UNIT 6 ENZYMES, PIGMENTS AND DIETARY FIBRE

Structure

6.1 INTRODUCTION

In this unit, we will learn about enzymes, natural pigments, colourings and their application in food science and biotechnology. Once again, while studying this unit, we request you to refer back to the unit on enzymes in Nutritional Biochemistry and Advance Nutrition course. The structure and properties of enzymes have been dealt in great details in unit 3, block 1 of Nutritional Biochemistry course. Look up the content given there. It forms the basis of understanding the applications and uses of enzymes in the food industry, as discussed in this unit.

Objectives

After studying this unit, you will be able to:

- discuss the history of enzymes and its uses in food industry,
- classify the enzymes,
- describe the biotechnological applications of enzymes, and
- explain the natural pigments and colours used in foods.

6.2 INTRODUCTION TO ENZYMES

In the earlier times, processes such as the souring of milk and fermentation of sugar to alcohol could only take place through the action of a living organism. In 1833, the active agent breaking down the sugar was partially isolated and given the name ‘diastase’, now commonly known as the enzyme amylase. Later, a substance which digested dietary protein was extracted from gastric juice and was called ‘pepsin’. Subsequently, all active preparations were given the general name ‘ferments’. The term ferment was gradually replaced by the name ‘enzyme’, proposed in 1878. Today, enzyme still forms a major subject for academic research. Enzymes are still widely used in industry, continuing and extending many processes which have been used since the dawn of history.

Enzymes, as you may already know, are biological catalysts. They increase the rate of chemical reactions taking place within living cells without themselves suffering any overall change. The reactants of enzyme-catalyzed reactions are termed ‘substrates’ and each enzyme is quite specific in character, acting on a particular substrate or substrates to produce a particular product or products.

Note, all enzymes are proteins. Many enzyme proteins lack catalytic activity in the absence of a non-protein component, called a ‘co-factor’. In this case, the inactive protein component of an enzyme is termed the ‘apoenzyme’ and the active enzyme, including the cofactor, the ‘holoenzyme’. The cofactor may be an organic molecule, when it is known as a ‘coenzyme’ or it may be a metal ion. When a cofactor is bound so tightly that it is difficult to remove without damaging the enzyme, it is sometimes called a ‘prosthetic group’. With this basic introduction to enzymes, let us now learn about enzyme classification.

6.2.1 Classification of Enzymes

It has been a long tradition of giving enzymes names ending in 'ase'. The only major exception to this 'ase' is the *proteolytic enzymes*, whose names usually end with *'—in'*, *e.g. trypsin*. In the background of lack of consistency in the nomenclature, it becomes apparent as the list of known enzymes rapidly grew, that there was a need for a systematic way of naming and classifying enzymes. A Commission was appointed by the International Union of Biochemistry and its report, published in 1964 and updated in 1972, 1978, 1984 and 1992, forms the basics of the present accepted system which is summarized in box 1 herewith. A detailed classification of enzymes is presented in the unit 3, block 1 of the Nutritional Biochemistry course. We would want you to get it now and read it along with the study of this unit. You will see this will facilitate your understanding of enzymes.

Box 6.1

System of classification

The Enzyme Commission divided enzymes into 6 main classes, on the basis of the total reaction catalyzed. Each enzyme was assigned a code number; consisting of four elements, separated by dots. The first digit shows to which of the main classes the enzyme belongs, as follows:

First digit	*Enzyme class*	*Type of reaction catalyzed*
1	Oxido-reductases	Oxidation/reduction reactions
2	Transferases	Transfer of an atom or group between two molecules (excluding reactions in other classes)
3	Hydrolases	Hydrolysis reactions
4	Lyases	Removal of a group from substrate (not be hydrolysis)
5	Isomerases	Isomerization reactions
6	Ligases	The synthetic joining of two molecules, coupled with the breakdown of

	pyrophosphate bond in a nucleoside triphosphate

The system of the nomenclature and the classification of enzymes is based exclusively on the reaction that is catalyzed and does not consider their origin or multiplicity. Enzymes catalyzing the same reaction, but isolated from different species, will have varying amino acid sequences so that they may be distinguished by electrophoretic methods. They may have different sizes and net negative charges and they may even differ in their catalytic behaviour.

