
UNIT 11 FOOD COMPONENTS OTHER THAN ESSENTIAL NUTRIENTS

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11.1 INTRODUCTION

In the previous units so far, we have read about the six major nutrients, which are essential for us. Though these vary in their requirements and roles, they have significance in our daily diets. Apart from these nutrients, there are certain health-promoting essential nutritional factors which have protective and preventive functions. In this unit, we will be studying about what are these and their beneficial, as well as, adverse health effects.

The idea that food can be health-promoting beyond nutritional value is gaining a wide acceptance. This broad view of nutrition has led to the concept of ‘functionality’. The American Dietetic Association (ADA) has taken the position that specific substance in food e.g. phytochemicals may benefit health when consumed as a part of a varied diet. Many common foods contain non-nutritive components that may provide protection against chronic diseases. It is believed that ‘nutraceuticals’ or ‘*functional foods*’ will help to provide an increased ‘health span’, that is, medical benefits, including the prevention and treatment of disease.

These substances are bioactive (extra-nutritional) constituents of foods which can act as ‘chemopreventers’ i.e. have anticarcinogenic and other beneficial properties evoking

physiological, behavioural and immunologic effects. This unit deals with such health promotive nutritional factors and bioactive constituents—their potential health implications and mechanisms of action to the extent that has been elucidated so far. While reading this unit you should bear in mind that despite animal research and some clinical trials, there is still uncertainty about the absolute safety and long-term benefits of supplementing the diet with some of these constituents. While functional foods in general have promoting properties, some of them may have an adverse effect on absorption or utilization of certain nutrients. We shall learn about these effects in this unit.

Objectives

After going through this unit, you will be able to:

- describe what are functional foods/nutraceuticals,
- classify the functional foods,
- discuss the potential health implications and mechanisms of action of functional foods, and
- explain the various adverse effects of these substances.

11.2 FUNCTIONAL FOODS

The term ‘functional food’ was born in Japan. Functional foods are actually products formulated with naturally occurring chemicals or a combination of these. They are found in many fruits, vegetables, grains, herbs and spices to provide a health benefit, lower the risk of certain diseases or affect a particular body process. To be precise, these are *the food substances, beyond basic nutrients that are designed to lower the risk or delay the onset of certain diseases*. The Japanese were the first to observe that food could have a role beyond nutrient supply. Thus, a functional food must be a food and not a drug. Beneficial effects should be obtained by consuming normal amounts that is within the parameters of a ‘normal’ diet.

In Japan, several functional foods are available in the market. It was the first country to legislate these products (*FOSHU stamp—Foods of Specified Health Use*). Europe and the American countries incorporated later the concept of an added value of food. There is no consensus between Europe and the USA regarding a concrete definition. Therefore, we have several different terms such as: *nutraceutical, designer food, vita foods, pharma food, medicinal foods, prescriptive foods, therapeutic foods, super foods, foodiceuticals and medifoods*. Nutraceutical is the preferred term in USA. European experts decided to adopt the term ‘functional food’ with a consensus definition. You may refer to Box 11.1 and Box 11.2 for a detailed European and Japanese perspective on functional foods.

Box 11.1	Functional Food in Europe: The Functional Food Science in Europe (FUFOSE, ILSI Europe, 1999)
	Functional foods are: • conventional/everyday foods consumed as a part of the normal diet; • composed of naturally-occurring components, sometimes in increased concentration or present in foods that would not normally supply them; • scientifically demonstrated to promote positive effects on target functions beyond basic nutrition; • thought to provide enhancement of the state of well-being and health in order to improve the quality of life and/or reduce the risk of disease; and • advertised by authorized claims.

Box 11.2 Japanese 'FOSHU' Criteria for Functional Food

The Japanese criteria for functional foods include:

- They are food (not capsules, pills/powder) on the basis of naturally occurring food components.
- They can and should be consumed as part of normal daily diet.
- They have a defined function on the human organism to:
 - improve immune function,
 - prevent specific disease,
 - support recovery from specific diseases,
 - control physical and psychic complaints, and
 - slow down the ageing process.

According to Japanese criteria, functional foods are not capsules, pills/powder, however, in some countries functional foods (prebiotic products) are marketed in the form of powder or liquid suspension. Now then having gone through the European and Japanese perspective on functional food, it must be coming to your mind that what is present in a food that makes it to be classified as a functional food. Let us find out.

So far, the most important components that have been identified and can be added to food are:

- **Probiotics:** A mono or mixed culture of living organisms, which when ingested in certain amounts, has a positive impact on host health, beyond conventional nutritional effects. You may recall reading about them earlier in the Food Microbiology and Safety Course (MFN-003) as well in Unit 1. These stimulate the growth of certain other bacteria in the colon, thereby improving health. Bacteria most often used as probiotics are *Lactobacilli* and *Bifidobacteria* which can be given along with the fermented foods e.g. yoghurt, fermented vegetables/meat.
- **Prebiotics:** Ingredients/compounds that have a beneficial effect on microflora in the large intestine of the host e.g. fibre, fructo oligosaccharides, lactulose, sugar alcohols. Generally, they are carbohydrates that may be fermented in the large bowel and stimulate growth of potentially beneficial bifidobacteria.

Several beneficial effects of functional foods have been reported, which include effects such as antioxidant, anticarcinogenic, blood glucose/lipid-lowering, regulation of intestinal transit, prebiotic effect, anti-bacterial, anti-viral and immunopotentiating. A reduced risk of several chronic diseases such as CVD, cancer, diabetes, hypertension, osteoporosis has also been reported.

A wide variety of functional foods are available in the market. Functional foods can be identified/selected on the basis of their properties, clinical significance or composition. Discussed below are some of the common methods employed for classifying functional foods.

11.2.1 Classification

Functional foods may be classified in various ways. From the nutritional viewpoint, they can be categorized as *nutrients* and *non-nutrients*. Have a look at the Table 11.1, which presents this classification.

Table 11.1: Classification of functional foods

Nutrients	Non-Nutrients
• <i>Lipids</i> n-3 fatty acids Conjugated linoleic acid • <i>Vitamins</i> Folates Vitamin E Carotenoids: β-carotene and α-carotene Vitamin C • <i>Minerals</i> Selenium	• <i>Fibre</i> Insoluble and soluble fibre • <i>Phenolic compounds</i> Phenolic acids Flavonoids Isoflavones Catechins Tannins • <i>Non-digestible Oligosaccharides (NDO)</i> Fructans Galacto oligosaccharides Isomalto oligosaccharides Xylo oligosaccharides Soy oligosaccharides • <i>Phytosterols</i> • <i>Glucosinolates</i> • <i>Carotenoids</i> Lutein Cryptoxanthine • <i>Lycopene</i> • <i>Organosulphur compounds</i>

Here we will not be discussing nutrients classified as functional foods, since these have already been dealt with in the earlier units of this course. We will only focus on the non-nutrient category. But first we shall look at the other classifications of functional foods.

Another classification is by the *target organ/system benefited*. This is of clinical relevance especially with regards to treatment and management of various diseases. Examples of functional foods, on the basis of their beneficial impact on different organ systems, are given in Table 11.2.

Table 11.2: Classification based on organ/organ system

Organ/Organ System	Food Component
Gastrointestinal tract	Prebiotics (NDOs), soluble fibre Insoluble fibre Probiotics Polyphenols Phytate n-3 fatty acids Micronutrients
Cardiovascular system	n-3 fatty acids Polyphenols Micronutrients
Immune system	Soluble fibre Prebiotics Probiotics Nutrients n-3 fatty acids Polyphenols
Skeletal system	Fructans
Kidney	Fructans

Besides classification based on organ/organ systems, another classification is based on *origin* or *source* i.e. plant, animal or microbial. Some compounds/substances included in each group are listed herein Table 11.3.

Table 11.3: Classification based on origin/source

Source	Components
Animal	Conjugated linolenic acid (dairy products)
	Chitosan
	Fish oils (ω L-fatty acids)
Microbial	Probiotics
Plant	Fibres (all components)
	Polyphenols
	Fructans
	n-3 fatty acids
	Phytates
	Carotenoids
	Non-glycerides in edible oils

Some foods are inherently functional and do not require much modification whereas others do. Foods may be made functional by:

- elimination of components e.g. toxins or allergenic proteins,
- increasing the concentration of a natural component e.g. fortification,
- addition of components with beneficial effects e.g. non-vitamin antioxidants,
- addition of beneficial microbes e.g. some yeasts, bacteria,
- replacement of a component, usually a macronutrient e.g. fat replaced with modified or emulsified carbohydrates, and
- enhancement of bioavailability of components.

