UNIT 5: VITAMINS AND MINERALS

Structure

5.1 INTRODUCTION

In this unit we will study about vitamins and minerals. You may have already studied the structure and physico-chemical properties of vitamins and minerals in the course on Food Biochemistry. The course Advance Nutrition also gives you the information on vitamins and minerals. We suggest, like in the other units you have studied so far, please organize the study of this unit along with the respective units on vitamins and minerals in the other two courses. This will give you a comprehensive understanding of the different aspects of vitamins and minerals.

Here in this unit our focus is on application of vitamins, minerals in the food and/or pharmaceutical industry. In this context, you will find a brief description about each of the vitamin and mineral, in terms of its characteristics, occurrence, importance and determination. This information will assist in your understanding of these vital substances.

Objectives:

After studying this unit you will be able to:

- highlight the importance of vitamins and minerals,
- discuss the occurrence and determination/estimate of vitamins/ minerals in food or in test sample, and
- describe the functional role of vitamins and minerals, particularly in the food industry.

5.2 VITAMINS

Vitamins, as you may already know, derive its name from the word 'Vital Amine'. These are the organic compounds required for normal metabolism, growth and development, and regulation of the cell functions. Vitamins classified as water soluble or fat soluble as highlighted in Figure 5.1. In this section, we will learn about the characteristics, importance and application of vitamins, both water soluble and fat soluble. We begin with Vitamin A.

Figure 5.1 Classifications of Vitamins

5.2.1 Vitamin A (Retinol)

Purified vitamin A is a viscous yellowish oil. It is freely soluble in most organic solvents such as methanol, ether, acetone, chloroform and petroleum ether as well as in fatty oils like olive, sesame and groundnut. However, Vitamin A is insoluble in water. It is highly susceptible to the action of atmospheric oxygen and oxygen carriers, further to oxidizing agents and metal ions. Vitamin A is also rapidly destroyed by UV light. Even in diffuse day light, the vitamin A content of solutions is markedly reduced within a few hours. You must remember that vitamin A should be stored in a cool place, protected from light under vacuum or

inert gas in air-tight containers or sealed ampules. You must understand that vitamin A itself is very unstable against external influences. For its use in foods and as therapeutics, therefore, only those derivatives with improved stability are used. Thus, esterification of the alcoholic group of vitamin A with acetic or palmitic acid produces compounds which are somewhat more stable. Vitamin A, therefore, is practically sold and used only in the form of concentrates and oily solutions of the acetate and palmitate and their formulations.

The importance of vitamin A is undisputable. You may already be aware about the functions/role of vitamin A in our body. Vitamin A is absolutely necessary for normal vision, both black and white and colour. Vitamin A deficiency in childhood leads to blindness. Vitamin A is highly important for the normal condition and functional maintenance of epithelial tissues. Vitamin A deficiency symptoms are manifest in characteristic changes of skin and mucosae. With prolonged vitamin A deficiency, cornification of the mucous membranes of the respiratory tract, the digestive tract and the urogential organs develops. Site of action of vitamin A is probably the protein metabolism of the epithelial cells. An early symptom of vitamin A deficiency is night blindness. The interrelationship between vitamin A and the individual hormones is also important. The sex organs show a high vitamin A content and ceases to function in vitamin A deficiency. Vitamin A and thyroxine levels are interrelated. Normally, vitamin A in the presence of fats and bile is absorbed mainly from the intestine and stored in the liver.

In addition, you will find information on how to determine / estimate vitamins / minerals in foods or in any test sample. As a food scientist, you will find this information very useful. The effort of processing on mineral content of food is another aspect covered in this unit. As advised for other units here too we will recommend that you look up the units on vitamins and minerals in the courses Nutritional Biochemistry and Applied Nutrition. Now let us learn about the occurrence of Vitamin A.

Occurrence

In the vegetable kingdom, vitamin A probably occurs in the form of its provitamins which belong to the group of carotenes. Carrots, spinach, lettuce,

celery leaves,mangoes, papaya and dried apricots are especially rich in carotenes and carotene isomers. In the human beings and animals, you should know, the provitamins (carotenes) are converted into vitamin A in the intestinal mucosa. The most important provitamin is β-carotene. Other important sources of preformed vitamin A are the fish-liver oils (like codfish, Salmon, tunny fish and whale), butter, milk, egg yolk and animal liver.

Determination

Vitamin A assay is carried out by chemical method. This method is called Carr Price Reaction.

Vitamin A and the carotenoids produce an intense blue colour upon addition of a saturated solution of antimony trichloride in anhydrous and alcohol-free chloroform. This reaction is called Carr Price Reaction and used to determine Vitamin A. The absorption maxima is around 620 nm.

In another method, absorption of vitamin A dissolved in isopropyl alcohol is determined at wavelengths of 310, 325 and 334 nm. This method is suitable for the determination of pure vitamin A (or of the ester after their saponification), such as oily solutions as well as in tablets, coated tablets, capsules and other formulations.

Applications

- *Vitamin A dry powder*

Vitamin A dry powder is used in the (manufacture of dry mixtures) for which the oily ester concentrates are unsuitable or undesirable. Mainly, they are incorporated in dry formulations such as (coated tablets and capsules). Even in animal feed industry, vitamin A dry powder is preferred. The stability of vitamin A in the dry powder is increased by matrix coating of the esters with gelatin, thus protecting them from direct oxidation.

- *Water soluble Vitamin A*

Water soluble vitamin A is a yellowish green, slightly turbid, fluorescent liquid of faint characteristic odour. The taste is at first faintly sweet and then bitter. Water

soluble vitamin A is especially suitable for the manufacture of solutions for oral administration. This preparation contains the palmitate of vitamin A. This ester which otherwise is only fat soluble, has been rendered miscible with water and aqueous liquids by a solubilizer. The stabilizer added to the preparation moreover delays possible loss of activity due to atmospheric oxidation.

5.2.2 Vitamin B Complex

The vitamin B complex comprises the vitamins B_1, B_2, B_6 and B_{12} as well as the vitamin B factors biotin, folic acid, nicotinic acid and its amide as well as pantothenic acid. Besides, inositol, choline and adenine (vitamin B_4) are considered as vitamins of the B complex. In nature, the active principles of the vitamin B complex mostly occur together.