6.2.2 Structure of Enzymes

Since all enzymes are proteins, you will realize that knowledge of protein structure is clearly a pre-requisite to any understanding of enzymes. Yes, as you can see all proteins consist of amino acid units, joined in a series. You have already learnt about the structure of proteins, amino acids in Unit 4 in this block and in the Nutritional Biochemistry course. Look up the protein structure once again. As you can see all proteins consist of amino acid units joined in series.

Two distinct types of proteins are known: fibrous and globular proteins. Fibrous proteins are insoluble in water and are physically tough, which enables them to play a structural role. In contrast, globular proteins are generally soluble in water and may be crystallized from solution. They have a functional role in living organisms, *all enzymes being globular proteins.*

Based on the structure, enzymes can be classified as monomeric or oligomeric. Let's learn how the two types of enzymes differ from each other.

Monomeric enzymes

Monomeric enzymes are those which consist of only a single polypeptide chain, so they cannot be dissociated into smaller units. Very few monomeric enzymes are known and all

of these catalyze hydrolytic reactions. In general, they contain between 100 and 300 amino acid residues and have molecular weights in the range of 13 kDa to 35 kDa. Some, for e.g. *carboxypeptidase A*, are associated with a metal ion, but most act without the help of any cofactor.

A number of monomeric enzymes are *proteases* (or proteolytic enzymes), i.e. they catalyze the hydrolysis of peptide bonds in other proteins. In order to prevent them doing generalized damage to all cellular proteins, they are often synthesized in an inactive form known as a 'proenzyme' or 'zymogen' and activated as required. Such enzymes include the *serine proteases*, so called because of the presence in the active site of an essential serine residue, i.e. a serine residue whose presence is essential for the enzymic activity. The serine proteases, chymotrypsin, trypsin and elastase, which are produced in an inactive form by the mammalian pancreas, form a closely related group of enzymes.

Other monomeric enzymes include*, pepsin,* like the pancreatic serine proteases, plays a role in the digestion of proteins eaten by mammals. It is called an acid protease because it functions at the low pH values found in the stomach. Peptide fragments are removed from the inactive form, pepsinogen, by the action of acid or other pepsin molecules to produce the active enzyme. Another acid protease found in the stomach is *chymosin (rennin)*.

A group of *thiol proteases*, similar in structure to each other, are found in plants. These include *papain* from the papaya fruit and *ficain* from figs. Other thiol proteases, of different structures are found in bacteria and mammalian lysosomes. The essential cysteine residue in each of these enzymes plays a similar role to that of serine in the serine proteases.

Several exopeptidases, which remove terminal amino acid residues from polypeptide chains, are well known. Bovine pancreatic carboxypeptidase A, a monomeric enzyme containing one zinc ion per molecule, will break the peptide bonds linking C-terminal non-polar amino acids to the rest of the chain. It is produced when trypsin removes

peptide fragments from the zymogen, procarboxypeptidase A. A very similar enzyme, carboxypeptidase B, which has specificity for C-terminal amino acids with basic side chains, is also secreted as a zymogen by bovine pancreas.

Oligomeric enzymes

Oligomeric proteins consist of two or more polypeptide chains, which are usually linked to each other by non-covalent interactions and never by peptide bonds. The component polypeptide chains are termed sub-units and may be identical to or different from each other, if they are identical, they are sometimes called protomers. Dimeric proteins consist of two, trimeric proteins of three and tetrameric proteins of four subunits. The molecular weight is usually in excess of 35000 Da. The vast majority of known enzymes are oligomeric, which include *lactate dehydrogenase, lactose synthase, tryptophan synthase and pyruvate dehydrogenase*

Check Your Progress Exercise 1

1. Fill in the blanks:

a) Enzymes are termed as catalyst because they----------

b) The non-protein component of an enzyme is---------- in which active part is----------and non-active part is termed as----------

c) The system of nomenclature and classification of enzymes is based on----------

d) Two distinct types of proteins are----------and---------- which differ in the property of ----------

e) Proteases are synthesized in an inactive form as ----------to prevent----------

2. How can the enzymes be classified? Explain giving examples.

Having understood the classification of enzymes, let us move on to learning about the applications of enzymes in food industry.

