, this did not affect the accumulation of reducing sugars during 4°C storage.

Other studies suggest that starch breakdown in potato tubers during cold storage is not solely due to the activity of starch phosphorylase. Cochrane and coworkers, using a modified amylase assay, found that α - and β -amylases and α -glucosidase activities were much higher in tubers stored at the colder temperature (4°C) than those stored at 10°C, and in cultivars known to be more susceptible to LTS. It was considered inappropriate to correlate reducing sugar content and amylase activity, because the formation of reducing sugars is influenced by many other cold labile processes in the tuber. Reducing sugar content and the activities of α - and β -amylases and debranching enzymes were measured by Cottrell et al. over 139 days in five cultivars of potato tuber stored at 4 and 10°C. The activities of these enzymes were always greater at 4 than at 10°C, but cultivars that accumulated high levels of reducing sugars did not always display the greatest level of hydrolytic enzyme activity. It has been reported that the onset of sugar accumulation in tubers during low temperature storage coincided with an increase in the activity of one specific isoform of amylase, the β -amylase in the cultivar Désirée. Amylase activity was present at low levels in tubers stored at 20°C, and increased from four- to fivefold within 10 days of storage at 3°C. However, no specific role has been established for this cold induced β -amylase in LTS.

Effect of Inorganic Phosphates: The intracellular compartmentalization of Pi has been suggested to influence carbon partitioning in nonphotosynthetic potato tubers in a manner similar to its role in photosynthetic tissues. It has been observed that increased inorganic phosphate in the amyloplast shifted metabolic activities toward starch degradation rather than accumulation. Increased Pi concentration inhibits ADPGPase and enhances starch breakdown by α -glucan phosphorylase. A high concentration of Pi was found in tuber amyloplasts, and Pi in cold stored tubers was later found to be cleaved off from starch. Higher levels of Pi were found in Russet Burbank potatoes stored at 5.5 than at 15.5°C. A highly significant correlation was found between the Pi content and the accumulation of reducing sugars. Amyloplasts were found to have high concentrations of Pi, citrate, Cl⁻, and K⁺. It was

suggested that Pi leaks from the amyloplast to the cytoplasm during cold storage and induces higher sugar concentration levels in tubers during LTS.

Another source of Pi in plant cells is the vacuole. The major portion of Pi is stored in potato tuber vacuoles where it is compartmentalized away from the cytoplasm. Loughman examined the respiratory changes of potato tuber slices and found that the larger part of Pi in the cell was localized in the vacuole, and did not take part in the steady state metabolism of the cell. However, the Pi in the vacuole may become available for metabolism in the cytoplasm during cold storage when ionic pumps that utilize ATP in the tonoplast become unable to maintain ionic gradients. This scenario could happen by passive leakage of Pi into the cytoplasm or when the membrane becomes leaky due to changes in the properties of the lipid bilayer. Increased Pi in the cytoplasm could affect the metabolism by mobilizing carbon from the amyloplast into the cytoplasm in exchange for Pi transported into the amyloplast by the hexose phosphate translocator. Inside the amyloplast stroma, Pi can serve as a substrate for the continued phosphorylation of starch, via α -glucan phosphorylase, forming additional molecules of G-1-P. Elevated cytoplasmic levels of Pi initiated by leaky membranes, coupled with reduced levels of fructose 2,6-bisphosphate during cold stress, would direct carbons away from glycolysis and favor the buildup of hexose phosphates for gluconeogenic reactions.

Module 175: Starch-Sugar Balance

The close relationship between starch and sugar levels when potatoes are cooled from 10 to 2°C and then rewarmed from 2 to 10°C after an interval, has given rise to a misleading concept of a starch to sugar balance in which the overall change between the two compounds is seen as being reversible. All the available evidence suggests that sucrose is formed from starch by an irreversible pathway, and that starch is formed from sucrose by separate, but likewise irreversible, routes. The very close relationship between starch and sucrose in stored potatoes may be due to the fact that starch is the only possible source of carbon for sugar synthesis in the cold. There is strong evidence to indicate that G-6-P is transported into the amyloplast of potato tubers to support starch synthesis. Thus the pathways of starch and sugar biosynthesis compete for the same pool of precursors.