In the human and animal metabolism, they form a functional unit, since, as components of the various coenzymes, they are essential members of reaction chains. They play a part in numerous metabolic processes such as cellular respiration, and energy production, carbohydrate conversion and the synthesis and breakdown of fats and amino acids. Because of their role in energetic metabolic processes, the vitamins are essential for the maintenance of vital processes. Because of this close interdependence and the multiplicity of functions, it is readily understood that the absence of one B complex factor will impair the efficacy of the other vitamins. Therefore, the symptoms associated with vitamin B complex deficiency may be manifold. They mainly affect growth, skin and mucous membranes, blood and nervous system. We will discuss the characteristics, importance and application of each of the B-complex vitamins one by one in this section.

5.2.2.1 Vitamin B_1 (Thiamine hydrochloride)

Thiamine hydrochloride is a white, crystalline powder, slightly hygroscopic, of an odour resembling yeast and of bitter to taste. It is freely soluble in water. Thiamine hydrochloride is soluble in glycerin, sparingly soluble in methanol and ethanol (95%), almost insoluble in absolute alcohol, acetone, ether, chloroform, benzene, fats and fatty oils.

Occurrence

Vitamin B_1 occurs widely in the vegetable kingdom. The richest sources for vitamin B_1, as you may already know, are yeast, grain germs and rice polish. Whole grain bread and potatoes are vitamin B_1 sources of considerable dietary importance. Animal tissues rich in vitamin B_1 are liver, kidney, brain and heart. Aqueous solutions of thiamine hydrochloride are unstable in the presence of alkalis and substances with alkaline reactions, atmospheric oxygen, metal ions and UV light. Moreover, the solutions are affected by reducing and oxidizing agents, especially in the neutral and alkaline ranges.

Determination

Vitamin B_1 is determined by chemical method and microbiological assay. In the chemical method, vitamin B_1 is oxidized in alkaline solution by potassium ferricyanide solution to form thiochrome. The latter may be extracted with isobutanol and the resulting vivid blue fluorescence measured.

In the microbiological assay, turbidimetric growth measurement of the specific culture of *Lactobacillus fermenti* ATCC 9338 and/or *Saccharomyces cerevisiae* ATCC 7753 is performed by titration against 0.1 N NaOH.

Applications

The steadily increasing consumption of white flours (insufficiently ground and thus low in vitamin content) by large sections of the population involves the risk of vitamin B_1 deficiency. By fortification of white flours with chemically pure vitamins of the B group, the population may retain its most important source of vitamin B.

Due to the manifold functions performed by vitamin B_1 in the metabolism, inadequate supply is manifest by a number of deficiency symptoms. Vitamin B_1 hydrochloride is used for the preparation of drug formulations of all types, especially of oral drop solutions, drops, tablets and others and also for the vitamin fortification of foods.

5.2.2.2 Vitamin B_2 (Riboflavin)

Riboflavin is a yellow to orange-yellow, crystalline powder of faint odour and intensely bitter taste. Its solubility in water is very slight and depends on the crystalline structure. The saturated aqueous solution of riboflavin shows neutral reactions to litmus (pH about 6.0). Solubility is markedly increased in the presence of various substances, especially sodium chloride, urea and nicotinamide. Riboflavin is also soluble in alkali hydroxide solutions, but will decompose. In the common organic solvents riboflavin is practically insoluble.

Aqueous solution shows a pronounced green-yellow fluorescence, which is maximal at a pH of about, 6-7 and disappears upon the addition of acids and alkalis. Riboflavin shows amphoteric reaction. The isoelectric point of riboflavin is around 6.0. The melting point is approximately 280-285°C. Riboflavin is inactivated by exposure to day light and UV light. Irradiation of an alkaline solution produces yellowish-green lumiflavin. In neutral or acidic solutions, the colourless lumichrome is formed along with varying amounts of lumiflavin.

In the presence of alkalis and materials giving alkaline reaction, aqueous riboflavin solutions are unstable. Also in the presence of strong reducing agents, metal ions and upon exposure to day light and UV light, the solution is not stable.

Importance

As a prosthetic group of the flavin enzymes, vitamin B_2 is involved in the reactions of almost all nutrients of plants and animals. They are designed to regulate redox processes. Riboflavin, thus, catalyzes the reactions of carbohydrates, fats, proteins and nucleic acids.

Occurrence

Vitamin B_2 (riboflavin) occurs in nature almost exclusively in a combined form i.e. esterified with phosphoric acid as riboflavin-5'-phosphoric acid ester. Vitamin B_2 occurs in a combined form in any plant and animal cell - yeasts, wheat, white bread, rye bread, fruits, nuts, spinach, cabbage, milk, cheese, egg yolk, fish, meat, rice bran, potatoes and others.

Determination

In the chemical method, the yellow-green fluorescence of aqueous solution of riboflavin is measured. In the microbiological assay, turbidimetric growth measurement or determination of lactic acid formed by *Lactobacillus casei* ATCC 7469 is performed.

Applications

Vitamin B_2 is used as a colour and nutrient in various food commodities such as baked foods, noodles and macaroni products, ice creams and soup mixes.

5.2.2.3 Vitamin B_6 (Pyridoxine hydrochloride)

Pyridoxine hydrochloride is a white, crystalline powder, practically odourless. The dry substance is sufficiently stable in air. With prolonged exposure to day light and UV light, it will change gradually. Pyridoxine hydrochloride is easily soluble in water. A 10% (w/v) aqueous solution shows a pH of about 3.0. Pyridoxine hydrochloride is moreover soluble in 95% ethanol. It is slightly soluble in acetone and practically insoluble in ether. The melting point is 206-212°C. Aqueous solutions of pyridoxine hydrochloride are incompatible in particular with oxidants, metal ions as well as day light and UV light.

Importance

In the human and animal organisms, vitamin B_6 acts in the form of pyridoxal. In the form of 5'-orthophosphate (Pyridoxal-5'-phosphate), pyridoxal is the prosthetic group of numerous enzymes of essential importance in the protein metabolism.

