



iJRASET

International Journal For Research in
Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 9 Issue: XII Month of publication: December 2021

DOI: <https://doi.org/10.22214/ijraset.2021.39552>

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Nutraceuticals and its Impact on Health Care

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I. INTRODUCTION

Food and drugs from nature place a quite significant role in public healthcaresystem throughout the world¹. Human inquisitiveness and search for specific constituents of plants animals minerals and microbial origin which are beneficial to our overall health have cost coining of terminologies such as functional food or nutraceuticals. Nutraceuticals have evolved from the recognition of the link between food and health.

The term nutraceuticals was coined from "nutrition" and "pharmaceutical" in 1989 by Stephen Defelice MD, founder and chairman of the foundation for innovation in medicine (FIM) Cranford, NewJersy According Defelice nutraceutuicals can be defined as a food (or part of a food) that provides medical or health benefits including the prevention and/or treatment of a disease³.

American association of nutritional chemist mentioned nutraceuticals as a products that has been isolated or purified from food and generally solved in medicinal forms not usually associated with food¹. When food is been cooked or prepared using "scientific intelligence" with or without knowledge of how or why it is being used, the food is called functional food³.

OR

Functional foods are ordinary foods that have components, ingredients, incorporated in them to give them a specific medicinal or physiological benefit other than a purely nutritional effect. As we approach towards the 21st century nutritional science has an essential factor by all manufactures and consumers placing for more emphasis on the benefits to be derived from food¹. Designing a proper food maintain proper health has gained recognition and acceptance worldwide. Due to this the food industries in many countries are modifying their products as a response to consumer demands. In recent years many of the natural products from India are gaining popularity in USA and Europe has nutraceuticals. Nutraceuticals or functional foods have been found to be associated with the prevention and/or treatment of many chronic diseases and ailments such as cancer, diabetes, heart diseases, hypertension, arthritis, osteoporosis etc. Statistical data indicates that 35% of all cancer are related to the food that we eat and also associated with certain dietary habits. It is certainly makes the old saying "you are what you eat". More relevant in context of the health benefits of the food. As the importance of dietary changes to optimize health in gaining recognition and acceptance, the food industry is responding to consumer demands for more healthful nutrient rich food products.

II. CLASSIFICATION

Nutraceuticals or functional foods can be classified on the basis of their natural sources, pharmacological conditions or as per chemical constitution of the products. On the basis of natural source it can be classified as the products obtained from plants, animals, minerals or microbial sources. The classification of nutraceuticals based upon its therapeutical utility for the treatment or prevention of specific condition may produce a big list. Some of the important conditions in which the nutraceuticals are specially directed for its treatment, prevention or support is given in Table 1.

Table 1: Nutraceuticals used in various disease conditions

Conditions	Nutraceuticals
Allergy relief	Ginkgo biloba
Arthritis support	Glucosamine
Cancer prevention	Flax seeds, green tea
Cardiac diseases	Garlic
Cholesterol lowering	Garlic
Digestive support	Digestive enzymes
Diabetic support	Garlic, momordica
Female hormone support	Black conosh, false unicorn
Immunomodulators	Ginseng
Prostate support	Tomoto lycopenes

A systematic classification on the basis of therapeutically important compounds of the nutraceuticals products responsible for the specific health benefit can be done as given in Table 2.

Sr.No	Class	Examples
1	Inorganic mineral supplements	Minerals
2	Vitamin supplements	Vitamins
3	Digestive enzymes	Enzymes
4	Probiotics	Lactobacillus acidophilus
5	Prebiotics	Digestive enzymes
6	Dietary fibres	Fibres
7	Cereals and grains	Fibres
8	Health drinks	Fibre
9	Antioxidants	Natural antioxidants
10	Herbs as a functional foods	Soya proteins

III. INORGANIC MINERAL SUPPLEMENTS

Large number of elements control variety of physiological and biochemical functions of human body. Most of these minerals are provided through the diet but their deficiency in diet may develop variety of health related problems and diseases.