Thus, a number of substances or components can be added to food to make it more appropriate for the treatment of a disease or management of health. Next, we move on to the study of functional foods. We will begin with the bioactive substances especially those present in protein-rich foods.

11.3 BIOACTIVE SUBSTANCES FROM PROTEIN FOODS

Bioactive substances are derived from living organisms that can be used by humans for a variety of applications. They are constituents in foods or dietary supplements, other than those needed to meet basic human nutritional needs and are responsible for changes in health status. Let us in this section learn about these as present in protein foods and their possible health effects.

The main function of dietary protein, as we know, is to supply the body with essential amino acids and organic nitrogen. However, proteins also supply bioactive peptides that are released by proteolysis *in vivo* (within the living body) and *in vitro* (in an artificial environment outside the living). Casein-derived peptides have found applications as dietary supplements and pharmaceutical preparations.

Peptides originating from food proteins should be considered as potential modulators of various regulatory processes in the body. A number of *in vitro* studies have shown anti-carcinogenic and anti-tumor activity for many of these in addition to the antimicrobial activity of the whey proteins.

Let us next get to know more about edible oils and fats and how they act as bioactive components.

11.4 NON-GLYCERIDES IN EDIBLE OILS

You may be aware of the fact that the glyceride fraction (an ester of glycerol and fatty acids that occurs naturally as fats and fatty oils) in edible oils and fats is about 90-98% and the remaining 2-10% is the non-glyceride fraction and that it contains a variety of components. These are: *Sterols, terpene alcohols, tocopherols, hydrocarbons, long chain alcohols including waxes, carotenoid pigments, sulfur- and nitrogen-containing flavour compounds*. They possess nutritional and physiologic functions. These components are present in the crude extract and are destroyed/reduced during the process of concentration or chemically modified during refining.

A brief review on these non-glyceride fractions follows:

- *Sterols*: These constitute a major proportion of the non-glyceride component while tocopherols, carotene pigments and flavour compounds are minor compounds.
- *Aliphatic alcohols*: These are water-insoluble, with their content varying from 0.5-7% of the unsaponifiable matter. Rice bran oil contains a mixture of ferulic acid, esters of sterols and triterpene alcohols, 'Oryzanol' which is considered to have a hypocholesterolemic effect.
- *Terpene alcohols*: Partly free, partly esterified e.g. ferulic acid; these are found in significant amounts in rice bran oil, wheat germ oil, soybean oil, linseed and olive oil. Commonly used edible oils e.g. groundnut, sesame, safflower, corn, coconut, sunflower and palm oils contain only ~10-20% of the amount found in rice bran oil. A hypocholesterolemic effect has been seen in animals and humans. Blends of rice bran oil and safflower oil have been shown to reduce cholesterol and LDL aside from the effects of PUFA and MUFA.

So far we have read about functional foods, bioactive substances present in protein foods and the non-glyceride fractions in edible oils which have been found to have health promotive properties. We shall learn about some other food components in our subsequent discussions. However, before we proceed, it would be a good exercise to recapitulate about the concepts learnt so far by answering the questions mentioned in the check your progress exercise 1.

Check Your Progress Exercise 1

- 1) What are functional foods? Where are these found?

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- 2) What are the functions of functional foods, as per the Japanese 'FOSHU' criteria?

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3) List the important components that can be added to food.

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4) Enumerate:

a) Any five ‘non-nutrient functional foods’.

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b) The food components for benefiting the immune system.

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c) Any three plant-based functional foods.

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5) What are the different ways by which foods can be made functional?

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6) What are the major components of non-glyceride fraction in edible oils and fats?

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Let us now move to the next section on probiotics and prebiotics. We have earlier briefly discussed about these. Let us now get to know about these in detail.

11.5 PROBIOTICS AND PREBIOTICS

We all have read or heard about *probiotics* and *prebiotics* at some point of time. However, what exactly do we mean by these terms? When were they identified? What are the benefits of these components and which foods/food products would contain them? These are a few questions which shall be answered through the discussions to be followed next.

Well, during the initial stages, research and development of functional foods was confined to mostly those to which certain components such as micronutrients were added. However, one of the most promising current targets for functional food development is the gastrointestinal tract (GIT). The human large intestine is inhabited

by very large numbers of microorganisms. The resident microflora through their metabolic activities exerts important physiological action relevant to health and disease. This and other possibly beneficial effects to other target organs have led to an intensive study of probiotics and prebiotics.

We shall now begin our study about pro and prebiotics by first defining these terms.

11.5.1 Definition and Characteristics

An early definition of *probiotics* is ‘organisms and substances which contribute to intestinal microbial balance’. Later the word ‘substances’ was removed since these could include antibiotics and microbial stimulants that are categorized as prebiotics. The revised definition is *a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance*. It has been suggested that the definition should be expanded to include other areas of the body e.g. skin, vagina and respiratory tract. Thus, the beneficial effect of probiotics can be mediated through the gut microflora by ingesting viable microorganisms. According to the definition, probiotics include not only preparations but also traditional yoghurts and other fermented foods.

Prebiotics, on the other hand, are *non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon*.

Let us now move on to the understanding of the characteristics of probiotics and prebiotics.

Characteristics of Probiotics and Prebiotics

The characteristics of probiotics and prebiotics are highlighted in Table 11.4.

Table 11.4 : Characteristics of probiotics and prebiotics

Probiotics	Prebiotics
Are microorganisms	Are non-digestible dietary/food ingredients
Promote a healthy intestinal microflora	Are not hydrolyzed/absorbed in the upper GIT, reach the large intestine in an intact form and have a specific metabolism therein – directed towards beneficial/advantageous bacteria than those which are adverse/detrimental for host health
Ensure colonization resistance to pathogens	Fermented in colon
Destruction of genotoxins and mutagens	Alter composition of colonic microbiota towards a healthier community
Immune system function	May induce systemic effects beneficial to host

Next, we shall study about the mode of action, usage in foods and resistance properties of probiotics.

11.5.2 Probiotics: Dietary Sources and their Mode of Action/Effects

We can have specific preparations designed for probiotic use, as well as, foods. Probiotic products which are marketed are in the form of powders, tablets and liquid suspensions. Among the various organisms, *lactic acid* bacteria, *streptococci* and *bifidobacteria* are commonly used.

It is not clear how probiotics influence the flora and produce a beneficial effect. However, colonization of the gut appears to be a prerequisite for the probiotic effect. Important relevant features may be:

- resistance to low pH and bile acids, and
- ability to adhere to intestinal epithelium.

However, ability to adhere to intestinal epithelium does not ensure that an organism will permanently colonize the gut. Also, there is a continuous interchange of species with one strain being replaced by another, which is possibly better suited. Since some strains are more suitable than others. Organisms such as *Saccharomyces boulardii* are effective although they do not grow in the gut. For such organisms, continuous administration is required to ensure the presence of large numbers of metabolizing cells in the GIT.

As a dietitian, you must be now curious to know which foods contain probiotics. Let us read further and find out.

Probiotics in Foods

Yoghurts have been supplemented with probiotic strains of *bifidobacteria* and *lactobacilli*. Milks fermented solely by intestinal isolates of lactic acid bacteria have also been developed. Other fermented milk products like cottage cheese, sour cream etc. contain viable organisms and may have an incidental probiotic effect.

We will now examine the health effects of probiotics.

Health Effects related to Probiotics:

Several lines of evidence support the conclusion that normal gut microflora are involved in *resistance to disease*, especially gastrointestinal infections. These are:

- germ-free animals are more susceptible to infection than animals having complete gut flora,
- orally administered antibiotics increase susceptibility of animals to infection (antibiotics are known to destroy gut microflora), and
- administration of enemas of faecal suspensions from a healthy adult can control antibiotic-associated diarrhoea (AAD).

Let us briefly examine the effects that have been reported (remember most of it has been on animals):

- In animals, probiotics may control tumour production.
- In humans, probiotics can reduce incidence of intestinal infections. The possible mechanism of action is: the probiotic has some antagonistic effect (direct/indirect) on the pathogen: either through direct chemical antagonism, competition for nutrients or an indirect effect via the immune system by competition for receptors on the epithelial surface.
- 50% reduction in AAD observed in patients given *Saccharomyces boulardii*.
- Significant reduction in diarrhoea incidence among children aged 4-45 months after administration of *L.casei* GG. The effect was more pronounced in patients with confirmed rotavirus infection.
- Can have an antifungal effect. During chemotherapy for leukemia. Treatment

with a milk preparation containing *L. acidophilus* and *Bifidobacterium sp.* markedly reduced the faecal count of *Candida*.

Now that we have understood the benefits and usage of probiotics, let us look at the details of prebiotics in the next sub-section.