Occurrence

Vitamin B_6 activity is attributed to the 3 compounds–pyridoxol (pyridoxine), pyridoxal and pyridoxamine, generally comprised in the vitamin B_6 group. They occur in any living cell. While the quantitative share of pyridoxal is proportionately largest in the vegetable kingdom, the phosphates of pyridoxal and pyridoxamine predominate in the animal cell and in yeasts, vitamin B_6 is found

abundantly in green plants, molasses, brewer's yeast, egg yolk and several animal organs such as liver and kidneys.

Determination

Colorimetric determination of indophenol dye using 2,6-dichloroquinone chloraimide as colour reagent or diethyl-p-phenylenediamine in the presence of an oxidizing agent. Microbiological determination is preferably made by turbidimetric measurement of the growth of *Saccharomyces carlsbergensis* ATCC 9080.

Applications

The heating processes employed in the industry for the sterilization of milk based formulations will greatly reduce their vitamin B_6 content. Possibly, as yet, unknown decomposition products of vitamin B_6 are formed/it is inactivated in these products during manufacture and storage, with the loss of vitamin activity.

An interesting fact of practical importance is the observation that the vitamin B_6 hydrochloride added to milk or milk preparation is more resistant to thermal sterilization than the vitamin B_6 naturally present in the milk. Loss of vitamin B_6 loss due to thermal sterilization of milk based preparation may thus be compensated by external addition of vitamin B_6 hydrochloride.

Check Your Progress Exercise 1

1. Fill in the blanks:

(i) Vitamin A is susceptible to the action of ----------

(ii) Vitamin A destruction can be prevented by----------

(iii) Vitamin A is used and sold as----------

(iv) Richest sources of Vitamin B, are ----------

(v) The most convenient way to prevent the risk of vitamin B, deficiency is ----------

(vi) Riboflavin is unstable in the presence of----------

(vii) Vitamin B_6 group comprises of ----------

2. Complete the following table by listing the application of the vitamins in food industry.

Sr.No.	Vitamins	Applications in food industry
1.	Vitamin A dry form	
2.	Vitamin A water-soluble	
3.	B_1	
4.	B_2	

5.2.2.4 Vitamin B_{12} (Cyanocobalamin)

Vitamin B_{12} refers to a group of Cobalt-containing corrinoids known as cobalamins. It is also called antipernicious- anemia factor, extrinsic factor. In human and animals, derivatives like hydroxycobalamin, adenosyl cobalamin and methyl cobalamin are the major compounds which are metabolically active. The latter two are the active co-enzymic forms. Cyanocobalamin is a synthetic form of Vitamin B_{12} that is widely used clinically due to its availability and stability which is transformed into active form in the body.

Importance

Vitamin B_{12} deficiency is manifest by the symptoms of pernicious anaemia (Addison-Biermer's disease). An irreversible atrophy of the gastric mucose is primarily responsible for pernicious anaemia, causing loss of the intrinsic factor, which is predominantly produced in stomach. Vitamin B_{12} from the ingested food is bound by intrinsic factor that reaches intestine for absorption. In addition, vitamin B_{12} acts as a mediator in the formation of labile methyl groups for the synthesis of methionine. This explains its lipotropic and protein saving effect. In various animal species, increased growth may be produced by administration of

vitamin B_{12}. Also, in children, the growth promoting activity of vitamin B_{12} has been observed.

Occurrence

Vitamin B_{12} is one of the cobalamin, a group of active principles widely occurring in nature. Vitamin B_{12} is present especially in liver, kidney, heart, brain, spleen, thymus and muscles. Larger amounts may also be detected in the intestine. In addition, considerable amounts are found in the culture media of various *Streptomyces* species and also in residue of streptomycin manufacture. Foods of plant origin are essentially devoid of Vitamin $B_{12.}$

Cyanocobalamin occurs as dark red crystals or as a crystalline hygroscopic powder of the same colour without odour and taste. Upon standing in the air, the anhydrous substance absorbs up to 12% water. Cyanocobalamin is soluble in water and 95% ethanol, but almost insoluble in ether, chloroform and petroleum ether. In the presence of alkalis and reactive alkaline compounds, reducing and oxidizing agents, aqueous solutions of cyanocobalamin are not stable. Cyanocobalamin substance and solution gradually decompose with discolouration by the action of day light and UV light. The activity of cyanocobalamin solutions may also be considerably affected by microbial growth. Cyanocobalamin should be kept in air tight containers protected against light and moisture in a cool place. To be protected against mold growth during storage.

Determination

Spectrophotometric determination of the characteristic ultraviolet absorption is suitable for the determination of pure vitamin B_{12} or of pure aqueous solution in the absence of interfering impurities. Microbiological assay is performed by any of the following:

- Determination of the diffusion in the cup-plate test using a mutant of *Escherichia coli* ATCC 10799 as the test organism
- Titrimetric determination of the acid produced in the tube test using *Lactobacillus lecihmannii* ATCC 7830.

Applications

Cyanocobalamin is used in the preparation of liquid and dry drug formulations of all kinds. In the animal feed industry, usually cobalamin concentrates are being used.

5.2.2.5 Biotin

Biotin forms long, colourless needles or a white crystalline powder. It is sparingly soluble in cold water, but more soluble in hot water and dilute alkalis. Biotin is sparingly soluble to insoluble in common organic solvents. Aqueous solutions of biotin are sensitive against the action of oxidants and UV light. In the presence of strong acids and concentrated alkalis, biotin is not stable. Biotin solutions are susceptible to mold growth.

Importance

Biotin is involved in a number of important metabolic reactions, probably as coenzymes. Deficiency symptoms are manifest as degenerative changes of the skin and pelt of various animal species such as rats, mice, chickens and pigs. In addition, degenerative processes of the muscles and various glands have been observed. Resistance against various infections is reduced.

Occurrence

Biotin occurs in nature very likely in all living cells, although usually in minute concentrations. In animal organs and in yeast, biotin is mainly contained in bound form. However, in vegetables, rice bran and in milk, it is present in free form.

Determination

Microbiological methods are suitable only for the detection of free biotin. We shall not go into the details of the method used for determination of biotin here since it is not within the perview of the unit.

Applications

Biotin is mainly used in the preparation of injection, and also in the manufacture of vitamin B complex and multi-vitamin preparations. It is used in the manufacture of baking yeasts.