A net flux of carbon from starch synthesis into sucrose occurs in cold stored tubers as evidenced from the use of radiolabels in experiments. The coexistence of the pathways of sucrose synthesis and starch breakdown in stored tubers may be regulated by fine control mechanisms. In potato discs incubated with ^{14}C glucose at 3°C and 15°C, a large proportion

of label is recovered in starch. At low temperature, in a cold sensitive, high sugar-accumulating cultivar, the ratio of ¹⁴C recovered in sucrose to that recovered in starch increased, but was unaffected in a cold tolerant, low sugar-accumulating cultivar. This suggests that genotypic variation in the capacity to maintain an active starch synthesizing system may help in alleviating the rate of sucrose accumulation.

Sucrose Metabolism: Sucrose is the first sugar to accumulate during LTS. Its accumulation in potato tubers has been recorded within hours of their placement at LTS inducing temperatures, with the accumulation of reducing sugars occurring a few days later. Sucrose synthesis occurs in the cytoplasm of the tuber either by sucrose 6-P synthase (SPS), or by sucrose synthase and the hexose phosphates required for this are transported from the amyloplast via a phosphate translocator.

Pressey reported that SS activities decreased after harvest, and continued to do so under low temperature storage conditions. SPS activity also decreased if tubers were held at warm temperatures but rapidly increased when tubers were held at low temperatures. This observation indicates that SPS is the enzyme responsible for sucrose synthesis at low temperatures. Pollock and ap Rees reported that sucrose synthesis during LTS is catalyzed by SPS and not by SS. This was also confirmed by ¹³C NMR studies.

The increase in sucrose synthesis upon transferring the tubers to low temperature has been associated with the increased expression of an isoform of SPS (SPS-1b, 127 kDa). The cold induced increase in the SPS-1b isoform was found to correlate well with the change in the kinetic properties of the enzyme. The major isoform found in tubers stored at room temperature is a 125-kDa protein (SPS-1a). Reconditioning of the tubers at 20°C resulted in the disappearance of the cold induced SPS isoform after 2–4 days. An increase in the total amount of SPS transcript was observed at low temperature in each of these studies. SPS from potato tubers has been shown to be subject to fine regulation by allosteric effectors and protein phosphorylation. Potato tuber SPS is allosterically activated by G-6-P and inhibited by protein phosphorylation.

Antisense technology has been used as an effective tool to investigate the roles of enzymes that lead to the production of sucrose, as well as reducing hexoses such as glucose and fructose in LTS. Many researchers investigating LTS mechanisms have used this technology to substantiate the role of enzymes in the carbohydrate metabolic pathway. For example, in experiments involving transgenic tubers where the SPS activity was reduced by 70–80%

either by antisense or cosuppression, cold sweetening was reduced by inhibiting the increase of the cold induced isoform of SPS. The authors also observed that the V_{max} of SPS was 50 times higher than the net rate of sugar accumulation in wild type tubers, and found that SPS is strongly substrate limited, particularly for UDP-G. These results indicate that the rate of cold sweetening in wild type tubers is not strongly controlled by the overall SPS activity or the overall amount of SPS protein. Alterations in the kinetic properties of SPS during cold temperature storage were more effective in stimulating sucrose synthesis than changes in SPS expression. The observation that changes in the kinetic properties of potato tuber SPS coincide with the onset of sugar accumulation points to the fact that the fine regulation of SPS may be more important than coarse regulation in controlling the ability of a cultivar to sweeten during cold storage.

However, it should be noted that SPS may not be the only candidate that regulates sugar accumulation during LTS, because other factors that affect the availability of hexose phosphates, such as glycolysis and the pentose phosphate pathway, may have key roles to play.

UDP-glucose pyrophosphorylase (UGPase) is a cytosolic enzyme that catalyzes the formation of UDP-G, one of the substrates required for the synthesis of sucrose. Depending on the physiological state of the tubers (i.e., growth or post harvest storage), the UGPase reaction may be directed toward the synthesis or degradation of starch. During the process of cold sweetening, it has been suggested that UDP-G and PP_i have regulatory roles in directing carbon flux into glycolysis, starch synthesis, hexose formation, or a combination of the three. The activity of UGPase has been correlated with the amount of glucose that tubers of different cultivars accumulate in cold storage, leading to the assumption that this enzyme might be a control point for low temperature sweetening, as it regulates the rate of SPS and sucrose synthesis by controlling the levels of UDP-G.