11.5.3 Prebiotics: Dietary Sources and their Mode of Action/Health Effects

We have already seen how prebiotics are defined. Let us go a little in-depth about them. Prebiotic fermentation should favour growth of potentially health promoting bacteria especially *Lactobacilli* and *Bifidobacteria* which are indigenous to the gastrointestinal tract. In a way, the approach is similar to that of dietary fibre or resistant starch. However, prebiotics will affect bacterial growth selectively. Also, efficient prebiotics will reduce/suppress the numbers and activities of possibly pathogenic microorganisms.

Since prebiotics are fermented by the gut microflora, they may have physiological effects on the GIT such as:

- control of transit time and motility,
- regulation of epithelial cell proliferation,
- influence on nutrient bioavailability,
- modulation of immune function, and
- modulation of endocrine function.

In addition, they may exert systemic effect by influencing carbohydrate or lipid homeostasis.

Our subsequent discussion would be pertaining to some important types of prebiotics such as Non-digestible oligosaccharides. Let us read about them.

Non-Digestible Oligosaccharides (NDO)

Among the various food components, the best prebiotic effects seem to be exerted by the NDOs. They are *oligomeric carbohydrates, which are resistant to hydrolysis by intestinal digestive enzymes but NDOs can be metabolized by colonic bacteria*. What will be the products of bacterial fermentation? Well, these are:

- short chain carboxylic acids (short chain fatty acids),
- gases, and
- organisms also increase metabolic energy, growth and proliferation.

Generally, these carbohydrates are a mixture of oligomers of differing chain lengths. Oligosaccharides with prebiotic effect are:

- fructooligosaccharides (FOS) or inulin-type fructans
- soybean oligosaccharides e.g. stachyose and raffinose
- galactooligosaccharides
- maltooligosaccharides
- galactosylsucrose
- palatinose condensates
- xylooligosaccharides

Many prebiotic oligosaccharides are being used in Japan. Among these, FOS have been the most studied. We will now highlight some interesting features of fructo-oligosaccharides or fructans.

Fructans

It is a general term for any carbohydrate in which one/more fructosyl fructose link constitutes the majority of glycosidic bonds. They are linear or branched fructose oligopolymers i.e. β -2,1 fructosyl fructose linked inulins or β -2,6 linked *levans*. Inulins are mainly of plant origin, containing 2-70 units, whereas levans are mostly produced by some fungi and many bacteria. Fungal/bacterial inulins have a much higher degree of polymerisation (DP) (upto 150). Oligofructose (DP 2-10) is produced by enzymatic hydrolysis of inulin. Thus, as food ingredients, they are available either as native inulin or high molecular weight inulin, or the enzymatically produced hydrolysate oligofructose.

Synthetic fructans are mainly β -2,1 linked fructose with DP 2-4, industrially obtained by enzymatic synthesis from sucrose. Inulin type fructans are present in significant amounts in a variety of edible fruits and vegetables e.g. plant families such as *Lilliaceae*, *Amaryllidiaceae*, *Graminae*, *Compositae* e.g. asparagus, artichoke, garlic, leek onion, banana, chicory roots.

Fructans can be ingested by consuming these foods, e.g. in cereals, aerial parts of many *Graminae* particularly young seedlings contain upto 70% of their dry weight. Estimated daily intake in Western countries is 3-11g/day, depending on the type of food consumed.

Because of their β configuration, inulin-type fructans are resistant to human digestive enzymes, and in the ileum due to microbial activity and/or enzymatic hydrolysis, with the remaining 86-88% of the ingested fructans being left undigested. Isomaltooligosaccharides are partially hydrolyzed by isomaltase in the jejunum whereas the soybean oligosaccharides raffinose, stachyose and palatinose are not hydrolyzed much. Galactooligosaccharides appear to be metabolized.

The functional/technological attributes that give them a potential for incorporation into foods and the health benefits of fructans are enumerated next.

A) Health Benefits of Fructans

The health benefits of fructans include:

- **Bifidogenic Effect:** Fructans selectively stimulate the growth of *Bifidobacteria* and *Lactobacilli*, while decreasing concentrations of *E.coli*, *Clostridia* and bacteroides. Predominance of these bacteria is achieved within two weeks, with effects lasting as long as the fructans are consumed. Daily doses of 4-40 g increased the bifidobacteria upto $10^{9.5}$ per gm of faeces.

But what are the benefits of increasing the growth of bifidobacteria?

Bifidobacteria displaces potential pathogens selectively, showing an antibiotic like effect, which is unrelated to the changes in short chain fatty acids (SCFA) and pH. Due to fermentation, the carboxylic acids produced—acetate, propionate, butyrate—have both systemic and physiological effects. Also, these end products of fermentation allow the host to salvage a part of the energy of NDO and may play a role in regulating cellular metabolism, cell division and cell differentiation. Look at Table 11.5, which presents the physiological effects of bacterial growth and acid produced.

Table 11.5 : Physiological effects of fermentation

Acid Causes:	Bacterial Growth Causes:
• decrease in faecal pH • hyperplasia of intestinal mucosa • increased wall thickness in small intestine and cecum • increased blood flow in the tissue • the lowered pH in a micro niche makes it unfavourable for pathogens	• increased bacterial biomass • increased faecal mass • competition effects towards colonization sites and available nutrients • excrete natural anti-microbial agents which can affect a variety of organisms • may themselves function as anti-infective agents through occupation of pathogen colonization or receptor sites

Thus, prebiotics help to attain an appropriate balance of microflora and help in resisting the effects exerted by the various pathogens.

- *Mineral absorption*: NDOs affect mainly calcium/magnesium absorption and balance. Acidification of colonic contents increases the concentration of ionized minerals, particularly calcium and magnesium, thus favouring passive diffusion. Formation of soluble salts of organic acids and colonic hypertrophy facilitates increased absorption of these minerals. Similar effects have been observed in animals (but not yet in humans) for iron and zinc.
- *Glycemia/Insulinemia*: Preliminary research evidence indicates that there may be beneficial effects in terms of decreased fasting blood glucose/hepatic glucose production. This may be due to delayed gastric emptying, increased transit time, which may be dose-dependent. Alternatively, hepatic metabolism of glucose may be modified, mediated by the SCFA, especially propionate. Propionate inhibits gluconeogenesis probably via its conversion to methylmalonyl CoA and succinyl CoA, both of which inhibit pyruvate carboxylase. Propionate enhances glycolysis and may lower plasma fatty acid concentrations.
- *Lipid Metabolism*: Animal studies consistently show a hypotriglyceridemic effect although equivocal results have been seen with healthy humans. Hypotriglyceridemia is due to decreased VLDL concentrations. Animal studies show a decreased *de novo* lipogenesis in the liver due to reduced activity (almost 50%) of all lipogenic enzymes, possibly through modification of lipogenic enzyme gene expression. Inhibition is mainly attributable to propionate. Limited studies show possible lowering of serum total and LDL cholesterol.
- *Uremia and Nitrogen Disposal*: In animals, faecal and renal nitrogen were enhanced and decreased uremia was seen in normal and nephrectomised animals. The mechanisms proposed are:
 - increased colonic biomass and consequent nitrogen fixation with acidification and conversion of diffusible ammonia into less diffusible ammonium ion,
 - due to NDO's osmotic effect, urea transfers into distal ileum and large intestine is accelerated. In the large intestine, ureolytic microflora may act. When fermentable carbohydrate intake is high, the amount of ammonia required may become insufficient and blood urea is used as a substrate, and
 - inhibition of ureagenesis in the liver by propionate.

Once again it should be emphasized here that these are inferences drawn from animal studies and verification in humans will have to wait until relevant studies are carried out.

Enumerated below are the potential uses of fructans.

B) *Fructans as Food Ingredients*

Oligosaccharides have several functional/technological attributes that give them a potential for incorporation into foods. They can:

- modify freezing point and moisture content,
- have variable stability to acid, on storage and processing,
- have bacteriostatic properties,
- may stabilize proteins,
- retain flavour, aroma,
- affect colour formation,
- variable sweetening power, thus variable energy density and carcinogenicity,
- can be used as fat replacers since they confer mouth feel and texture similar to fat, and
- can be used as bulking agents.

Thus, to summarize, we can say that a good prebiotic should have the following properties:

- be active at a nutritionally feasible, as low a dose as possible,
- lack side effects,
- be able to exert fine control of microflora modulation,
- persist throughout the colon including distal areas,
- have varying viscosity,
- have good storage/processing stability,
- have differing sweetness, and
- inhibit adhesion of pathogens.