5.2.2.6 Folic acid

Folic acid occurs as a yellow or orange-yellow, microcrystalline powder, almost without odour and taste. Water content of folic acid is up to 8.5%. Folic acid is very slightly soluble in water. Solubility increases with the temperature and the pH. Folic acid is sparingly soluble to insoluble in the usual organic solvents. The substance is without characteristic melting point, heating above 250°C will produce darkening and decomposition. Aqueous solutions of folic acid are not stable in the presence of reducing and oxidizing agents, heavy metal ions as well as sunlight and UV light.

Importance

In general, folic acid is known to be involved in a number of vital metabolic reactions.

Occurrence

Folic acid is an active principle widely occurring in the animal and vegetable kingdom. Richest sources are liver, dark green leafy vegetables, beans, wheat germ and yeast. Most dietary folates exist in the polyglutamate form which is converted in the small intestine to the monoglutamate form before absorption in the blood stream. Enzymes present in the liver catalyses the liberation of folic acid from these compounds. Bacteria, yeasts and molds contain abundant amounts of folic acid or its component 4-aminobenzoic acid; both these compounds stimulate their growth.

Determination

Reductive hydrolysis of folic acid produces 4-aminobenzoyl glutamic acid which is determined photometrically after diazotization and coupling with N-(1-naphthyl)-ethylenediamine.

In the microbiological assay, it is either by the turbidimetric growth measurement of *Streptococcus faecalis* ATCC 8043 or titrimetric determination of lactic acid produced by *Lactobacillus casei* ATCC 7469.

Applications

Folic acid is mainly used in the manufacture of dry drug formulations and also in the vitamin fortification of animal feeds.

5.2.2.7 Nicotinic acid (Niacin)

Niacin refers to both nicotinic acid and its amide derivative. Nicotinic acid occurs as white or almost white crystals or as a crystalline powder of the same colour without odour and is of weakly acid taste. Nicotinic acid is freely soluble in boiling water and sparingly soluble in water at about 20°C. Nicotinic acid is freely soluble with salt formation at low temperatures in acids and alkalis. The solubility of nicotinic acid in ethanol corresponds to that in water. Acetone and ether are also good solvents for nicotinic acid.

Importance

Nicotinic acid is successfully used in the therapy of a number of vascular disorders such as vascular spasms, angina pectoris, arthritis, hypertensive complaints, moreover as supportive therapy in migraine, headache and others. In the animals, Niacin is involved in reactions that generate energy from carbohydrate, fats and protein catabolites. It is essential for the synthesis of hormones.

Occurrence

Nictoinic acid occurs widely in the animal and vegetable kingdom in free as well as in bound form (in enzymes). Several animal organs such as liver, kidney and myocardium show a high nicotinic acid content. Yeasts and mushrooms are also very rich in nicotinic acid. In animals, it is predominantly found as nicotinamide.

Determination

The chemical methods of assay are based on colour reactions of pyridine. Nicotinic acid and nicotinamide are converted by cyanogen bromide into a derivative of the glutacone aldehyde, which is condensed with aromatic amines to form polymethine dyes (yellow colouring reactions).

The microbiological assay involves the titrimetric determination of lactic acid produced by *Lactobacillus arabinosus* ATCC 8014.

Applications

Nicotinic acid is mainly used in the vitamin fortification of flour, macaroni and noodle products. Independent of its vitamin efficacy, nicotinic acid is used in the meat industry, sometimes in combination with ascorbic acid to retain the colour in minced and unpickled meats.

5.2.2.8 Pantothenic acid (Calcium pantothenate)

Calcium pantothenate is a white, loose, faintly hygroscopic powder without odour and of bitter taste. It is easily soluble in water, glycerin, sparingly soluble in ethanol, acetone and ether; insoluble in chloroform and benzene. A 5% aqueous solution has a pH of 7.2-8.0. Pantothenic acid is stable under neutral conditions but is readily destroyed by heat in alkaline or acidic solution.

Importance

One of the active forms of pantothenic acid is coenzyme A, which is involved in numerous metabolic functions. It is involved in the synthesis of vital compounds like sterols, hormones, neurotransmitters, phospholipids, antibodies and in reactions that supply energy.

Occurrence

Pantothenic acid is a ubiquitous active principle occurring in free as well as in the bound form. In the bound form, pantothenic acid is incorporated into the coenzyme A which is an important compound that initiates several biological reactions in the living cells. Small amounts are synthesized in the intestines.

Determination

Hydrolysis of calcium pantothenate in acidic medium produces α,γ-dihydroxy-β,β-dimethylbutyrolactone, which reacts with hydroxylamine in the presence of alkalis to produce the corresponding hydroxamic acid. After addition of ferric chloride solution, the purple colour produced is measured at 500 nm.

The microbiological assay involves the titrimetric determination of lactic acid produced by *Lactobacillus arabinosus* ATCC 8014.

Applications

Calcium pantothenate is used in the preparation of drug formulations and the vitamin fortification of animal feeds.

Check Your Progress Exercise 2

1. Fill in the blanks:

(i) Vitamin B_{12} deficiency manifests as----------

(ii) Vitmain B_{12} is present in ----------

(iii) Deficiency symptoms of biotin are manifested as ----------

(iv) Biotin occurs as ----------in nature

(v) Folic acid is an active principle in ----------

(vi) ----------acts as a mediator in the methionine synthesis.

(vii) Nicotinic acid is used in the therapy of ----------

(viii) Nicotinic acid occurs in----------

(ix) The determination of nicotinic acid is based upon----------

(x) The active form of Pantothenic acid is which is involved in----------

2. Give the applications of the following vitamins:

a) Vitamin B_{12}

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b) Biotin

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c) Folic Acid

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d) Nicotinic Acid

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e) Pantothenic acid

5.2.3 Vitamin C (Ascorbic acid)

Vitamin C, ascorbic acid is also called anti-scorbutic vitamin. Ascorbic acid occurs as white crystals or white crystalline powder without odour and of citric acid like taste. Turns pale yellow on prolonged storage. Ascorbic acid is easily soluble in water, soluble in methanol, ethanol and glycerin and insoluble in ether, chloroform, benzene, petroleum ether, fats and fatty oils. The pH values of aqueous ascorbic acid solutions are:

1% (w/v) solution is about pH 2.8; 5% (w/v) solution is about pH 2.5; 10% (w/v) solution is about pH 2.4; 20% (w/v) solution is about pH 2.2

Ascorbic acid reacts like strong, monobasic acid. The action of atmospheric oxygen as well as exposure to daylight and UV light causes rapid destruction of ascorbic acid in aqueous solutions. Ascorbic acid is also destroyed by the action of oxidizing agents and heavy metal ions and in the presence of alkalies.