Genetic manipulation to down regulate the expression of UGPase in potato tubers has resulted in contrasting results based on the physiological stage of the tubers. In two separate experiments in which the UGPase activity was reduced by 30–50% compared to their wild types, the transgenic tubers accumulated lower levels of sucrose during storage relative to wild type tubers at 4°C and 12°C (67) and at 6°C and 10°C. It has been suggested that by limiting the rate of UDP-G synthesis, UGPase may exert control over the flux of carbon toward sucrose during the cold storage of tubers. These observations are supported by the

results of Hill et al. who observed that following the initiation of cold sweetening, the concentration of UDP-G changed in parallel with the concentration of sucrose.

In contrast to the above results, Zrenner observed that carbohydrate metabolism of growing tubers was not affected when the transgenic plants had a 96% reduction in UGPase activity as compared with the wild type plants. No significant changes were observed in the levels of fresh mass, dry mass, starch, hexose phosphates, or UDP-G at harvest relative to the wild type tubers. It was reported that 4–5% of UGPase activity was still in considerable excess compared to the activity of other glycolytic enzymes in the tuber, and the antisense construct may have to reduce UGPase to negligible levels in transgenic potatoes before any phenotypic differences are noticeable.

It should be noted that the flow of carbon is different based on the physiological state of the tuber. In the growing tuber, most of the incoming sucrose is used for the synthesis of starch, while in the stored tuber the hexose-phosphate produced from starch degradation is converted into sucrose. In cold stored tubers, when the rate of starch breakdown exceeds the rates of glycolysis and respiration, the conversion of G-1-P to UDP-G is the only means of controlling the level of hexose phosphates. Hence it is possible that a significant effect of reduced UGPase activity may be observed only in tubers acting in the direction of sucrose synthesis, such as during post harvest storage.

Two UGPase alleles have been identified in potato tubers: UgpA and UgpB. In a survey conducted on a number of American and European cultivars and selections stored at 4°C, it was observed that a relationship existed between the allelic polymorphism of UGPase and the degree of sweetening. The genotypes that resist sweetening during cold storage have demonstrated a predominance of the allele UgpA; the genotypes susceptible to sweetening have a predominance of the allele UgpB.

Sucrose Degradation

Sucrose plays a pivotal role in plant growth and development because of its function in translocation and storage, and the increasing evidence that sucrose (or some metabolite derived from it) may play a nonnutritive role as a regulator of cellular metabolism, possibly by acting at the level of gene expression. As mentioned earlier, sucrose is the first sugar to form during LTS, and the source of glucose and fructose accumulation appears to be the degradation of sucrose. Sucrose is broken down by two types of enzymes in plants. By

invertase action, it is hydrolyzed into glucose and fructose; whereas by the action of SS, it is converted into UDP-G and fructose in the presence of UDP.

Potato tubers are known to possess both alkaline and acid invertases. Acid invertase is localized in vacuoles, whereas alkaline (neutral) invertase is localized in the cytoplasm. Acid invertase isoforms that are ionically bound to the cell wall have also been identified. Alkaline invertases are sucrose specific, while acid invertases cleave sucrose at the fructose residue but can also hydrolyze other -fructose containing oligosaccharides such as raffinose and stachyose.

Based on several observations of sucrose synthase and acid invertase activities in developing, mature, and cold stored tubers, and given the fact that sucrose is stored mainly in the vacuole, it is believed that sucrose synthase is responsible for sucrose degradation in developing tubers, whereas acid invertase is the principal enzyme responsible for the breakdown of sucrose into hexoses during LTS. Based on the widely established inverse correlation between sucrose content and vacuolar acid invertase activity, it is strongly believed that sucrose is broken down by acid invertase in the vacuole and the resulting glucose and fructose are transported into the cytosol for the formation of hexose phosphate by hexokinase. It has been reported that glucose concentrations are frequently higher than fructose concentrations in stored potato tubers. Zrenner evaluated the glucose to fructose ratio of 24 different cultivars and found that the ratios were between 1.1 and 1.6, which is a strong indicator that invertase is the key enzyme responsible for the conversion of sucrose to hexose.