In this section, we learnt about pro- and prebiotics. The discussions highlighted the different types of these substances, their properties and uses in the food industry. It must have been interesting to learn about non-digestible oligosaccharides particularly, fructans. We shall continue to learn further about some more bioactive non-nutritional components such as polyphenols. However, let us first revise our concepts by performing the following exercise given in check your progress exercise 2.

Check Your Progress Exercise 2

1) Define the following terms, giving suitable examples.

a) Probiotics

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b) Prebiotics

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c) NDOs

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2) Enlist the characteristics of Probiotics and Prebiotics.

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3) What is the possible mechanism by which probiotics act?

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4) Describe any two physiological effects of prebiotics and probiotics.

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5) Discuss the benefits attributed to the intake of NDOs.

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6) What characteristics of Fructans give them a potential for incorporation into foods?

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11.6 POLYPHENOLS

We now come to a group of compounds, which were until recently considered to adversely influence nutrient absorption or giving colour to foods e.g. anthocyanins. Today they are among the most popular and commonly consumed nutraceuticals. More than 4000 phenolic phytochemicals have been identified so far including tocopherols and tocotrienols. They are ubiquitous in all plant organs and therefore are an integral part of the human diet. Let us find out what are these.

11.6.1 Definition and Classification

Polyphenols are *the compounds that contain an –OH group attached to the benzene ring*. The main classes are: flavonoids, phenolic acids and polyphenols (tannins). They arise from common intermediates phenylalanine or its precursor shikimic acid. Natural phenolic compounds range from simple ones like hydroxybenzoic acid to highly polymerized ones e.g. tannins. They are glycosylated by one or more sugar residues. The OH groups linked can be glucose, galactose, rhamnose, xylose or arabinose, glucuronic or galacturonic acids.

The various classes are:

- a) Phenolic acids and derivatives
- b) Flavonoids such as Flavonols and Flavones, Isoflavones and Flavanols
- c) Tannins
- d) Stilbenes
- e) Lignans

We will now describe each of these one by one.

A) *Phenolic Acids and Derivatives*

Two families of phenolic acids are widely distributed in plants: a range of benzoic acid derivatives and those derived from cinnamic acid, both occurring in a conjugated or an esterified form. Examples are:

- *Hydroxybenzoic acid*: e.g. ellagic and gallic acids, which are hydrolyzable tannins, present in berries and nuts.
- *Hydroxycinnamic acid*: e.g. caffeic and ferulic acids, which are heat-sensitive. Caffeic acid is a precursor of lignin. Caffeic with quinic acid gives chlorogenic acid. They occur in fruits, vegetables notably seeds, coffee beans, grains, sunflower seeds. Among these, curcumin and chlorogenic acid have been studied more. Anticarcinogenic effects are attributed to caffeic and ferulic acid. They may act in two ways:
 - a) prevent formation of carcinogens from precursors, and
 - b) block reaction of carcinogens with critical cellular macromolecules.

Next, let us get to know about the flavonoids.

B) *Flavonoids*

Flavonoids constitute the largest group of plant polyphenols, generally are the compounds of low molecular weight, bound to sugar molecules. You may be familiar with some of these plant pigments. *Anthocyanins* (red, blue and purple pigments) are flavonoids. We also have *anthoxanthins*, which include flavonols, flavones, flavanols and isoflavones. All of these are colourless/white/yellow. We will look at each briefly:

- *Flavonols and Flavones*: These are the most widely distributed among the flavonoids. The most commonly occurring are *Quercetin*, *Myricetin* and *Kaempferol*. Quercetin is quantitatively the most important and is present in onions, apples, kale and tea. It is concentrated in the outer exposed parts of plants and its concentration is proportional to the greenness. Losses vary; average cooking methods result in less than 20% losses whereas other processing methods lead up to 50% losses.

- **Flavanols:** Examples are *catechin* and *epicatechin*. You may have heard of the benefits of tea. Some of them are due to gallic acid, which is combined with epicatechin. Flavanols constitute 30% of dry matter from tea solutions. In fruits, legumes and grains, they exist as condensed polymers and are concentrated more in immature fruits. Fruits stored over winter lost approximately half their content.
- **Isoflavones:** These are usually treated separately from the other five subclasses and are an area of considerable research interest. Isoflavones are found almost exclusively in the legume family but occur in high amounts only in soybeans. *Genistein* and *Daidzein* are found in soybeans. They are heat stable, slightly water-soluble. The content in dry soybeans ranges from 1600-2400 mg/kg.

The third polyphenol covered in this section are tannins.

C) **Tannins**

Long known for their inhibitory effects on iron absorption, recent research indicates that tannins do have beneficial effects. Tannins are compounds of high molecular weight. In fact, the astringent taste that you find in some foods is due to the reaction of tannins with mouth proteins. Figure 11.1 highlights some of their characteristic features.

Tannins are



Condensed	Hydrolyzable
• polymers of catechins/epicatechins • structurally more complex than hydrolyzable tannins • referred to as 'Leucoanthocyanidins' because they form cyanidin upon acid hydrolysis • found mainly in fruits, grains, legumes • usually accumulate in outer layers of plants • removed by dehulling or heating in water • not degraded by acid treatment, but polymerize	• contain a central core of polyhydric alcohol e.g. glucose or hydroxyl groups • polymers (esterified partially or wholly) of gallic/ellagic acid • found in berries/nuts • can be easily hydrolyzed by acids, bases or certain enzymes

Figure 11.1: Characteristics of different forms of tannins

D) **Stilbenes**

Stilbenes contain 3 phenyl compounds connected by a 2-carbon methylene bridge and occur in nature in restricted distribution. In plants, they act as anti-fungal phytoalexins, usually synthesized only in response to infection/injury. The most extensively studied is trans-resveratrol, present in grapes, wine and peanuts. Red wine contains 1.5-3.0 mg/L.

Finally, let us get to know the lignans.

E) **Lignans**

Lignans are diphenolic compounds formed by dimerization of 2 cinnamic acid residues. Most lignans apparently pass through the GIT as fibre. Some lignans may be converted by intestinal microflora to mammalian lignans-enterodiol and enterolactone, which are absorbed through enterohepatic circulation. Flaxseed meal and flour are said to be the richest sources of 2 lignans- *Secoisolariciresinol diglycoside (SDG)* and *matairesinol*. Other good sources of lignans are pumpkin seeds and sunflower seeds.

Next, we shall read about the absorption/bioavailability of polyphenols.

11.6.2 Bioavailability of Polyphenols

What do you mean by the term 'bioavailability'? You would recall reading about it earlier in this course. We suggest you to go back and refer to its definition in Unit 7 and 8 and then proceed further.

We need to consider bioavailability before studying the functions because their nutritional significance depends on their behaviour in the digestive tract. Some of the salient aspects related to bioavailability have been elucidated herewith:

- i) their digestion and utilization depends on their structure, degree of glycosylation/acylation, conjugation with other phenolics, molecular size and stability,
- ii) *in vitro* studies and animal experiments show that upto 1/3rd of extractable polyphenols are excreted, however, the percentage of non-extractable polyphenols excreted are twice or three times more,
- iii) some phenolics like aglycones, simple phenols and flavonoids are directly absorbed into the small intestines. Glycosylated polyphenols are hydrolyzed by glycosidases present in food/produced by the GIT mucosa or they are acted upon by microflora, after which they are absorbed in the colon. Intestinal bacteria have been found to solubilize non-extractable polyphenols, and
- iv) absorption in rats is low (20-34%). In humans, the absorption is apparently influenced by the chemical nature, thus the extent varies from as much as 24-52% for Quercetin, 9-21% for isoflavones to as little as 0.2-0.9% for tea catechin.

Human studies on bioavailability indicate that plasma levels of flavonoids are close to 0.55 $\mu\text{mol/L}$ but do not exceed 1 $\mu\text{mol/L}$ when the dose was close to dietary intakes.

With a brief understanding about the bioavailability of polyphenols, next we shall study the influence of polyphenols on macronutrients and minerals.

11.6.3 Influence of Polyphenols on Macronutrients and Minerals

Let us study in this sub-section how the polyphenols affect the digestion and absorption of various macronutrients such as proteins, carbohydrates, lipids and minerals.

A) *Proteins*

You know that tannins are considered as anti-nutritional because their presence is usually accompanied by a reduced protein digestibility and a subsequent increase in the faecal nitrogen. Decreased protein digestibility may be due to the formation of 'protein-tannin complex', as well as, inhibition of proteolytic enzymes like trypsin by polyphenols. Polyphenols have exhibited a highly significant and negative correlation with *in vitro* digestibility of proteins.