6.3 BIOTECHNOLOGICAL APPLICATIONS OF ENZYMES

Although enzymes have been used in certain industrial processes for centuries, their precise role or even their identity was not known over most of this period. Often, they were utilized as components of intact cells, e.g. yeasts in the baking and brewing industries. The first enzyme to be made commercially available in a partially purified form was the acid protease, rennin (chymosin) as rennet, a crude preparation obtained from the fourth stomach of young calves, used to curdle milk in cheese production. In view of the varied problems faced, the trend has been towards increased utilization of microorganisms as sources of enzymes.

More recently, procedures involving recombinant DNA technology has been used to increase the yield of enzymes already produced by the microorganisms, e.g., β-galactosidase by *E. coli* or to produce completely different enzymes including ones normally synthesized by eukaryotic cells. These techniques may even be used to produce enzymes of modified structure from suitably modified or synthesized genes. This is termed as *protein engineering.* Once a suitable strain of a potential microorganism has been identified, the same may be grown to produce larger amounts of enzyme. Most enzymes obtained commercially from microbial fermentation procedures are hydrolases. These are usually extracellular enzymes.

This technology, which involves the industrial use of biological processes to develop new products, is termed as *biotechnology.* The technique employs modification in genes, which you may already know, is a section of deoxyribonucleic acid i.e., DNA. You can find detailed information on enzymes and recombinant DNA technique in Box 6.2. Read it carefully. But first let us look at the various uses of enzymes in food industry.

6. 3.1 Enzymes utilization in food industry

Enzymes may be used in industry as components of living cells or after isolation in free or immobilized forms. All of them may be referred to as *biocatalysts.*

The traditional use of yeasts in the baking and brewing industries arose, because they contain the enzymes necessary to bring in desirable attributes.

A. Baking Industry

In the baking of bread, the preliminary process involves the mixing of wheat flour (mainly starch and proteins) with yeast and water. Starch consists of D-glucose units linked by α-1,4 glycosidic bonds with α-1,6 bonds at branching points, the enzymes α-amylase and β-amylase present in the flour cleave some of the α-1,4 bonds, the eventual products being glucose, maltose (a disaccharide) and some oligosaccharides, which cannot be broken down further, because of the presence of α-1,6 bonds. Glucose and maltose can then be metabolized by the yeast, and carbon dioxide is formed, which disintends the protein framework of the dough, ready for baking.

B. Brewing Industry

Here, the main starting material is malt, produced by allowing barley seeds to germinate under moist conditions. The reserve starch is broken down by the amylase present to give, among other products, glucose and maltose. The grains may then be roasted to prevent further growth and to add flavour, after which the soluble material present is extracted by water to produce the wort. This is then acted upon by the yeast to produce ethanol by alcoholic fermentation of the glucose and maltose.

Bacterial α-amylase (from *Bacillus subtilis*), which is even more heat stable than wheat α-amylase, is of increasing importance in the brewing industry. In the industrial production of glucose from starch, the latter is first solubilized and partly degraded by bacterial α-amylase and then treated with fungal amyloglucosidase. Glucose may also be obtained from cellulose-containing waste products by treatment with cellulose; as a

further possibility, it may be produced together with galactose by the action of β-galactosidase (lactase) on lactose, which is present in whey and so is a major by-product of cheese manufacturing.

Invert sugar, a mixture of glucose and fructose, is produced from sucrose by the action of yeast invertase (β-fructofuranosidase), an enzyme which can only be extracted by disruption of the yeast cell wall. Invert sugar may also be produced from glucose by the action of glucose isomerase (bacterial or fungal), an enzyme now thought to be identical to xylose isomerase.

The clarification of cider, wines and fruit juices is usually achieved by treatment with fungal pectinases. The pectins of fruits and vegetables play an important role in jam making and other processes by bringing about gel formation. However, they cause fruit drinks to be cloudy by preventing the flocculation of suspended particles. Pectinases are a group of enzymes including polygalacturonases, which break the main chain of pectins.

C. Cheese Production

Cheese production involves the conversion of the milk protein, k-casein to paracasein by a defined, limited hydrolysis, catalysed by chymosin (rennin). In the presence of Ca^{2+}, paracasein clots and may be separated from the whey, after which the clot is allowed to mature under controlled conditions to form cheese.

You will further read in detail about the uses of enzymes in food industry in the Food Microbiology Course, unit 5.