Module 176: Concept of Edible vaccines

Development of edible vaccines involves the process of incorporating the selected desired genes into plants and then enabling these altered plants to produce the encoded proteins. This process is known as transformation, and the altered plants are known as transgenic plants. Edible vaccines like traditional subunit vaccines consist of antigenic proteins and are devoid of pathogenic genes. Despite this advantage, traditional subunit vaccines are unaffordable and technology-intensive, require purification, refrigeration and produce poor mucosal response. Unlikely, edible vaccines would eliminate the need for trained medical personnel required for oral administration particularly in children. Production of edible vaccines is effective process and can be easily scaled up. Edible vaccines offer numerous advantages like they possess good genetic and heat stability and do not need cold-chain maintenance. Edible vaccines can be stored at the site of use thus avoiding long-distance transportation. Syringes and needles are

also not required, thus reduces the incidence of various infections. Important advantage of edible vaccines is elimination of contamination with animal viruses-like the mad cow disease, which is a hazard in vaccines developed from cultured mammalian cells, as plant viruses cannot infect humans. Edible vaccines act by stimulating the mucosal as well as systemic immunity, as soon they meet the digestive tract lining. This dual mechanism of action of edible vaccines provide first-line defense against pathogens attacking via mucosa, like *Mycobacterium tuberculosis* and carriers causing diarrhea, pneumonia, STDs, HIV etc. Oral administration of edible vaccines to mothers might prove to be useful in immunizing the fetus-in-utero by transplacental movement of maternal antibodies or the infant through breast-feeding. Edible vaccines enable the process of seroconversion in the presence of maternal antibodies, thus playing a possible role in protecting children against diseases like group-B *Streptococcus*, respiratory syncytial virus (RSV), etc. At present edible vaccines are produced for various human and animal diseases (measles, cholera, foot and mouth disease and hepatitis B, C and E). They can also be used to prevent exceptional diseases like dengue, hookworm, rabies, etc. by combining with other vaccination programmes enabling multiple antigen delivery. Various foods under investigation for use in edible vaccines include banana, potato, tomato, lettuce, rice, etc

Developing an Edible Vaccine

The selected gene obtained from the microbes encoding specific antigen can be handled in two different ways:

Suitable plant virus is genetically engineered to produce the desired peptides/proteins. The recombinant virus is then incorporated into the plant, which enables it to produce a huge number of new plants from which chimeric virions are isolated and purified. The consequential edible plant vaccine can then be used for immunological applications.

In another method, the desirable gene is incorporated with plant vector by transformation. Many other approaches have been utilized which can be categorized into following groups:

Agrobacterium mediated gene transfer

In this method, the suitable gene (recombinant DNA) is incorporated into the T-region of a disarmed Ti plasmid of *Agrobacterium*; a plant pathogen, which is co-cultured with the plant cells, or tissues that needs to be transformed. This approach is slow with lower yield however; it showed satisfactory results in dicotyledonous plants like potato, tomato and

tobacco. Researches in some fields have proven this approach good in expressing the desirable traits by selected genes in several experimental animals and plants.

Biolistic method

This sophisticated method involves the use of gene gun that fires the gene containing DNA coated metal (e.g. gold, tungsten) particles at the plant cells. Plant cells are then permitted to grow in new plants, which are later on cloned to produce ample number of crop with similar genetic composition. This approach is highly attractive due to its undependability on regeneration ability of the species as DNA is directly incorporated into cells of plant. However, requirement of expensive device particle gun adds to the major drawback to this method.

Electroporation

In this method DNA is inserted into the cells after which they are exposed to high voltage electrical pulse which is believed to produce transient pores within the plasma lemma. This approach requires the additional effort of weakening the cell wall as it acts as an effective barrier against entry of DNA into cell cytoplasm hence, it requires mild enzymatic treatment.

Module 177: Examples of Edible Vaccines

1. Transgenic potatoes for diarrhea

The first successful human trial for an edible vaccine was conducted in year 1997 in which volunteers were fed transgenic potatoes, which possessed the b-subunit of the E. coli heat-labile toxin, responsible for diarrhea. A 4-fold increase in serum antibodies 1999 was manifested in ten out of the 11 volunteers. Next clinical trial took place at the Boyce Thompson Institute at Cornell University, USA, in which 20 volunteers ate the potatoes containing the Norwalk virus (responsible for vomiting and diarrhea), out of which 19 showed an immune response. Potato-based edible vaccine has a major drawback that it needs to be eaten as raw because cooking causes denaturation of protein and makes it ineffective.

2. Transgenic tomatoes against diarrhea

Transgenic tomatoes were produced at the Cornell University, in the US, against the Norwalk virus, responsible agent for severe diarrhea. The transgenic tomatoes are capable to produce

surface protein specific to the virus and it has been shown that mice fed with transgenic tomatoes showed an immune response towards the virus.