However, this may have a beneficial effect in overweight or obesity, since tannins bind to endogenous proteins in the intestinal tract such as digestive enzymes like lipase and amylase and inhibit them, resulting in lower energy availability.

B) *Carbohydrates*

Binding of proteins may indirectly affect carbohydrate absorption. Inhibition of amylolytic enzymes and subsequent reduction of dietary carbohydrate hydrolysis can decrease the postprandial glycemic response, which can have a beneficial effect for diabetics.

C) *Lipids*

The effect of food polyphenols on lipid metabolism has not been extensively studied. Both soluble polyphenols and condensed tannins have been shown to decrease the apparent digestibility of lipids, lipase activity and increase faecal fat excretion.

D) *Bioavailability of Minerals*

Polyphenols can form complexes with metal cations through their carboxylic or hydroxylic groups, and thus interfere with the intestinal absorption of minerals like iron, copper, sodium and aluminum. The important negative interaction in the Indian setting is the iron absorption vs. polyphenols, as Indian diets are deficient in iron.

In view of the impact of polyphenols on the metabolism of several macro and micro nutrients, research has been carried out to explore their potential in the treatment, management and prevention of various diseases. Enumerated next are some of their health benefits.

11.6.4 Health Benefits of Polyphenols

Various health benefits have been attributed to polyphenols. These include:

- anti-microbial
- anti-viral
- anti-oxidant
- hypotensive
- hypoglycemic
- anti-carcinogenic
- anti-mutagenic
- oestrogenic
- anti-ulcer
- anti-inflammatory
- prevent expression of adhesion molecule
- inhibit replication of HIV

Most of these properties (health benefits) are due to their anti-oxidant activity. Both in vitro and in vivo studies have been conducted to elucidate these effects. Let us read further to understand the various properties of polyphenols in detail.

A) *Antioxidant Action of Polyphenols*

The properties of polyphenols due to which they have been identified to act as antioxidant is because they:

- act as terminators of free radicals by rapid donation of H atoms,
- chelate metal ions like iron and copper,
- act as terminators of propagation,
- quench reactive oxygen species and reactive N₂ species,
- inhibit lipoxygenase and cyclo-oxygenase enzymes, and
- inhibit damage by haemprotein/peroxide mixtures.

The antioxidant function depends on the number of hydroxyl groups and their positions. Among the various derivatives, flavonoids are the most potent and retain their scavenging capacity even after chelation with the metal ions. Non-extractable polyphenols are apparently 15-30 times more effective than simple phenols. A point to note is that their ability to chelate metals also makes them act as pro-oxidants-in situation when tissue injury causes release of iron/copper.

The most important antioxidant role of polyphenols identified so far has been with respect to the treatment, management and prevention of several degenerative diseases particularly, heart diseases and cancer. We will read about these in detail.

B) Cardiovascular Effects

You must have heard of the 'French Paradox'. The lower incidence of heart attacks in certain areas of France despite higher prevalence of factors like smoking and high fat intake was attributed to the red wine consumption in these areas. Subsequently, the 'Zutphen study' in the Netherlands showed an inverse relation between the incidence of CHD/ stroke in elderly men with the dietary intake of flavonoids especially quercetin, which originated mainly from tea, fruits and vegetables. Average total daily consumption of flavonoids was at least 23 mg (16 mg quercetin/day) and the average vitamin E intake was 7-10 mg.

Phenols have been found to consistently decrease several risk factors/CVD indicators – plasma LDLc was less susceptible to oxidation, contained lower amount of lipid peroxides. There was lower plaque formation in spontaneously hypertensive rats, consumption of wine polyphenolic extracts improved aortic blood pressure and vessel mechanical properties.

Next, we move over to the prevention and inhibition of cancer.

C) Cancer and Inhibition of Tumorigenesis

Polyphenols appear to play a preventive role although the molecular mechanisms of action and applicability to human cancer prevention are unclear. Various studies appear to suggest that they may be effective when given in the post initiation period i.e. they are more helpful in inhibiting tumor promotion and progression.

Most studies have been conducted either in vitro or in vivo in animals. Hence, we must be cautious in extrapolating these observations to humans. The observations from studies are summarized as:

- i) caffeic acid, ferulic acid, chlorogenic acid and curcumin inhibited tumor promotion –curcumin inhibited colon tumorigenesis.
- ii) multiplicity of invasive and non-invasive adenocarcinoma was lower.
- iii) apoptosis of colonic tumor cells was higher.
- iv) expression of oncoprotein was decreased.
- v) in mammary tumors, these compounds cause inhibition directly or by modulating oestrogen action.

These effects have been observed with tea catechins and related compounds, caffeine, quercetin and other flavonoids, isoflavones and lignans, resveratrol and other grape constituents. How do they confer beneficial effects? Well, this is through the following mechanisms:

- inhibit activation of carcinogens,
- facilitate elimination of certain carcinogens or their reactive intermediates,
- inhibit the metabolism of arachidonic acid–decreased production of pro-inflammatory or mitogenic metabolites like certain prostaglandins or reactive oxygen species,
- modulate hormone-dependent carcinogenesis,
- modulate different oncogenes, suppressor genes, signal transduction pathways. Thus, they inhibit cell proliferation, transformation and induce apoptosis, and
- promote terminal differentiation of human tumors.

It must be evident to you by now as to why polyphenols have become popular nutraceuticals world wide and are being consumed frequently as an over the counter food supplement. Let us now move on to the next group of compounds, phytoestrogens.

But before that, you need to quickly review what we have learnt till now by answering the questions included in the check your progress exercise 3.

Check Your Progress Exercise 3

- 1) What are Polyphenols? Name the compounds from which they arise.

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- 2) List the salient aspects of bioavailability of polyphenols.

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- 3) Briefly discuss the influence of Polyphenols on:

- a) Minerals

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- b) Macronutrients

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- 4) Enumerate any five important health benefits of polyphenols.

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- 5) What characteristics of polyphenols are responsible for their anti-oxidant action?

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6) Match the following:

A

- i) Phenolic Acid
- ii) Flavonols
- iii) Isoflavones
- iv) Lignans
- v) Stilbenes

B

- a) Genistein and Daidezin
- b) SDG and Matairesinol
- c) Hydroxybenzoic acid, Hydroxyannamic acid
- d) trans-reservatrol
- e) Catechin and epicatechin

11.7 PHYTOESTROGENS

You may be aware of the surging interest in phytoestrogens (PE) especially in connection with osteoporosis. This section briefly discusses these compounds.

As the term implies, they are *the oestrogenic compounds found in plants*. They exert oestrogenic effects on the central nervous system, induce estrus and stimulate growth of the genital tract of female animals. In a broader sense, they are chemicals showing effects suggestive of oestrogenicity e.g. binding to oestrogen receptor (ER), inducing specific oestrogen-responsive gene products and stimulating ER-positive breast cancer cell growth.

Phytoestrogens can be divided into 3 main classes: *isoflavones*, *coumestans* and *lignans*. All are diphenolic compounds with structural similarities to natural and synthetic oestrogens and anti-oestrogens. Our subsequent discussions highlight the major forms of phytoestrogens and their food sources.

11.7.1 Dietary Sources and Chemical Forms

Isoflavones and *coumestans* are the most common compounds. Soybeans and soyfoods are the most important sources, containing approx. 0.2-1.6 mg per gram dry weight; although chick peas and other legumes also contain them. Second generation soy foods e.g. products like tofu yoghurt contain 6-20% of the isoflavones present in the whole beans.

Isoflavones may be present in unconjugated form (aglycones) e.g. daidzein, genistein, or as glycosides such as daidzin, genistin.

Non-fermented soy foods like tofu contain greater levels of glucosides whereas fermented foods e.g. tempeh have higher amounts of aglycones due to enzymatic hydrolysis during the fermentation process.

A large number of coumestans e.g. coumestrol in alfalfa have been isolated from plants but only a few possess uterotrophic (stimulating growth of the uterus) activity. The highest amounts of coumestans are present in clover and alfalfa sprouts (5.6 and 0.7 mg/g dry weight). Other sources are split peas, kala chana, lima beans and soy bean sprouts (15-80 mcg/g dry weight).

Lignans do not induce estrus but are considered to be phytoestrogens because they have oestrogen-like actions. They are present in plant foods and human biological fluids, plant lignans being converted by bacterial action to mammalian lignans in the GIT. Secoisolariciresinol and matairesinol are plant dietary precursors of the mammalian lignans, enterodiols and enterolactone.

The content of lignans in various foods is shown in the Table 11.6, oilseeds being the richest plant sources.