Box 6.2 Enzymes and recombinant DNA technology

Recombinant DNA technology, also called as *genetic engineering* makes use of a variety of enzymes, particularly restriction endonucleases (from bacteria) and DNA ligases to insert extra genes into cells with the help of vehicles termed ‘vectors’. One important group of vectors is ‘plasmids’, which are small, circular, cytoplasmic molecules of DNA, acting as extrachromosomal genes in bacteria. It is possible to extract and purify plasmids and to insert extra genes into the circles. These altered plasmids can then be taken up

again from the medium by the microorganism. Then as the bacterium is grown in culture, the inserted gene will be replicated together with the vector. The production of identical copies is termed as 'cloning. You will learn about cloning in the Nutritional Biochemistry course, unit 9, block 3 as well.

Another group of vectors are the variants of a bacterial virus known as λ phage. The phage DNA is about 45 kb long, of which the middle third has no role in the infection process and can be replaced by another piece of DNA of about the same length (again by means of restriction endonuclease and ligase enzymes) without affecting the ability of the phage to infect bacteria and be reproduced in abundance. Genes or gene fragments of about 15 kb length can be inserted and cloned in this way, in contrast to the limit of 10 kb when plasmids are used as vectors. Irrespective of the vector used to insert new DNA into bacteria, this DNA can specify the synthesis of a protein by transcription/translation, which enables in the large scale production of enzymes.

You have so far learnt about enzymes and its uses in food industry and biotechnology. Further, you would be surprised to learn that analysis of enzymes in foods can provide useful information regarding a process or condition. How? Read the next section and find out.

6.3.2 Enzymatic analysis in foods: applications in food industry

Did you know that the degree of bacterial contamination of foods or freshness of food, particularly, meat etc. can be determined through a simple enzymatic analysis in foods. Besides these, the other uses of enzymatic analysis are many.

In the food industry, the activity of certain enzymes may be determined before and after pasteurization/sterilization procedures to ascertain the efficient completion of the process.

For e.g. alkaline phosphatase and invertase present in milk are inactivated within the same temperature range as is required for pasteurization, so the activities of these enzymes at the end of the process gives an indication of its effectiveness.

Similarly, the degree of bacterial contamination of foods can be estimated by the assay of microbial enzymes not normally present in foods, for e.g. milk should contain small amounts of reductases, but bacteria produce large amounts of reductases. Reductases may be easily assayed, because they catalyze the reduction of methylene blue to colourless leuco-methylene blue under anaerobic conditions. A test strip (Bacto-strip) incorporating 2,3,4-triphenyl tetrazolium salts provides a convenient way of testing for the presence of bacteria, a red formazan dye being produced as a result of the action of reductase.

Enzyme assay may also be used to determine whether stored plant products are suitable for use as food commodities, for e.g. α-amylase should be present in relatively low amounts in stored wheat seeds. If there is sprouting/germination of a stored crop, then the α-amylase content increases. Once the flour has been produced, its amylase content may again be assayed to give an indication of the amount of starch breakdown which can be expected to take place when the dough is prepared.

The freshness of meat may be determined by the use of monoamine oxidase to detect amines formed during degradation. Besides, enzyme assay is used in the investigation of diseases in plants, for e.g. it has been found that an injury (either mechanical or pathogenic) results in a marked and localized increase in the activity of glucose-6-phosphate dehydrogenase, but not of glucose phosphate isomerase.

Enzyme assay is also used for research into such processes as the browning of plant products which poses problems during value addition. The browning process involves the cyanide-resistant uptake of oxygen, which oxidizes phenols to quinines resulting in the formation of dark melanins. In wine preparation, the concentration of malic acid is sometimes determined by a method involving malate dehydrogenase.

The discussion above highlighted the various applications of enzymes and enzyme analysis in food industry. With this we come to the end of our study about enzymes. Next we shall focus on pigments and natural colours in foods.