3. Other transgenic plants

Presently, banana is being exploited as a good source for edible vaccine production because of its two major advantages it does not require cooking and is locally grown plant. However, the protein expression in transgenic banana is tissue specific promoter dependent. Several other examples involve rabies glycoprotein expressed by viral vectors in spinach and hepatitis B surface antigen in case of lettuce and potato.

Module 178: Merits and Demerits of Edible Vaccines

Merits of Edible Vaccines:

- 1 Edible vaccines have efficient mode of action for immunization, as they do not require subsidiary elements to stimulate immune response.
- 2 Edible vaccine unlike traditional vaccines brings forth mucosal immunity.
- 3 Edible vaccines are comparatively cost effective, as they do not require cold chain storage like traditional vaccines.
- 4 Edible vaccines offer greater storage opportunities as they seeds of transgenic plants contain lesser moisture content and can be easily dried. In addition, plants with oil or their aqueous extracts possess more storage opportunities.
- 5 Edible vaccines do not need sophisticated equipments and machines as they could be easily grown on rich soils and the method is economical compared to cell culture grown in fermenters.
- 6 Edible vaccines are widely accepted as they are orally administered unlike traditional vaccines that are injectable. Thus, they eliminate the requirement of trained medical personnel and the risk of contamination is reduced as they do not need premises and manufacturing area to be sterilized.
- 7 Edible vaccines offer greater opportunity for second-generation vaccines by integrating numerous antigens, which approach M cells simultaneously

8. Edible vaccines are safe as they do not contain heat-killed pathogens and hence do not present any risk of proteins to reform into infectious organism.

9. Edible vaccine production process can be scaled up rapidly by breeding.

Demerits of Edible Vaccines:

3. Following are some major drawbacks of edible vaccines. Individual may develop immune tolerance to the particular vaccine protein or peptide.

4. Dosage required varies from generation to generation and, plant to plant, protein content, patient's age, weight, ripeness of the fruit and quantity of the food eaten.

5. Edible vaccine administration requires methods for standardization of plant material/product as low doses may produce lesser number of antibodies and high doses are responsible for immune tolerance.

6. Edible vaccines are dependent on plant stability as certain foods cannot be eaten raw (e.g. potato) and need cooking that causes denaturation or weakens the protein present in it.

7. Edible vaccines are prone to get microbial infestation e.g. potatoes containing vaccine can last long if stored at 4°C while a tomato cannot last long.

8. Proper demarcation line is necessary between 'vaccine fruit' and 'normal fruit' to avoid misadministration of vaccine, which can lead to vaccine tolerance.

9. Edible vaccine function can be hampered due to vast differences in the glycosylation pattern of plants and humans.

FOOD MARKETING**Module 178: Food marketing principles**

Marketing activities are generally carried out in all types of industries. To a large extent, the kinds of marketing activities carried out are common across all industries. However, the nature of the industry does lead to some differences in the activities. This implies that the methodology of marketing can be applied to many industries, albeit with some modifications. Marketing can also be applied to the 'food' industry. There exist marketing books that offer information about the general methodologies and theories of marketing based on studies of food companies. Basic text-books will contain case studies on Nestle, Unilever, Coca-Cola, etc. McDonald's is a popular case in recent years. Food companies contribute to the development of marketing theory as objects of case study.

However, discussions on a common marketing methodology for the entire food industry are confronted with one difficulty. 'Food' is a general term for edible products that human beings ingest in daily life. The companies mentioned above produce different kinds of food. Nestle is famous for dairy products, chocolate, coffee, etc. Unilever makes margarine, Coca-Cola is a soft drink company and McDonald's is a hamburger chain. All these companies are included under the broad classification of the 'food industry' despite the fact that they produce different kinds of food. In addition, companies that produce perishable food, canned food, frozen food, processed food, drinking water, alcoholic beverages, etc. are also classified under the same umbrella. The food industry comprises plural sub-industries. Therefore, there is a need to consider many types of marketing applications that correspond to the different products in the food industry.

Module 179: Marketing Principles

Our first question is 'What is marketing?' You are aware that TV commercials and big signboards on the roofs of buildings are a part of marketing activities. When you read newspapers and magazines, you come across a huge amount of advertising. Advertising is an important part of marketing. Sometimes, you are requested to sample new products, such as a new flavour of cheese or a new taste of beer, in a supermarket. Sales people explain to you how the new sample differs from existing products. This selling activity is also a part of

marketing. Thus, we see some examples of marketing activity in our daily lives. However, advertising and selling constitute only a part of marketing.