Importance

Vitamin C is involved in redox processes in the organisms. It is possible, that vitamin C exerts its physiological function as a redox substance in combination with an enzyme group. Vitamin C affects the formation of the mesenchymal tissues, bones, cartilage and teeth. Vitamin C is implicated in the metabolism of aromatic amino acids. Also vitamin C promotes the conversion of folic acid into its active form, the citrovorum factor. While most animals are capable of synthesizing ascorbic acid themselves, man, monkeys and guinea pigs depend on the supply of ascorbic acid with their food.

Occurrence

Vitamin C (ascorbic acid) is an active ingredient present in any animal or vegetable cell which occurs in the plant in free form and also bound to protein as ascorbigen. Vitamin C amounts contained in the various plant and animal tissues differ considerably. All fresh fruits, various vegetables and also milk are important nutritional physiological carriers of vitamin C. Considerable vitamin C is lost by cooking, preservation, drying and storage of the food commodities.

Determination

Majority of chemical methods of determination is based on the rapid oxidation of ascorbic acid and therefore not too highly specific. Titration of ascorbic acid with 2, 6-dichlorophenolindophenol is one of the commonest methods.

Applications

Ascorbic acid is used for the preparation of various drug formulations and for the vitamin fortification of foods and beverages.

5.2.4 Vitamin D (Calciferol – vitamin D_2)

The main forms are Vitamin D_2 (ergocalciferol-plant origin) and vitamin D_3 (cholecalciferol-animal origin). Vitamin D_2 forms colourless, acicular crystals or a crystalline powder without taste and of only faint odour. Vitamin D_2 is easily soluble in either chloroform or benzene, ethanol, acetone and fatty oils and insoluble in water. Vitamin D_2 is sensitive against atmospheric oxygen. It is not stable either in the presence of oxidizing substances and oxygen carriers. Moreover, vitamin D_2 is destroyed by acids, metal ions as well as UV and visible light.

Importance

Vitamin D is mainly involved in the calcium and phosphate metabolism. It usually promotes absorption of calcium and inorganic phosphate from the intestine. It participates in the transport of calcium and phosphate and also in the deposition of these mineral substances in the organic bone matrix.

Occurrence

Cholecalciferol is synthesized in the skin by the action of ultraviolet light on 7-dehydrocholesterol which is widely distributed in animal fat. Vitamin D is relatively stable in foods. Storage, processing and cooking have little effect on its activity although in fortified milk upto 40% of added vitamin may cost as a result of exposure to light.

Determination

Spectrophotometric determination of the orange yellow colour developed with antimony chloride in chloroform, with an absorption maximum at 500 nm.

Applications

Vitamin D_2 is mainly used in the preparation of various drug formulations. For the animal feed industry, a mineral stable dry powder under the name of vitamin D_2 is available. In many countries, milk and milk products, margarine and vegetable oils fortified with vitamin D serve as a major dietary source of vitamin.

5.2.5 Vitamin E (DL-α-Tocopherol)

DL-α-Tocopherol is a yellow to red-brown, clear, viscous oil almost without odour which decolourise when exposed to light and air. DL-α-Tocopherol is soluble in ethanol, acetone, ether, chloroform, petroleum ether and in fats and fatty oils. It is insoluble in water. By the action of oxidants (such as oils and fats containing peroxide, atmospheric oxygen) DL-α-Tocopherol will readily lose its potency. This process is accelerated by irradiation. Contact with heavy metals should be avoided, if possible (catalytic effect on oxidation processes). Light, heat and oxygen are detrimental factors which reduces the stability.

Importance

Vitamin E has a pronounced antioxidizing effect. Because of this property, vitamin E decreases the basic metabolism of the tissues or the oxygen consumption and protects readily oxidizable compounds like vitamin A and carotenoids in the intestine and the tissues against oxidation. In the same manner, vitamin E also protects the easily oxidable unsaturated fatty acids. It also promotes utilization of these essential fatty acids and prevents the formation of toxic lipoperoxides which have a detrimental effect on tissues.

Occurrence

The tocopherols are widely found in animal and vegetable materials, like nuts and seeds. Considerable amounts are found in a number of vegetable oils, wheat and corn germs.

Determination

DL-α-Tocopherol is oxidized with ferric chloride and the ferrous ions formed are measured colorimetrially with the aid of α,α'-dipyridyl (bright red colour).

Applications

DL-α-Tocopherol is mainly used as an antioxidant in stabilizing edible oils and fats and fat-containing food commodities, pharmaceutical preparations and cosmetics.

5.2.6 Vitamin K (Menadione – Vitamin K_3, oil soluble)

Menadione is a yellow, lustrous, crystalline powder. It is easily soluble in benzene, soluble in fats and fatty oils. It is sparingly soluble in ethanol and chloroform and very sparingly soluble in water. Melting point is between 105 and 108°C. Menadione is not stable in the presence of acid as well as of alkalis and substances with alkaline reaction and in the presence of reducing agents. Exposure to light and ultraviolet light will decompose menadione with brown discolouration.

Importance

The site of action of vitamin K activity is the highly complex mechanism of blood coagulation. Due to its effect on prothrombin, vitamin K is also involved in the immunological processes as prothrombin is a component of that complement the blood fibre of which is raised by administration of vitamin K.

Occurrence

Vitamin K is found naturally in plants and vitamin K2 is synthesized by the bacteria in the intestines in humans. In nature, only two naphthoquinone derivatives with antihaemorrhagic action – vitamins K_1 and K_2 have been found so far. In addition, there are a number of synthetic naphthoquinone compounds with vitamin K activity. Of these, especially vitamin K_3 (menadione) and some esters of vitamin K_4 (menadiol), such as 2-methyl-1,4-naphthohydroquinone dibutyrate are distinguished by valuable therapeutic properties. Other dietary sources include green leafy vegetables, cabbage, lettuce, soyabeans, beef liver and green tea. Egg yolk, milk, butter and cheese also contain vitamin K.