Check Your Progress Exercise 2

1. Name the enzymes that are used in :

a) Baking of bread ----------

b) Brewing ----------

c) Clarification of fruit juices and wines----------

d) Cheese production----------

2. Define the following terms:

a) Genetic engineering

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b) Biotechnology

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3. Describe how enzymes assay is helpful in determining the extent of freshness in:

a) Wheat seeds

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b) Milk

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c) Meat

6.4 NATURAL PIGMENTS

What is a pigment? Scientifically, a chemical that can impart colour and is insoluble in the solvent in which it is used, is referred to as a 'pigment'. Well, you would agree that colour has a remarkable influence on food selection, consumption and overall enjoyment. Although the colouring of foods with natural compounds is considered to be desirable for various reasons, their use is limited at present. A few substances, notably the carotenoids, have been successfully incorporated into specific products, but natural colourants offer neither the range of colour nor the stability of synthetic dyes. Attempts to improve the situation have taken various forms. There has been an extensive search of the microbial, plant and animal kingdoms for pigments that possess both high tinctorial power/strength (a measure of the potential colouring power of a colourant) and stability, but so far, no exceptional candidates have emerged. The instability of the common natural colourants in food has been studied to seek the means of stabilization and the environment in which the pigments exist in nature, has been investigated for the same reason. Recent work on these approaches is reviewed, but any solution to the problem must be reconciled with the legislative and economic constraints governing the use of colourants in foods.

Currently, the use of natural colourants is limited due to their instability, low tinctorial power or price disadvantage. The trend towards natural ingredients in foodstuffs is continuing and this is evidenced by the consumer's acceptance of 'natural' foods and the various national regulations which completely or selectively ban artificial colours from food. Let us get to know a bit more about these natural colours used in foods.

6.4.1 Natural Colors Used In Foods

Natural colourants produced for use in an analogous way to the coal-tar dyes are crude extracts of pigments, which are basically unstable. The apparent stability of some food

products owes more to the amount of pigment present than to the tinctorial power of the pigment itself. For example, beetroot even after prolonged cooking retains an attractive deep red colour, but the extracted pigment is unstable. Anthocyanin preparations have found use in some products, but their colour variation with pH has restricted their use, mainly to acidic products. However, in nature, the flavonoids (Flavanoids are antioxidant molecules found in plant sources such as fruit, flowers, roots, stems, tea, wine, grains and vegetables. They are often responsible for the beautiful coloring of plant structures) produce colours from white through yellow, red and blue to black at the pH of cell sap. The potential colouring power of flavonoids is, therefore, great.

Carotenoids are relatively stable and there is a sufficient demand to make complex chemical syntheses of 'nature-identical' carotenoids. Their colour range is limited to yellow/orange/red and they are naturally fat soluble, although water soluble forms are also available.

Chlorophylls are used as colourants in a range of foodstuffs and both natural chlorophyll (containing magnesium as the central metal ion) and 'copper chlorophylls' (copper substituted for magnesium) are available. Both chlorophyll and copper chlorophyll are manufactured in fat soluble and water soluble forms by selective retention or hydrolysis of the phytol side chain. Apart from these three plant pigments, there are others such as red beet extract (betanin), cochineal, turmeric extract and others which have found use in food. As alternates to the above, there has been a search among the plant, animal and microbial kingdoms for pigments which are stable under conditions prevailing in foods (i.e., pH of 2 to 8; temperature of -20 to 110°C; presence of additives and preservatives). Another approach has been to prevent the colour loss from foods. Both chemical and physical methods have been used to prevent destruction of natural colours in foodstuffs. As a means to achieve the desirable attributes of natural colours, it has been shown that biotechnological approaches appear to hold a lot of promise in the application of natural colours in foods. What are the sources of natural colourants? The next section details the sources of natural colourant.

6.4.2 Novel Sources of Natural Colourants

In the search for a colourant that has the properties desired for food use, practically every part of the biosphere has been investigated. The following are some of the sources:

A. Microbial sources

Production of materials by microbial cultures has several advantages. The rapid growth of microbes cuts the production time to a matter of days and the process lends itself to a continuous operation. Compared to plant or animal sources, the production is flexible and can easily be controlled. Microbes produce a variety of colourants such as chlorophyll and carotenoids as well as some unique pigments. By incorporating suitable genetic material into selected microbes (recombinant DNA technology), it may be possible to produce pigments of choice, both qualitative and quantitative approaches. Most of the reports of food colourants from microbial sources involve *Monascus* species, particularly *Monascus purpureus*, followed by *Rhodotorula* species, *Chlorella*, *Nocardia* and red algae. Often, instead of extracting the pigment from *Monascus*, a few researchers have attempted in adding the whole coloured substrate to foods for the purpose of providing colouration. Careful control of the culture conditions selectively improves the yield of various pigments elaborated by the specific microbial culture.