Today, marketing is considered to have a broader and deeper meaning than in the past. Kotler and Armstrong define marketing thus:

‘Marketing is the process by which companies create value for customers and build strong customer relationships in order to capture value from customers in return.’

This definition comprises two parts. The first part is ‘to create value for customers’.

Marketing begins with understanding the customer’s needs and wants.

Module 180: The Marketing Concept

Finding potential needs and wants that the customer is still unaware of also plays a key role. In order to create value for customers, it is necessary to realize customers’ needs and wants. Knorr’s soup stock was accepted by customers since it realized one such customer need: it not only reduced the soup to powder but also met the demand for quick and easy soup preparation.

The second part of the definition is ‘to build strong customer relationships’. This part suggests that the goal of marketing is to capture value from the customer. In order to deliver the value created to the customer, many kinds of activities are carried out, such as advertising and sales promotion. Further, once a customer buys a product, some measures must be taken to ensure that he/she makes repeat purchases. It is important to establish the brand name for the customer to recognize the product.

The marketing process

As mentioned above, the aim of marketing is to capture value from the customer. To arrive at this value, marketers go through five steps, which are collectively referred to as the ‘marketing process’:

- Understand the marketplace and customer needs and wants.
- Design a customer-driven marketing strategy.
- Construct a marketing programme that delivers superior value.
- Build profitable relationships and create customer delight.

- Capture value from customers to create profits and customer equity.

The first step of the marketing process requires marketers to obtain an understanding of their marketplace and customers. Marketing research is one method to understand the market. Marketers need to understand the main influences on the marketing activities within the macro and micro environments and on customer behaviour. Further, they need to collect, analyse and evaluate information and data by using different methods.

The second step is to design a customer-driven marketing strategy. Marketing strategy formulation depends on the targeted customers; therefore, marketers are forced to decide who their customers are. In other words, it is important for marketers to carry out market segmentation and to deliver their goods to their target customers.

The third step is to prepare a practical marketing programme. The preparation of the marketing programme begins with planning the details of the marketing mix. The marketing mix is a combination of the firm's marketing tools – product, price, promotion and place.

These marketing tools are known as the 'four Ps'. By controlling these four Ps, firms need to draft a marketing programme that will meet the wants of the target market.

The fourth step consists of two parts. The first part is customer relationship management, and the second part is partner relationship management. The former pertains to the manner in which firms build relationships with their customers, while the latter pertains to how firms build relationships with their trade partners.

The final step of the marketing process is aimed at capturing value from the customer. Firms attempt to build customer loyalty by enhancing customer satisfaction. They focus not only on increasing their market share but also on increasing customers' mind share. By building customer loyalty, firms are able to increase their profits substantially.

Module 181: The Marketing Process

The market research process in the food market

1. Recognize problems;
2. Describe the general needs;
3. Specify the products;

4. Seek suppliers;
5. Solicit proposals;
6. Select the supplier;
7. Order the desired specification;
8. Review the performance.

Module 182: Application of social, legal, ethical and environmental principles to marketing situations

‘The idea of social responsibility supposes that the corporation has not only economic and legal obligations, but also certain responsibilities to society which extend beyond these obligations.’

The economic responsibilities demand that the corporation should produce goods and services that society wants and sell them at fair prices. Fair prices do not naturally imply low prices. Rather, a fair price is one that includes dividends to the investors and sufficient profits for the continuance of the business. In recent years, legal responsibilities have come to the fore due to the keen market competition. Compliance with laws is an important aspect of a business's responsibilities. However, a company's ethical responsibilities toward society go beyond its economic and legal obligations. Ethical responsibilities embrace those activities and practices that are expected or prohibited by societal members even though they are not codified into law..

The application of CSR to marketing situations is linked to producing a positive image that the company contributes to society and that its existence is valuable to society. The positive image is not a tangible asset of the company but is stored in the minds of customers. Thus, neglecting CSR can lead to major problems. For example, when Snow Brand Milk failed to prioritize CSR, it not only destroyed its favourable reputation in the Japanese market but also incurred huge losses in its milk business. In 2000, low-fat milk produced by Snow Brand Milk led to rampant food poisoning. The ensuing investigation revealed that the production process, although compliant with the existing laws, was unacceptable to consumers. However, Snow Brand Milk failed to take appropriate measures to regain consumer confidence in its products. As a result, its brand value plummeted and it lost market share.