Determination

Colorimetric determination of menadione in oily solutions with dinitrophenyl hydrazine.

Applications

Menadione is used in the preparation of drug formulations and vitamin K supplementation of feed mixes and infant formula.

Check Your Progress Exercise 3

1. Fill in the blanks:

(i) Vitamin D_2 is sensitive against----------

(ii) The Vitamin D can be determined by----------

(iii) Vitamin E loses in efficacy by----------

(iv) Antioxidizing effect of vitamin E leads to----------

(v) The destruction of ascorbic acid is caused due to----------

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(vi) One of the common methods to determine ascorbic acid is----------

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(iv) The protein-bound form of vitamin C in plants and animal cells is ----------

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(v) The Vitamin K derivatives which have valuable therapeutic properties are-

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2. Give the roles of following vitamins in food industry:

a) Vitamin C

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b) Vitamin D

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c) Vitamin E

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d) Vitamin K

5.3 MINERALS

We are all familiar with the term minerals. Definition of minerals, as it applies to food and nutrition usually refers to the elements other than carbon, hydrogen, oxygen and nitrogen that are present in food. All foods contain minerals in relatively low concentrations. Minerals play key functional roles in health and nutrition of humans. Ninety chemical elements occur naturally in the earth's crust. About 25 of them are known to be essential to life and thus are present in living cells. Since our food is ultimately derived from living plants or animals, we can expect to find these 25 elements in our food. The minerals in foods are usually determined by ashing or incineration. This destroys the organic compounds and leaves the minerals behind. Minerals, as you may recall studying earlier, are mainly classified into two categories. What are these categories? The next sub-section presents a brief summary for you to read and refresh your memory.

5.3.1 Classification of Minerals

Minerals can be divided into two main categories, namely, main elements (calcium, potassium, phosphorous, chlorine, sodium, magnesium) and trace elements (iron, zinc, copper, manganese and iodine etc). According to their biological roles, they may also be divided into essential elements that have known

biological roles; non-essential elements with unknown functions; and toxic elements. There are 20 essential nutritive elements e.g. sodium, potassium, phosphorous, iron, calcium, chromium, zinc, selenium, magnesium, copper, tin, cobalt, manganese and fluorine. Non nutritive non toxic elements are boron and aluminium; and non nutritive, toxic elements are mercury, lead and cadmium.

What is the role of these substances? The nutritional and functional role of minerals is discussed herewith.

5.3.2 Nutritional and functional role of minerals in foods

Essential elements including the main elements and a number of trace elements fulfill various functions: as electrolytes, as enzyme constituents and as building materials in bones and teeth. In addition to their nutritional and physiological role, minerals contribute to food flavour. They activate or inhibit the enzyme catalyzed and other reactions. In some foods, minerals also affect the texture. Even though minerals are present in low concentrations, they often affect the physical and chemical properties of food because of their interactions with other food components. The functional role of few of the minerals are highlighted in tabular form herewith.

Table 5.2 Nutritional/Functional role of minerals

Minerals	Food Source	Nutritional Functional role
Aluminium	Low and variable in foods, component of some antacids and leavening agents	Possibly essential, evidence not conclusive, deficiency unknown. *Leavening agent:* As sodium aluminium sulfate (Na_2SO_4. $Al_2(SO_4)_3$) *Texture modifier*
Bromine	Brominated flour	Not known to be essential to humans. *Dough improver:* $KBrO_3$ improves baking quality of wheat flour. It is the most used dough improver.
Calcium	Dairy products, green	*Essential nutrient:* Deficiency leads to

	leafy vegetables, tofu, fish bones	osteoporosis in later life. Texture modifier: Forms gel with negatively charged macromolecules such as alginates, low-methoxy pectins, soy proteins, caseins, etc. firms canned vegetables when added to canning brine.
Copper	Organ meats, sea foods, nuts, seeds	*Essential nutrient:* Deficiency is rare. Catalyst: Lipid peroxidation, ascorbic acid oxidation, non enzymatic oxidative browning. *Colour modifier:* May cause black discoloration in canned, cured meats. *Enzyme cofactor:* Polyphenoloxidase. Texture stabilizer: Stabilizes egg white foams.
Iodine	Iodised salt, sea food, plants and animals grown in areas where soil iodine is not depleted.	*Essential nutrient:* Deficiency produces goiter and cretinism. *Dough improver:* KIO_3 improves baking quality of wheat flour.
Iron	Cereals, legumes, meat contamination from iron utensils and soil	*Essential nutrient:* Deficiency leads to anemia, impaired immune response, reduced worker productivity, impaired cognitive development in children. Excessive iron stores may increase risk of cancer and heart disease. Catalyst: Fe^{2+} and Fe^{3+} catalyse lipid peroxidation in foods. *Colour modifier:* Colour of fresh meat depends on valency of Fe in myoglobin and haemoglobin: Fe^{2+} is red, Fe^{3+} is brown. Forms green, blue or black complexes with poly phenolic compounds. Reacts with S^{2-} to form black FeS in canned foods. *Enzyme cofactor:* Lipoxygenase, cytochromes, ribonucleotide reductase, etc.
Magnesium	Whole grains, nuts, legumes, green leafy vegetables	*Essential nutrient:* Deficiency is rare *Colour modifier:* Removal of Mg from chlorophyll changes color from green to olive-brown
Manganese	Whole grains, fruits, vegetables	*Essential nutrient:* Deficiency extremely rare. *Enzyme cofactor:* pyruvate carboxylase, superoxide dismutase
Nickel	Plant foods	*Essential nutrient:* Deficiency in humans unknown. *Catalyst:* hydrogenation in vegetable oils – finely divided, elemental Ni is the most widely used catalyst for this process