Although cultivation of microorganisms for the production of food colourants has attractions, these must be measured against the financial legislative and user constraints. It is desirable, that the microbial cultures which produce colourants would have to be proved pathogen free and also free from any toxic components. There is still no substantial evidence to suggest that the pigments from *Monascus* are superior to other natural colourants. Potentially its orange or red colour is suitable for food use, its long usage in Oriental foods is also in their favour and the property of reacting with amino-containing compounds suggests that they could easily be incorporated into food systems. They appear to be stable in the pH range 2-10 although below pH 2, precipitation occurs on standing.

B. Animal sources

The most common animal pigments of use as food colourants are those based on the haem structure. In nature, haem is combined with proteins and occurs mainly as haemoglobin and myoglobin. Although the appearance of these two components is attractive when they are oxygenated (bright red), the colour produced on heating is typically brown (e.g. cooked meat) and removal of oxygen in the native state gives rise to the blue/purplish colour of venous blood. The colour changes are due to the oxidation state of the central iron atom in the haem portion of the molecule and the nature of ligands surrounding the iron atom. The bright red colour of freshly cut meat is due to oxygen binding as a ligand to the iron atom which is in the ferrous state. However, oxygen does not bind very strongly and it is known that other ligands bind more strongly, stabilizing the molecule and preserving the red colour.

Ligands suggested for the stabilization of haem pigments are imidazole (and its derivatives), S-nitrosocysteine and nitrite. While searching for alternatives to nitrite in the preparation of fish sausages, imidazole, 5(4)-aminoimidazole-4(5)-carboxamide (AICA) and various amino acid derivatives were used and found to impart colour to the finished product. Imidazole gave a red/pink colour with an orange tint, which faded on the surface of the produce unless an antioxidant was present. Animals appear to be a poor source of colourants.

C. Plant sources

Although there is a multitude of colours in the plant kingdom, their extraction and use in food systems is not an easy task. Unless the colourants have some outstanding advantage, e.g. good stability in food or very high tinctorial power, it is generally not worth continuing. Assuming that the source provides a colourant with exceptional properties, further considerations need to be taken into account. A major problem with the plant sources is their availability, as most plants are seasonal. Many of the plant sources listed in literature would require major planting programmes to provide sufficient material for production purposes. Another consideration is the cost of extraction and processing,

which is obviously minimal for an aqueous extraction, but will be greater, if organic solvents are used and subsequently recovered.

Usually, the natural food colourants from plant sources are classified into 5 types: Anthocyanins, Betalaines, Carotenoids, Chlorophylls and Curcumin. Literature has been well documented, with a lot of research work going into varied aspects of colourants from the above plant sources. A variety of fruits, vegetables and flowers have also been studied as potential sources of colourants. One of the novel sources has been tissue culture of grape varieties. The callus induced from the anther of grape plants is transplanted to a liquid culture medium and cultured aerobically. Pigment is then extracted from the culture which can be manipulated to give maximum colour yield during growth, by adding various chemicals to the medium or irradiating with light.

Colourant production from by-products has the advantages that there is normally an abundance of the source, it is cheap and in a defined state. Further, treatment of the by-product reduces waste and enhances the profitability of a process. The citrus industry produces large amounts of waste and the extraction of colourants from cold pressed citrus oils has been described. The colourants (presumably carotenoids) are extracted from the oil by solvent and purified.

The volume of patent literature concerning novel microbial, animal and plant sources of colourants indicates the potential of the field, but only a few of them are workable. Many pigment extracts are no better than the presently available natural pigments and the availability and/or economics of the source material or extraction procedure causes rejection of others.

Stability of natural colourants is one of the major issues in food industry. There is a lot of interest and work being undertaken to ensure stability of natural colourants in food. Are these efforts successful? Let’s get to learn more about this issue. Microbial sources are also important like *Rhodotorula* and *Monascus* but needs to be commercialized.