In the 1980s, there emerged the so-called global environmental problems, such as global warming, depletion of the ozone layer, reckless deforestation and sea pollution. However, environment protection has rarely been discussed in the context of marketing. In 1992, Peattie suggested the concept of 'green marketing'. Green marketing proposes the development of a new marketing method to cope with both the pursuit of benefits and a reduction in the environmental load. For companies to build their reputation in the market, it is important that they carry out green marketing. In addition, customers who are very sensitive to global environmental problems constitute an important market that firms cannot afford to ignore.

Module 183: Marketing Research

As the first step of the marketing process, it is important for marketers to understand their marketplace and customer needs and wants. Therefore, marketers research markets and customers, analyse consumer behaviour and establish methods of collecting and analysing many kinds of data.

Module 184: Consumer Behavior

As markets are classified into consumer markets and business markets, buying behaviour is also classified into consumer buyer behaviour in the consumer market and business buyer behaviour in the business market. In order to understand consumer buyer behaviour, marketers have to consider two things: factors influencing consumers and the buyer decision process. As for factors influencing consumers, the following four are well known:

- Cultural social
- Personal
- Psychological

These four factors are presented in a kind of progression, from factors that have a broad influence to those that have a personal influence.

Marketers need to understand the three components of the buyer decision process. Specifically, they need to understand the buying decision maker, types of buyer decisions and the steps in the buyer decision process. First, the buying decision maker has a role in the buying decision process. Consider the example of a man who purchases daily food items

from a supermarket by referring to a list. He is the purchaser. His partner, who made the list, is the decision maker of these purchases. We can picture the partner asking the man what he would like to have for dinner while drawing up the list. In this case, the purchaser plays the role of the influencer. Many kinds of roles exist in the buying process. Marketers have to identify the buying decision maker and approach him/her.

Second, the decision-making process of a purchaser depends on what he/she wants to buy. The types of buyer decisions are classified along two axes: the commitment level to the buying process and the differences between brands. There is a difference between the wine purchased for a Christmas dinner and that purchased for daily consumption. In the case of the latter, the consumer customarily buys the usual wine. The buying process seldom takes a long time. Moreover, in the process, the buyer emphasizes custom over the brand. However, in the process of buying wine for Christmas, the purchaser takes a long time and selects a good brand.

Third, the following model depicts the five steps in the buyer decision process (for example, The buyer:

- recognizes a need;
- seeks information;
- evaluates alternatives;
- decides the purchase; and
- makes a post-purchase assessment.

The buying process begins long before the actual purchase and continues long after it is completed. Therefore, marketers need to focus on the entire buying process rather than on merely the purchase decision.

We now discuss business buyer behaviour. The business market consists of organizations that purchase other companies' products and services in order to produce their goods. Some characteristics of a business market differ from those of a consumer market (Kotler, 2000):

The business market has fewer buyers than a consumer market.

The buyers in a business market are bigger than those in a consumer market.

There exists a close relationship between the supplier and the customer.

Similar to consumer buyer behaviour, business buyer behaviour is influenced by various factors. The most important of these are environmental, organizational, interpersonal and individual factors. These factors include many factors that differ from those that influence the general public and those that are related to individual transactions.

1 In a business market, marketers also need to understand the three components of the buyer decision process, that is, the buying decision maker, types of buyer decisions and the steps in the buyer decision process.

First, in business purchasing, the decision is made in the buying centre. The buying centre includes organization members who play five roles in the purchase decision process: ‘users’ use the product or service; ‘influencers’ help to prepare the specifications; ‘buyers’ select the supplier for routine buying; ‘deciders’ have the final authority to select suppliers; and ‘gatekeepers’ control the information flow into the buying centre.

2 Second, there are three orientations in the case of the buyer decision. The ‘purchasing orientation’ is a market transaction; the buyer aims to buy goods that are cheaper. The ‘sourcing orientation’ builds more cooperative relationships with suppliers; by building close relationships, the buyer aims to simultaneously achieve quality improvement and lower costs. Further, in the case of ‘supply management orientation’, the buying centre plays a broader role than in other orientations; the company aims to enhance value through the entire value chain that it formulates.

Third, the following eight steps are suggested to constitute the buyer decision process. Business buyers and subsequent actions. Appropriate information is distributed through the system and helps marketing managers in their decision-making. MIS need not necessarily be a computer system. Marketing managers should be able to utilize MIS in any form.