Table 11.6: Lignan content of selected foods

Plant Source	Content (mg per 100 g wet wt)
Flaxseed	0.8 mg secoisolariresinol per g dry wt
Unhulled soybeans	20.5
Dried sea weed	0.9
Whole legumes	0.6
Cereal brans	0.5
Legume hulls	0.4
Whole grain cereals	0.3
Vegetables	0.14
Fruits	0.03

The potential role of phytoestrogens with respect to maintaining good health is being discussed next.

11.7.2 Physiological Effects

Little is known about the biologic and physiologic effect of phytoestrogens in humans. Animal studies suggest the following physiological effects of phytoestrogens:

- hormonal effect, like stimulation of uterine growth in a manner similar to oestrogen (whether phytoestrogens have oestrogenic or anti-oestrogenic effect will depend on the amount of endogeneous oestrogen present), and
- antioxidant effects by increasing the activities of antioxidant enzymes such as catalase superoxide dismutase, GSH peroxidase and GSH reductase. Soya isoflavones may also act directly as anti-oxidant.

The health benefits ascribed to phytoestrogens are for protection against cancer, relieving symptoms of menopause like hot flushes, prevention of osteoporosis (through prevention of bone resorption and promoting increased bone density) and prevention of heart disease in postmenopausal women probably acting as oestrogen agonists (mimicking the effect of oestrogens), producing effects on lipoproteins similar to those caused by oestrogen.

Let us now look at the characteristic features of other food components with antinutritional effects. You may be familiar with the terms phytate and trypsin inhibitor. Let us discuss these and others.

11.8 OTHER DIETARY FACTORS WITH ANTINUTRITIONAL EFFECTS

In the above sections, we have learnt about a variety of food components having a host of health promotive properties. Let us now have a look at a few factors with antinutritional effects. These factors in foods include *protease inhibitors*, *physic acid*, *phenolic compounds*, *amylase inhibitors* and *saponins*. The well known anti nutritional effects of these factors are discussed first. These factors are present in foods that we consume regularly such as whole wheat, other whole grains, legumes and soyabeans. A few noteworthy points: The concentration of antinutritional factors is much lower in the refined cereals from which nearly all the bran and much of the endosperm has been removed. Further, some of the anti-nutritional factors such as, protease inhibitors in soyabean and some legumes, are heat labile and therefore proper cooking will inactivate them. Household processes such as soaking and fermentation will also remove many of the antinutritional factors. The traditional methods of cooking and processing all have the effect of reducing the antinutritional factors in our diets.

Therefore, you will notice that although several adverse effects are documented chiefly in animal models for these antinutritional factors, in actual practice, the cooking and processing of foods for human has evolved in such a way as to keep these antinutritional factors low in our diets so that we do not suffer from the ill effects. Occasionally, however, the consumption may reach levels at which ill effects become manifest. It is important, therefore, to be aware of these adverse effects. Following the adverse effects of each of these constituent, the currently known beneficial effects are listed. At the current time what we do not know is the amount of these to be consumed to maximize the beneficial effects and minimize the adverse effects.

So then let us begin our study of these factors with protease inhibitors.

11.8.1 Protease Inhibitors

These are protein in nature and are abundant in raw cereals and legumes, especially soybeans. It would be interesting to note here that since they are proteins, they can be denatured by heat. However, in commercial soy products, 5-20% of the activity has been detected.

What are the effects of protease inhibitors?

In experimental animals, they have been found to be associated with growth inhibition and pancreatic hypertrophy. A negative feedback mechanism in the small intestines has been postulated for these effects. In the presence of trypsin inhibitors, inactivation and loss of intestinal trypsin can take place. These can trigger the release of cholecystokinin (CCK) from the intestinal mucosa. CCK then can induce the pancreas to produce more trypsin. However, excess of trypsin inhibitors can reduce the protein available thus explaining the growth inhibition seen in animals, as well as, the pancreatic hypertrophy. Moreover, trypsin is rich in sulphur-containing amino acids; hence increased synthesis of trypsin (the more the inhibitor, the more trypsin that is made) increases the requirements for these amino acids, ultimately leading to weight loss. Also, the stress of the pancreas results in pancreatic hypertrophy and hyperplasia of the acinar (exocrine) cells.

Feeding purified trypsin inhibitor or raw soy flour containing protease inhibitors potentiated the effects of pancreatic carcinogens. It must be noted that most of the effects have been observed in some species of animals only, whose pancreas constitute a fairly higher percentage of body weight (0.29-0.8%) e.g. in rats, mice, chickens, hamsters and guinea pigs but not in large animals with a small pancreas (0.06-0.24 % of body weight) e.g. dogs, calves, monkeys.

In human subjects, provision of raw soy flour or purified trypsin inhibitor directly to the duodenum, increased secretion of pancreatic enzymes and serum level of CCK. This suggests that a negative feedback mechanism also exist in humans. However, it is interesting that among populations with fairly high intake of soybeans or other foods rich in protease inhibitors, e.g. in Japanese and Seventh Day Adventists, the incidence of pancreatic cancer is lower.

Another category of food components which may influence human health are the saponins. A few characteristic features of saponins are discussed below.

11.8.2 Saponins

These are a diverse group of compounds commonly found in legumes like soybean, lentils, chickpeas, peanuts and alfalfa sprouts. They are also present in some plants which we use as flavouring agents such as herbs and spices e.g. fenugreek, nutmeg, ginseng, sage and thyme.

Let us briefly understand their chemical nature.

Their structures are characterized by the presence of a steroid or triterpene group, i.e. an aglycone linked to one or more sugar molecules. Since these compounds have both polar (the sugar moiety) and non-polar (steroid or triterpene) group, obviously they will have surface-active properties. These properties are responsible for whatever effects saponins exert.

You should remember that saponins have both adverse, as well as, beneficial biological effects. Let us first list the adverse effects.

The adverse effects of saponins may be described as under:

- A well-known toxic effect is ‘erythrocyte lysis’ since they interact with the cholesterol in the erythrocyte membrane.
- In mammals, intravenous administration causes local inflammation.
- In large doses, it results in death due to massive release of erythrocyte debris.
- A reduction in oxygen-carrying capacity of blood.
- It can lyse other cells e.g. of the intestinal mucosa and thus affect nutrient absorption.
- High saponin intake results in decreased weight gain. This has been attributed to a number of reasons:
 - bitter taste
 - decreased nutrient absorption and utilization because of inhibition of metabolic and digestive enzymes such as amylase, lipase, protease and cholinesterase. These effects have been observed with soy saponin.

Alfalfa sprouts have been found to inhibit chymotrypsin, protease, succinoxidase, as well as, bind zinc.

Now let us study the beneficial effects of saponins. Saponins may have some anticarcinogenic effect by binding primary bile acids, formation of secondary bile acids and thus the promotion of tumorigenesis is reduced. They also enhance the immune response and have been used as adjuvants for oral vaccines.

Another group of substances of interest are the commonly consumed *amylase inhibitors*. These are most frequently consumed in view of the fact that they are present in several cereals and legumes and we know that Indian dietaries are principally cereal based.

11.8.3 Amylase Inhibitors

Inhibitors of the enzyme α -amylase are found in wheat, rye, beans, mango, legumes, potatoes, sorghum (jowar) and oats. Most amylase inhibitors from plants are active against animal amylase.

Let us see how these inhibitors act.

The inhibitor forms a complex with amylase, which in turn reduces starch digestion. Complex formation is influenced by pH, ionic strength, temperature, time of inactivation and inhibitor concentration. Other effects observed are pancreatic enlargement and hyperplasia.

When starch digestibility is reduced and in case starch intake is limited, you may expect growth to be adversely influenced. However, observations on animals provide conflicting data. It is possible that amylase inhibitors from different sources may have different effects due to pH sensitivity and sensitivity of the pancreas to different amylase inhibitors.

In humans, slightly different effects have been observed e.g. diarrhoea, nausea and vomiting. It is noteworthy that these effects were observed after intake of 'starch blockers' which may not be pure amylase inhibitors and other antinutritional factors like lectins and protease inhibitors also caused some of these effects.

Yet another category of food substances which are not conducive to good health include lectins and haemagglutinins. These terms may be relatively new to you. Let us understand some of their salient characteristic features.

11.8.4 Lectins or Haemagglutinins

These are sugar binding proteins, having the ability to bind and agglutinate red blood cells (RBCs). You will find that they occur in most plant foods including those, which may be eaten raw. Lectins are specific in the sugar they bind to and also in their toxicity. Of great interest would be the fact that lectins from horse gram, lima beans, kidney beans, mung, winged beans and castor beans are toxic when orally consumed but lectins from soybeans and peanuts are not.

What are their toxic effects?