6.4.3 Stability of Natural Colorants in Foods

Many attempts have been made to retain colouration by adding chemicals or modifying processing conditions. Ascorbic acid has been claimed as a stabilizer for natural colourants and conversely, its presence has been cited as the cause of pigment degradation. With anthocyanins, ascorbic acid sometimes has a protective effect, e.g. it absorbs available oxygen and thus prevents oxidation of the anthocyanin. On other hand, enzyme action on ascorbic acid results in the release of hydrogen peroxide, which oxidizes and decolourises the anthocyanins. Hence, addition of ascorbic acid to food products will not necessarily stabilize the colour.

The effect of metal ions on the colour and/or stability of food products has been the subject of several investigations. Traces of some metal ions, notably copper and iron, have a catalytic effect on the oxidation of ascorbic acid, which in turn leads to degradation of anthocyanins. Anthocyanins containing an *ortho*-dihydroxy grouping, chelate metal ions, which may alter the colour of the anthocyanin.

Red anthocyanin pigments from miracle fruit were isolated and tested in carbonated beverages, in combination with the organic acids. Pigments degradation occurred with all acids and malic acid caused the most rapid degradation. In grape juice, malic acid caused the greatest increase and also increased the colour stability as did valeric acid. Malonic and oxalic acids increased the colour initially, but fairly rapid decolourization occurred on storage.

Light does accelerates the degradation of natural colourants, especially anthocyanins, but is of secondary importance when compared to the losses of anthocyanin due to the effect of heat and/or oxidation. Anthocyanins had doubled resistance to sunlight fading when flavonoid sulphonates were used as co-pigments. While additives can improve the colour stability of natural pigments in specific cases, there are no general guidelines for their use.

We conclude our discussion noting the fact that ascorbic acid and metal ions can stabilize colour in some situations, but their use in unfavourable conditions may actually increase the rate of degradation. Some organic acids may be beneficial, but others enhance instability and the concentrations and/or nature of inorganic salts used to improve the stability of natural colourants are such as to limit their application.

Well, then are there any stabilized forms of natural colourants! Read and find out.

6.4.4 Stabilized forms of natural colourants

The mimicking of the native environment in which a natural colour exists is just one way of producing a stabilized form. In this case, the method of stabilization can be considered 'natural' whereas some of the modifications can be synthetic. The major emphasis has been on the stabilization of flavonoids and porphyrins. The present use of flavonoids as food colourants is limited to anthocyanin extracts from grape processing. These preparations are largely unsuitable for food use by themselves. A few of the following have been selected, as they appear feasible methods of stabilization within the constraints of retaining natural status:

Complex formation: Anthocyanin occurs naturally as complexes which are relatively stable. Studies on pectin/anthocyanin complexes may be useful.

Co-pigmentation: In formulating a product containing anthocyanin colourants, the inclusion of a co-pigment would augment and stabilize the colour.

Condensation: This seems the most likely way of producing acceptable, natural colourants for the reasons stated previously. Considerable research to study the production of the polymeric colours and their stability in food products needs to be carried out.

Chlorophyll is the porphyrin most widely used as a natural colourant. Its widespread occurrence in photosynthetic tissue and its breakdown character have prompted many investigations concerning stabilization of the molecule. Loss of magnesium follows denaturation of the protecting protein in the chloroplast. Several processing techniques to minimize chlorophyll loss involve alkaline blanching and soaking media. Attempts to stabilize chlorophyll have centered on the substitution of the normal magnesium ion.

Despite a wide search of novel sources throughout the world, attempts to find a naturally occurring pigment with all the desirable qualities of a food colourant have not been so successful on a commercial angle. Besides, the problem of securing an adequate supply of the successful colourant is a big question posed to the food industry. The stabilization of natural colours in foods is an extremely complex process and although some success has been there, each application needs to be looked into individually.

Check Your Progress Exercise 3

1. Fill in the blanks:

a) A pigment is----------

b) Colour in foods has a remarkable influence on ----------

c) Naturally-occuring colourants have limited use because of ----------
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d) The flavonoids produce colours such as----------

e) Chlorophylls, as food colourants are manufactured by----------

2. Name natural food colourants obtained from:

a) Microbial sources----------
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b) Animal sources----------
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c) Plant sources----------
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3. Discuss the effect of following on stability of enzymes:

a) Ascorbic acid

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b) Organic acids

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c) Metal ions

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d) Light

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4. Mention a few methods of stabilization of natural colourants

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6.5 LET US SUM UP

In this unit, you have learnt about enzymes, which are known as biological catalysts. All enzymes are proteins, which take part in various chemical reactions occuring within the living cells without themselves suffering or undergoing any chang.e do you remember the terms ‘co-factor, ‘apo-enzymes’ and ‘holoenzyme’?