Phosphates	Ubiquitous, animal products tend to be good sources	*Essential nutrient:* Deficiency is rare due to presence in virtually all foods. Acidulent: H_3PO_4 in soft drinks. *Leavening acid:* $Ca(HPO_4)_2$is a fast-acting leavening acid. *Moisture retention in meats:* Sodium tripolyphosphate improves moisture retention in cured meats. *Emulsification aid:* Phosphates are used to aid emulsification in comminuted meats and in processed cheeses.
Potassium	Fruits, vegetables, meats	*Essential nutrient:* Deficiency is rare. Salt substitute: KCl may be used as a salt substitute, may cause bitter flavour. *Leavening agent:* Potassium acid tartarate.
Selenium	Sea food, organ meats, cereals (levels vary depending on soil levels)	*Essential nutrient:* Keshan disease (endemic cardiomyopathy in China) was associated with selenium deficiency. Low selenium status may be associated with increased risk for cancer and heart disease. *Enzyme cofactor:* Glutathione peroxidase
Sodium	NaCl, MSG, other food additives, milk, low in most raw foods	*Essential nutrient:* Deficiency is rare; excessive intake may lead to hypertension. *Flavour modifier:* NaCl elicits the classic salty taste in foods. *Preservative:* NaCl may be used to lower water activity in foods. *Leavening agents:* Many leavening agents are sodium salts, e.g., sodium bicarbonate, sodium aluminium sulfate, sodium acid pyrophosphate.
Sulfur	Widely distributed	*Essential nutrient:* A constituent of the essential amino acids methionine and cystine. Sulfur amino acids may be limited in some diets. *Browning inhibitor:* Sulfur dioxide and sulfites inhibit both enzymatic and nonenzymatic browning. Widely used in dried fruits. *Antimicrobial:* Prevents, controls microbial growth. Widely used in wine making.
Zinc	Meats, cereals.	*Essential nutrient:* Deficiency produces loss of appetite, growth retardation, skin changes. ZnO is used in the *lining of cans* for proteinaceous foods to lessen

		formation of black FeS during heating. Zinc can be added to green beans to help stabilize the colour during canning.

From the table above, you would have got a fairly good idea about the functional role of minerals in foods in addition to their nutritional role in our body. Next, we will look at the bioavailability of minerals in foods.

5.3.3 Bioavailability of Minerals

It is well known that the concentration of a nutrient in a food is not a reliable indicator of its nutritional value. In other words, the entire quantity of the nutrient present in the food may not be utilized by the human body. *Bioavailability may be defined as the proportion of a nutrient in the ingested food that is available for utilization in metabolic processes*. In the case of mineral nutrients, bioavailability is determined primarily by the efficiency of absorption from the intestinal lumen into the blood. In some cases, the absorbed nutrient may be in a form that cannot be utilized. Bioavailability of mineral nutrients may vary from less than 1% for some forms of iron to greater than 90% for sodium and potassium. Chemical form of the mineral in food, formulation of chelates with minerals, redox activity of food components, mineral – mineral interactions and physiological state of the consumer are the factors that may influence the mineral bioavailability from foods. Processing the food can have an adverse effect on the minerals present in foods. What are these effects? We will dwell on this aspect in the next section, but first a word about how to estimate the mineral content in foods?

5.3.4 Estimation of minerals in foods

The minerals in foods are determined by ashing or incineration at temperatures in the region of 500°C following standard procedure. This destroys the organic compounds and leaves the minerals behind. Ash thus obtained provides an estimate of the total mineral content of foods. Minerals in the ash are in the form of metal oxides, sulphates, phosphates, nitrates and halides. Individual minerals are determined by dissolving the ash, usually in acid, and measuring the mineral concentration in the resulting solution. Atomic absorption spectroscopy is

generally used to estimate the mineral concentration. Nuclear Activation Analysis-NAA is a more sensitive method.

5.3.5 Effect of processing on mineral content of foods

Minerals are comparatively stable under processing conditions such as heat, light, use of oxidizing agents and extremes in pH. But, minerals can be removed from foods by leaching or physical separation. It has been reported that milling of cereals causes considerable mineral loss. Minerals are mainly concentrated in the bran layers and the germ. Thus, removal of bran and germ during milling leaves pure endosperm, which is mineral poor. For example, when wheat is milled to obtain refined flour, the losses in mineral content are 76% (iron); 78% (Zinc); 86% (manganese); 68% (copper); and 16% (selenium). Similar losses occur during milling of rice and other cereals. Cooking in water would result in some losses of minerals since many minerals have significant solubility in water. In general, boiling in water causes greater loss of minerals from vegetables as against steaming. Canned foods may take up metals from the container: tin and iron from the tin plate, and tin and lead from the solder. For example, this occurs in canned acid foods such as fruit juices; canned foods having sulphur containing amino acids.

Check Your Progress Exercise 4

1. Fill in the blanks:

(i) The term `Minerals' refers to the ----------

(ii) Bioavailability may be defined as----------

(iii) Ash contains minerals in the form of----------

(iv) ----------is used to estimate the mineral concentration

(v) Bioavailability of mineral nutrients varies from ----------to----------

2. Give the functional roles of essential elements in the food industry.

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3. Explain the effects of processing on mineral content.

5.4 LET US SUM UP

In this unit, you studied about different vitamin and minerals. Vitamins, as you would know, are of basically two types, fat-soluble (AD,E & K) and Water-soluble (B-complex and C) of these Vitamin B play a major role in the normal functioning and maintenance of biological processes. Both vitamin types are required for normal metabolism, growth and development and regulation of the cell function.

Next, we studied about different minerals and its classification. The nutritional and functional role of minerals in food was briefly discussed. Apart from these, certain other issues that were described included the concept of bioavailability, mineral estimation and processing effects on minerals in foods.

5.5 GLOSSARY

Acicular **:** narrow, long or pointed.

Amphoteric reaction **:** A double reaction possessed by certain fluids which have a combination of acid and alkaline properties.

Angina pectoris **:** A recurring pain or discomfort in the chest that happens when some part of the heart does not receive enough blood through narrowed, diseased coronary, arteries.

Antioxidants **:** A chemical compound or substance that is thought to protect body cells from damaging effects of oxidation.

Arthritis **:** An inflammation of a joint usually accompanied by pain, selling and stiffness, resulting from trauma infection, degenerative changes, metabolic disturbances or others

Atrophy **:** A wasting away from want of nourishment or to cause wasting due to disease of disuse

Bioavailability **:** This proportion of a nutrient in the ingested food that is available for utilization in metabolic processes.

Catalyst **:** A substance that increases/modifies the rate of a chemical reaction without being consumed in the reaction.

Chelates **:** To combine with a chemical compound to form a ring.