Well, these can cause growth inhibition in animals and diarrhoea, nausea, bloating and vomiting in case of human beings. When injected lectins agglutinate RBCs, haemolysis and death occurs in extreme cases. Several outbreaks in England after consuming improperly cooked beans have been related to lectins although other antinutritional factors may have played a role. Since lectins are proteins, they are denaturated by heat however, low temperature or slow cooking may not completely eliminate their toxicity.

Toxic effect of lectins also relates to their binding with specific receptor sites on intestinal mucosal cells. This results in lesions, disruption and abnormal development of microvilli. Other effects include reduced activity of brush border enzymes viz. peptidases, disaccharidases, alkaline phosphatase, as well as, pepsin and pancreatic/salivary amylase. Secretion of mucin and increased weight/number of intestinal mucosal cells in presence of lectin has also been observed.

Since the enzymes secretion/activity are affected, carbohydrates and proteins are not completely digested. When these substances reach the colon, they act as substrates for the colonic microflora, which subsequently ferment them and produce short chain fatty acids and gases. This is the underlying reason for some of the gastrointestinal symptoms associated with intake of raw beans/purified lectin.

Bacterial overgrowth may also occur and may contribute to lectin toxicity. This has been linked to thinning of the jejunal mucosa and damage to duodenal microvilli. Increase in bacterial colonization by lectin may be due to its polyvalent nature, which allows lectin to bind to both mucosal cells and bacteria simultaneously. This would 'fix' the bacteria close to the intestinal mucosa. If the mucosa is disrupted, it may allow translocation of the bacteria and/or endotoxin and cause a toxic response.

In addition, there are reports that lectins themselves may be internalized, causing systemic effects such as increasing protein catabolism, breakdown of stored glycogen and lipid, as well as, disturbing mineral metabolism.

Yet another type of food components which may influence nutrient absorption/health include phytates. You must have read about them before especially with respect to iron absorption. We will now discuss some salient features of phytates.

11.8.5 Phytates

We are familiar with phytates as an inhibitor of mineral absorption (calcium, iron etc.) especially in the vegetarian diets that are cereal-based. This sub-section deals with the well-known nutritional implications which have been long researched, as well as, the health benefits, which have been in focus more recently.

Let us begin our study with the chemistry and occurrence of phytates.

Chemistry and Occurrence

Most plant foodstuffs contain inositol which is present in the form of myo-inositol hexakisphosphate i.e. phytic acid and its salts. In seeds, inositol is the storage form of phosphate and 60-90% of phosphate is present as phytate. Phytic acid (PA) is generally complexed with minerals and/or proteins. The complexed form with minerals is known as 'Phytate'. Various cations can strongly chelate between two phosphate groups or weakly within a phosphate.

Our subsequent discussions will elucidate the interaction of phytates with nutrients, as well as, their health benefits. We will begin with influence of phytates on mineral absorption.

Nutritional Implications

We have already discussed about the influence of polyphenols on the various macronutrients and minerals earlier in Unit 9 and 10. Let us in this sub-section once again review the nutritional implications of these components.

Phytate and Mineral Interaction: Phytic acid occurs as a crystalline globoid inside protein bodies in the bran of cereal grains. It is negatively charged over a wide pH range. This will enable it to react with the positively charged ions. Thus, PA binds to mineral cations such as Ca^{2+} , Fe^{2+} , Fe^{3+} , Zn^{2+} and Cu^{2+} and forms strong complexes which render them unavailable for absorption. The effects of PA on mineral availability depends on numerous factors:

- the amount of PA present,
- concentration of the minerals,
- molar ratio of PA to minerals,
- size and valency,
- association of PA with protein,
- pH,
- presence of other metal ions,
- presence of other substances which can bind minerals, e.g. fibre, tannins, oxalic acid, and
- processing methods used.

At the pH values between 5 and 7 which are similar to those in the duodenum, PA binds and form insoluble chelates with divalent cations. At pH 7.4, the order of complexation in decreasing order is $\text{Cu} > \text{Zn} > \text{Co} > \text{Mn} > \text{Fe} > \text{Ca}$.

As phytates accumulates in various storage sites, other minerals apparently chelate to it forming the complex salt 'phytate'.

Studies on animals show that phytate inhibits zinc and calcium absorption, the maximum inhibitory effects being shown by 'myoinositol hexaphosphate.' The inhibitory effects appear to be greater for Zn than for calcium. It appears that at least 5 of the 6 possible sites need to be phosphorylated in order to exert its inhibitory effect. In vitro studies on availability of iron and zinc from North Indian diets indicate that it is very low—3.3 to 4.4% for iron and 7.8 to 8.7% for zinc, with a negative correlation between availability and PA content.

Next, we will discuss the influence of phytates on protein metabolism.

Phytate and Protein Interaction: You may be more familiar with phytate's role in mineral absorption. However phytates also bind with starch and protein. Depending on the pH, 2 types of complexes are formed—at acidic pH, a binary protein-phytate complex and as the pH approaches neutrality, a ternary protein-mineral-phytate complex. Strong electrostatic attraction between negatively charged phytates and proteins with a net positive charge results in the formation of protein-phytate complexes.

Apparently most such complexes are formed *de novo* in the gastrointestinal tract. Also phytates can interfere with digestive enzymes and their substrates, thus adversely affecting digestion of dietary protein and increasing endogenous losses of amino acids.

With this, we end our discussion of phytates. Our study on other factors with antinutritional effect, shall not be complete without a brief mention about the health benefits of these factors. Yes these factors can be beneficial to health. Let us quickly review the health benefits.

11.9 HEALTH BENEFITS OF OTHER DIETARY FACTORS WITH ANTINUTRITIONAL EFFECTS

Despite the predominantly nutritional antagonistic effects of the factors described above, there is some evidence accumulating to show that these may be associated with some health benefits as well. Some of the benefits noted with these factors appear to be similar to those observed for fibre e.g. lowering blood glucose/blood lipids and lowering the risk of developing cancer. These are briefly reviewed below.

- *Hypoglycemic effects:* Of the different carbohydrate foods tested for starch digestibility and blood glucose response, those rich in anti-nutrients e.g. legumes show the lowest values. Effects were best seen with the soluble whey fraction, which is rich in anti-nutrients like tannins, lectins and phytic acid. Amylase inhibitors can reduce blood glucose and raise insulin levels.
- *Reduction of blood lipids:* This effect have been especially observed with saponins. Diets containing saponin-rich foods (300-500 mg saponins/day) e.g. soya, alfalfa, chickpea, bean meal reduced plasma cholesterol by 16-24%. However, other substances in these foods like phytosterols, isoflavones, protein and fibre may also have contributed. Cholesterol reduction may be due to saponin binding dietary cholesterol thus preventing its absorption and/or binding with bile acids and increasing their faecal excretion.
- *Reduction in cancer risk:* Different cancers, especially colon and breast cancer, has been linked not only to phytates but also to protease inhibitors. In vitro studies show that they suppress malignant transformation of cells. Among the protease inhibitors, the most effective are those with chymotrypsin inhibitor activity in foods like soybean, chickpea and potato. Soybean also has the Bowman-Birk inhibitor which inhibits/prevents development of chemically induced liver cancer.

What are the mechanisms possibly responsible for these effects?

There are several possibilities: reduced availability of amino acids to cancer cells by reversing the carcinogen-induced change in oncogene expression, or by inhibiting formation of superoxide radicals, hydrogen peroxide formation by neutrophils which is induced by tumor promoters.

Research has indicated that phytates may have some beneficial aspects as well. Let us learn about them.

Beneficial effects of phytates: Some of the health promotive aspects of phytates include:

- 1) Phytic acid is a known antioxidant. Colonic bacteria produce oxygen radicals in appreciable accounts. Dietary phytic acid suppresses free radical mediated damage to intestinal epithelium.
- 2) Protective benefits are seen particularly in relation to carcinogenesis, particularly against colon cancers. Complexing of iron by phytate may reduce iron-catalyzed production of free radicals and reduce lipid peroxidation. Also, phytate-containing foods have a higher fibre content. Studies show that signal-transduction pathways, cell cycle regulatory and differentiation genes, oncogenes and tumor-suppressor genes may be involved in phytate's anti-neoplastic effects.
- 3) Lowering blood glucose and lipids.
- 4) Binding amylase and reducing starch digestion.
- 5) Lowering plasma cholesterol and triglycerides.

In the last two section(s) we read about certain food components such as protease/ amylase inhibitors, saponins, lectins and phytates, which are at times referred to as antinutritional factors. These food components have been found to inhibit nutrient absorption and a few substances also are health promotive. Research however needs to be conducted to further ascertain their health benefits especially with regards to their type and dosage. We hope that it must have been a good learning experience for you. A list of references has been mentioned at the end of the course for you to learn further.