Module 185: Market Research Process in Food Market

As a background for marketing managers’ utilization of MIS, we can point out that the managers need not only general information for decision-making but also information relevant to specific marketing situations that the organization is facing. This specific marketing information is obtained through marketing research.

The marketing research process has four steps :

- 1 defining the problem and research objectives;
- 2 developing the research plan;
- 3 implementing the research plan;
- 4 interpreting and reporting the findings.

The first step, defining the problem and research objectives, is the most difficult step in the process. Marketing managers are usually aware of the occurrence of some event in their market, but they are not always able to identify the specific cause. Marketing managers must think deeply about the causes through reflection and discussions with organization members. Defining the problem is the most important aspect. For example, Japanese sake companies are seeing a decrease in demand. The production volume of Japanese sake has been dwindling since 1996. The marketing managers of these companies pursued many possible causes, but they were unable to identify the specific determinants. However, they discovered that it was important to increase sake consumption among young people. Thus, the marketers defined the problem that they needed to solve.

Next, in order to define the problem, marketing managers must set the research objectives. There are three types of marketing research. The type selected depends on the research objectives. Exploratory research is selected when the objectives are to gather preliminary information that will help define the problem and suggest hypotheses. Descriptive research is opted for when the objectives are to better describe the marketing problems, situations or markets; this is equivalent to re-searching a potential market for new products, demographics, etc. In the third type, causal research, hypotheses on the cause-and-effect relationship are tested. Let us return to the case of Japanese sake. The problem that the sake companies face is the estrangement of young people from Japanese sake. In order to solve this problem, the marketing managers of Japanese companies can think of various ideas, such as developing new tastes and containers, finding new distribution channels and creating advertisements that portray a new image. Marketing managers set the research objectives after deciding which points they want to focus on.

The first step in the marketing research process is to define the problems and objectives. Therefore, the definition influences the final results of the marketing research. The second step is to develop the research plan for collecting information. The research plan outlines the sources of existing data and spells out the specific research approaches, contact methods,

sampling plans and instruments that researchers will use to gather new data. The research objectives are translated into the information required. For example, the marketing managers of the Japanese sake companies require information regarding the drinking behaviour, trends, fashions, etc. of young people. The research plan is presented in a written proposal. The proposal must cover the objectives of the marketing research and provide information that will help the managers in decision-making.

Information is classified into secondary and primary data. Secondary data are information that has been collected for another purpose. Such data are available free or for a fee. Government statistics are also secondary data. Secondary data must be collected first because such data offer a clue to the research and can be collected within a short period. However, since secondary data are collected for another purpose, managers do not always find the information that they seek from secondary data. On the other hand, primary data are collected for a specific purpose. They are needed data. However, collecting such data involves the expenditure of an immense amount of time and money. Observational research, survey research and experimental research are the well-known methods of primary data collection. In the case of observational research, primary data are gathered by observing relevant people, actions and situations. This method is used to gather information from the daily behaviour of test subjects. For example, consider a Japanese sake company's

marketing manager attempting to create opportunities to drink for young people. The manager can extract information about the drinking behaviour of Japanese youths from such observations. The second method of obtaining primary data, survey research, is the best-suited approach for gathering descriptive information. The company can learn about people's attitudes, preferences, buying behaviour, etc. by asking questions directly to them. The marketing manager of the Japanese sake company can explore the possibility of producing bottles of sake cocktail. This different kind of a bottled cocktail could become popular in the Japanese market. The young generation will find sake cocktail more appealing than ordinary Japanese sake. Thus, the manager can conduct a questionnaire survey to gather information on the preferences of bottled cocktail buyers. The third method of gathering primary data, experimental research, involves a comparison between two groups. Each group is treated differently, which clarifies the cause-and-effect relationship. The above-mentioned marketing manager can test the sake cocktail that his company has developed among different groups. This will clarify the preferences of each age group.

The third step of marketing research is to implement the research plan. This includes the collection, processing and analysis of information. Information collection has already been explained in the research methods described earlier. In the processing and analysis of information, it is essential to isolate the important information and findings easily. ☰

The fourth step is to interpret and report the findings. It is possible for a marketing manager to interpret some findings incorrectly. Past experience may prevent managers from accepting a new perspective. Marketing managers must discuss their interpretations with internal staff and external experts. After these discussions, they should be prepared to face the consequences of their decisions.

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