Co-factor is a non-protein component; apo-enzymes, the inactive protein component of an enzyme and holoenzyme is the active protein component

After, this, you also studied about the classification, functions and properties of enzymes. Finally, the various biotechnological aplications of enzymesin food industry and recombinant dna technlogy were discussed.

Use of colours and pigments, derived from natural and esynthetic source,s in food industry was described alongwith their stabiized forms as colourants.

6.6 GLOSSARY

Biotechnology : The industrial use of biotechnological processes, whereby the living organisms are used to develop new products.

DNA : Deoxyribonucleic Acid is a large molecule that contains genetic coding information within each cell.

Dye : A dye is a chemical that can impart colour and is soluble in the solvent in which it is used.

Flavanoids : Flavanoids are antioxidant molecules found in plant sources such as fruit, flowers, roots, stems, tea, wine, grains and vegetables. They are often responsible for the beautiful coloring of plant structures. Some 4000 flavanoids have been found. There are four main groups of flavanoids; 1) flavones, 2) flavanones, 3) catechins, and 4) anthocyanins. It is the flavones and catechins that appear to be important flavanoids in oxidation defenses.

Gene : A section of DNA which provides the genetic information needed to make one protein.

Porphyrins : Porphyrins are a ubiquitous class of naturally occurring compounds with many important biological representatives including hemes, chlorophylls, and several others.

Tinctorial strength : A measure of the potential colouring power of a colourant

6.7 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1.

a) They increase the rate of chemical reactions within living cells without themselves suffering any overall change.
b) Cafactor; apoenzyme, holoenzyme
c) The reaction that is catalyzed
d) Fibrous; globular; solubility in water
e) Zymogens/proenzyme; damage to all cellular proteins

2. Based on structure, enzymes can be classified into monomeric enzymes and oligomeric compounds.

- Monomeric enzymes: Enzymes which consist of only a single polypeptide chain, so they can't be dissociated into smaller units, for example, carboxypeptidase A, pepsin, chymosin etc.
- Oligomeric enzymes: Enzymes which consist of more than one polypeptide chain for example, lactate dehydrogenase, pyruvate dehydrogenase, tryptophan synthase.

Check Your Progress Exercise 2

1.

a) α-amylase and β-amlylase
b) Amylase
c) Pectinases
d) Chymosin/Rennin

2. a) Genetic engineering or recombinant DNA technology, involves the use of a variety of enzymes, such as restriction endonucleass and ligases to insert extra genes into cells with the help of vectors.

   b) The technology which involves the industrial use of biological processes to develop new products.

3. a) determination of α-amylase, content: an increase indicates sprouting/ germination of stored wheat;

   determination of reductase content: an increase indicates presence of bacteria

   b) Determination of alkaline phosphatase and invertase: activity level of these enzymes indicate the effectiveness of pasteruization.

   c) Determination of amines by the use of monoamine oxidase.

Check Your Progress Exercise 3

1. a) A chemical that can impart colour and is insoluble in the solvent in which it is used.

   b) Food Selection, Consumption, Enjoyment

   c) Instability, Low Tinctorial Power, Price

   d) White through yellow, red and blue to black at pH of cell sap.

   e) Selective retention or hydrolysis of the phytol side chain.

2. a) Monoscus sp., Rhodotorula sp., Chlorella, Nocardia and Red Algae

   b) Ligands of Haem Pigments – Imidazole, S- Nitrosocysteine and Nitrite

   c) Anthocyanins, Betalaines, Carotenoids, Chlorophyll, Curcurmin

3. a) It results in the release of hydrogen peroxide which oxidizes and decolorizes the natural colourants.

   b) These have a catalytic effect on the oxidation of ascorbic acid which leads to degradation and alteration of colour of the natural colourants.

   c) These degrade the pigments and rapidly decolorizes them on storage.

   d) It accelerates the degradation of natural colourants.

4.

- Complex formation
- Co-pigmentation
- Condensation