You may now need to perform the check your progress exercise 4 to recapitulate the details of the food components discussed in this section.

Check Your Progress Exercise 4

- 1) What are phytoestrogens? What are its classes and give examples of each class.

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- 2) Enlist the food sources of lignans.

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- 3) Discuss the toxic effects of:

- a) Saponins

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- b) Lectins

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4) Enumerate the health benefits of antinutritional factors.

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11.10 LET US SUM UP

In this unit, we studied about the health benefits adverse effects of several food components apart from the nutrients. We got to know about the concept of functional foods and which components make them healthier food choices. Subsequent discussions highlighted the characteristic features of several nutraceuticals/bioactive substances/food components such as probiotics, prebiotics, non-glyceride components of edible oils, phytoestrogens etc. Their mode of action, potential beneficial health effects food sources and clinical uses have been elucidated in this unit. We also saw their interactions with nutrients such as proteins and minerals, especially in the case of phytates.

In the subsequent sections, we learnt that the physiological effects of the various substances will depend on their level of intake, presence of other dietary constituents and the nutritional/health status. Benefits and risks need to be balanced. However, considerable work is required before we can issue recommendations in terms of amounts of these to be consumed for the population at large.

11.11 GLOSSARY

Adenocarcinoma	: cancer that begins in cells that line certain internal organs and that have secretory properties.
Angiogenesis	: the growth and proliferation of new blood vessels.
Apoptosis	: one of the types of programmed cell death that is the deliberate suicide of an unwanted cell in a multicellular organism.
Fructans	: a general term for any carbohydrate in which one/more fructosyl fructose link constitutes the majority of glycosidic bonds.
Functional foods	: the food substances beyond the basic nutrients that are designed to lower the risk or delay the onset of certain disease.
Lectins	: sugar binding proteins, having the ability to bind and agglutinate RBCs.
Non-digestible oligosaccharides	: oligomeric carbohydrates which are resistant to hydrolyzed by colonic bacteria.
Phytate	: complexed form of phytic acid with minerals.
Phytoestrogens	: oestrogenic compounds found in plants.
Polyphenols	: compounds containing an –OH groups attached to the benzene ring.

Prebiotics	: non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial in the colon.
Probiotics	: a live microbial feed supplement which beneficially affects the most animal by improving its intestinal microbial balance.
Tumorigenesis	: formation or production of tumors.

11.12 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

- 1) The food substances, beyond the basic nutrients that are designed to lower the risk or delay the onset of certain diseases are called the functional foods. They are found in mixed culture of living organisms, many fruits, vegetables, grains, herbs and spices.
- 2) As per the Japanese criteria, they have a defined function on the human organism to improve immune function, prevent specific disease, support recovery from specific diseases, control physical and psychic complaints, and slow down the ageing process.
- 3) Probiotics, Prebiotics.
- 4)
 - a) Fibre, Phenolic compounds, NDOs, Phytosterol, Lutein, Lycopene
 - b) Prebiotics, Probiotics, n-3 fatty acids and polyphenols
 - c) Any three of the following: Polyphenols, Fructans, n-3 Fatty Acids, Phytate, Carotenoids, Polyphenols, non-glycerides
- 5) The food can be made functional by the following ways:
elimination of components e.g. toxins or allergenic proteins; increasing the concentration of a natural component e.g. fortification; addition of components with beneficial effects e.g. non-vitamin antioxidants; addition of beneficial microbes e.g. some yeasts, bacteria; replacement of a component, usually a macro-nutrient e.g. fat replaced with modified or emulsified carbohydrates; and enhancement of biavailability of components.
- 6) The major components of non-glyceride fraction in edible oils and fats are sterols, terpene alcohols, tocopherols, hydrocarbons, long chain alcohols including waxes, carotenoid pigments, sulfur- and nitrogen -containing flavour compounds.

Check Your Progress Exercise 2

- 1)
 - a) Probiotic is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance for example, *bifidobacteria* and *lactobacilli*.
 - b) Prebiotic is a non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon for example, *bifidobacteria* and *lactobacilli*.
 - c) NDOs is oligomeric carbohydrates, which are resistant to hydrolysis by intestinal digestive enzymes but can be metabolized by colonic bacteria for example, FOS, Soybean Oligosaccharides.

- 2) Probiotics are microorganisms which promote healthy gut microflora while prebiotics are non-digestible food components which are fermented in the colon and alter the composition of microflora there. *Saccharomyces boulardii* is a probiotic while non-digestible oligosaccharides are examples of prebiotics.
- 3) Colonization of the gut appears to be a prerequisite for the probiotic effect. Important relevant features may be resistance to low pH and bile acids and ability to adhere to intestinal epithelium.
- 4) The physiological effects of probiotics are: (any two of the following)
 - In animals, probiotics may control tumour production; in humans, probiotics can reduce incidence of intestinal infections; 50% reduction in AAD observed in patients given *Saccharomyces boulardii*; significant reduction in diarrhoea incidence among children aged 4-45 months after administration of *L.casei* GG; and an antifungal effect.

The beneficial effects of prebiotics are as: (any two of the following)

- control of transit time and motility; regulation of epithelial cell proliferation; influence on nutrient bioavailability; modulation of immune function; and modulation of endocrine function.
- 5) The benefits attributed to intake of NDOs include: increases solubilization of minerals and hence calcium/magnesium absorption and balance, decreases fasting blood glucose and hepatic glucose production, a hypotriglyceridemic effect and decreased lipogenesis and decrease in uremia.
 - 6) Look up sub-section 11.5.3 under fructans and answer on your own.

Check Your Progress Exercises 3

- 1) Polyphenols contain an OH group attached to the benzene ring. They arise from phenylalanine or its precursor shikimic acid.
- 2) Look up sub-section 11.6.2 and answer on your own.
- 3) a) Influence of polyphenols on minerals include:
 - Reduced protein digestibility and a subsequent increase in the faecal nitrogen. Decreased protein digestibility may be due to the formation of protein tannin complex, as well as, inhibition of proteolytic enzymes like trypsin by polyphenols.
 - Inhibition of amylolytic enzymes and subsequent reduction of dietary carbohydrate hydrolysis can decrease the postprandial glycemic response.
 - Both soluble polyphenols and condensed tannins have been shown to decrease the apparent digestibility of lipids, lipase activity and increase faecal fat excretion.
- b) Polyphenols can form complexes with metal cations through their carboxylic or hydroxylic groups, and thus interfere with the intestinal absorption of minerals like iron, copper, sodium and aluminum.
- 4) Look up sub-section 11.6.4 and answer on your own.
- 5) The antioxidant action of polyphenols can be attributed to the following characteristics:
 - act as terminators of free radicals by rapid donation of H atoms,
 - chelate metal ions like iron and copper,
 - act as terminators of propagation,
 - quench reactive oxygen species and reactive N₂ species,
 - inhibit lipoxygenase and cyclooxygenase enzymes, and

- inhibit damage by haemprotein/peroxide mixtures.
- 6) i) - c)
- ii) - e)
- iii) - a)
- iv) - b)
- v) - d)

Check Your Progress Exercise 4

- 1) Phytoestrogens are oestrogenic compounds found in plants. They exert oestrogenic effects on the central nervous system, induce estrus and stimulate growth of the genital tract of female animals. PE can be divided into 3 main classes: isoflavones eg: daidzein and genistein, coumestans such as clover and alfalfa sprouts and lignans eg: secoisolariciresinol and matairesinol.
- 2) Flax seed, unhulled soyabean, dried sea weed, husk and bran of cereals and legumes, fruits and vegetables.
- 3) a) Toxic effects of saponins are:
 - A well-known toxic effect is ‘erythrocyte lysis’ since they interact with the cholesterol in the erythrocyte membrane
 - In mammals, intravenous administration causes local inflammation
 - In large doses, it results in death due to massive release of erythrocyte debris
 - A reduction in oxygen-carrying capacity of blood
 - It can lyse other cells
 - High saponin intake results in decreased weight gain.
- b) These are growth inhibition in animals and diarrhoea, nausea, bloating and vomiting in case of human beings. When injected lectins agglutinate RBCs, haemolysis and death occurs in extreme cases. Toxic effect of lectins also relates to their binding with specific receptor sites on intestinal mucosal cells resulting in lesions, disruption and abnormal development of microvilli.
- 4) Protective benefits are particularly in relation to carcinogenesis, particularly against colon cancers; lowering blood glucose and lipids; binding amylase and reducing starch digestion; lowering plasma cholesterol and triglycerides; hypoglycemic effects; reduction of blood lipids and reduction in cancer risk.