Cofactor **:** Inorganic ion or coenzyme necessary for the activity of an enzyme by forming a complex

Cornification **:** The conversion of squamous epithelial cells into a Keratinized horny material, such as hair, nails or feathers

Coupling **:** A connection between two things so they move together.

Decomposition **:** The act/ process of decay or dissolution consequent on the removal or alteration of some of the ingredients of a compound.

Esterification **:** A chemical reaction resulting in the formation of at least one ester product.

Flourescent **:** Emission of light during exposure to radiation from an external source.

Fortification **:** The addition of an ingredient for the purpose of increasing or improving the value of a product.

Hygroscopic **:** a substance that readily absorbs moisture, as from atmosphere.

Incineration **:** A treatment technology used to destroy waste by controlled burning at high temperatures.

Isoelectric point **:** The pH of a solution in which a protein has no net charge and does not migrate in an electric field.

Leavening agent **:** An organism or substance that when added to dough of flour and water causes it to rise by evolving carbon dioxide or other gases that become trapped as bubbles within the dough.

Mesenchymal tissues **:** Connective tissue, composed of star-shaped cells in an extracellular matrix.

Migraine **:** A vascular headache caused by blood flow and chemical changes in the brain leading to constriction of arteries supplying blood to the brain and release of certain brain chemicals, causing severe pain, stomach upset and other symptoms.

Miscible **:** Capable of being mixed; mixable

Oxidation **:** The act or process of oxidizing, or the state or result of being oxidized.

Pernicious anaemia **:** Condition caused by vitamin B_{12} deficiency and characterized by anaemia and spinal cord abnormalities, such as lesions of spinal cord, weakness, sore throat, numbness in the arms and legs, diarrhoea atc.

Prosthetic group **:** A tightly/covalently bound, specific non-polypeptide unit required for the biological function of some proteins

Redox Process **:** These are the electron transfer processes in which an oxidizing agent receives electrons and a reducing agent concedes electrons.

Stabilizer **:** A substance that renders or maintains a solution, mixture, suspension, or state resistant to chemical change.

Sterilization **:** The removal or destruction of all microorganisms, including pathogenic and other bacteria, vegetative forms and spores.

Thermostable **:** Unaffected by relatively high temperatures or capable of being heated to higher temperatures without loss of special properties.

Turbid **:** not clear; thick

Vascular Spasms **:** A sudden, brief tightening of a blood vessel. It can temporarily reduce blood flow to tissues supplied by that vessel.

5.7 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1.

(i) Atmospheric oxygen, oxygen carriers, oxidizing agents, metals ions and UV light.

(ii) Storage in cool place, under vaccum or inert gas in air-tight containers or sealed ampules.

(iii) Concentrates and oily solutions of the acetate and palmitate and their formulations

(iv) Yeasts, grain germs and rice polish

(v) Fortification of white flours with chemically pure vitamins of the B group.

(vi) Alkalis, materials giving alkaline reaction, strong reducing agents, metal ions and exposure to day light and UV light.

(vii) Pyridoxine, pyridoxal and pyridoxamine

2.

a) dry mixtures, coated tablets and capsules

b) drop solutions

c) drug formulations such as ampule solutions, drops and tablets

d) colour various food commodities such as baked foods, noodles and macaroni products, ice creams and soup mixes.

Check Your Progress Exercise 2

1.

(i) pernicious anemia

(ii) liver, spleen, thymus and muscles, intestine and streptomycin species

(iii) – degenerative changes of the skin and pelt of various glands.
- reduced resistance against various infections.

(iv) All living cells and animal organs and yeasts, vegetables rice bran and milk.

(v) animals and vegetable kingdom.

(vi) Vitamin B_{12}

(vii) Vascular disorders, migraine, headache, and others

(viii) Liver, kidney, myocardium, yeast and mushrooms

(ix) Colour reactions of pyridine

(x) Coenzyme A, metabolic functions

2.

(a) liquid and dry drug ascorbigen

(b) preparation of injections and manufacture of B-complex and multivitamin preparations

(c) dry drug formulations and animal feed

(d) vitamin fortification of flour macaroni and noodle products, retain colour of meat products

(e) drug formulations and vitamin fortification of animal feed.

Check Your Progress Exercise 3

1.

(i) atmospheric oxygen, oxidizing substances, oxygen carriers, acids, metal ions, daylight and UV light.

(ii) Spectrophotometry

(iii) Action of oxidants – oils and fats containing peroxide, atmospheric oxygen, irradiation, contact with heavy metals,

(iv) Decrease in basic metabolism of tissues or the oxygen consumption; protection or readily oxidizable vitamins in the intestines and tissues promotes utilization of essential fatty acids and prevents incidence of toxic lipoperoxides.

(v) Atmospheric oxygen, exposure to daylight and UV light, oxidizing agents, heavy metal ions and alkalis.

- Titration with 36 – dichlorophenolindophenol

2.

(a) drug formulations and vitamination of foods and beverages.

(b) various drug formulations.

(c) anti-oxidant in the food commodities and pharmaceutical industries.

(d) drug formulations and supplementation of feed mixes.

Check Your Progress Exercise 4

1.

(i) elements other than carbon, hydrogen, oxygen and nitrogen that are present in foods.

(ii) The proportion of a nutrient in the ingested food that is available for utilization in metabolic processes.

(iii) Metal oxides, sulphates, phosphates, nitrates and halides

(iv) Atomic absorption spectroscopy

(v) Less than 1% to greater than 90%.

2. Calcium: Texture modifier;
copper: catalyst, colour modifier, enzymes cofactor and texture stabilizer;
Iodine: dough improver;
Iron: Catalyst, colour modifier, Enzyme cofactor;
Magnesium: colour modifier;
Manganese: Enzyme Cofactor;
Nickel: Catalyst; Phosphates: acidulant, Leavening acid moisture retention in meals, emulsification aid; Potassium: salt
Sodium: Flavour modifier, preservative, leavening agent;
Sulphur: Browning inhibitor and anti-microbial agent;
Zinc: Browning Inhibitor and stabilizer

3. Minerals are comparatively stable under processing conditions such as heat, light use of oxidizing agents and extremes in pH, boiling and milling of cereals causes considerable mineral loss.