A. Calcium

Calcium is an important element in the treatment of decalcification of bone . Calcium deficiency is found in 25% of women, even though much higher percentages have osteopenia or osteoporosis. Prepuberty is the best time to begin supplementing the diet with calcium rich minerals along with exercise regimen. Sufficiently intake of calcium and vitamin D post menopausally can significantly reduce the risk for fracture.

B. Magnesium

Magnesium is an essential element involved in various enzymatic processes and critical in the proper use and maintenance of calcium. Many individuals with calcium deficiency are actually magnesium deficient which prevent proper use of calcium.

C. Manganese

Manganese is required in several enzymatic reactions and necessary for proper bone and cartilage formation.

D. Boron

Boron is reported to be helpful in supporting the calcium and estrogen level in post menopausal in women.

E. Copper

Copper is an essential element needed by all tissues in the body. Copper and Zinc must be in proper formation. Copper is best absorbed when bound to an aminoacids.

F. Zinc

Zinc is one of the most important trace mineral. Zinc supports the bodies overall antioxidant system by scavenging free radicals. It also perform many other vital functions.

G. Phosphorous

Phosphorous important in maintaining bone structure and modulating plasma and bone formation.

H. Silicon

Silicon is concentrated in the active growth areas of bone. It influences both for bone formation and calcification.

IV. DIGESTIVE ENZYMES

Much of the reflux is not caused by increased production of acid in the stomach but from poor digestion because of too little acid. As we grow older stomach cells responsible for acid production diminishes, this in turn slows the transit time of food in the stomach causing reflux of food from the oesophagus. So we have to use a variety of digestive enzymes to help absorption and digestion of food materials. There are animals as well as plant derived digestive enzymes.

The principle digestive enzyme in the pepsin, present in the gastric juices, which helps in the digestion of proteins. It is obtained from the glandular layer of fresh stomach of hog, *Sus scrofa var domestica* of family *Suidae*. Pancreatin, an enzyme obtained from pancreas of certain animals like hog (*Sus scrofa*) family *Suidae* or *Ox bostaurus* of family *Bovidae*. It is employed as digestive aid for converting starch into dextrin and sugar. Each gram contains not less than 12,000 units of amylase activity, 1000 units of protease activity and 15,000 unit lipase activity. Another important digestive enzymes of animal origin are Trypsin obtained from mammalian pancreas like *Ox bostaurus* of family *Bovidae*, Chymotrypsin also obtained from the same source. UROKINASE, FIBRINOLYSIN, DEOXY-RIBONUCLEASE, STREPTOKINASE are the other animal derived digestive enzymes.

Papain, the plant proteolytic enzyme obtained from *Carica papaya* fruits have anti-inflammatory activity. **BROMELAIN** derived from stem and fruits of pineapple plant *Ananas comosus* of family *Bromeliaceae* are used for the treatment of tissue inflammation and oedema due to surgery and injury.

V. DIETARY FIBRES

Dietary fibres play a critical role in keeping good health in human individuals and animals. They are rich in antioxidants. Fibres are those parts of the plant, leaves, stem, fruits and seeds.

There are two types of fibres

- 1) Water soluble
- 2) Water insoluble

The water insoluble fibres absorb water to a certain extent and mainly contribute to bulking of stool and allow quick passage of water through the alimentary canal.

Soluble fibres get dissolved in water and form a gel that binds the stool, also slows down the absorption of glucose and reduces blood cholesterol level.

It has been recommended that about 30-40 gm of dietary fibre should be consumed daily in order to obtain significant health benefits. The main sources of water insoluble fibres include whole grain cereals, whole wheat products, brown rice and fruits and vegetables with the peels. Sources of water soluble fibres are oats, dried beans, legumes, lentils, fruits and vegetables. Some of the processed food marketed in the form of soluble fibres are breads, breakfast cereals, high fibre beverages.

Cereals and grains are largely used throughout the world as the major food material. These can be used in the entire form and sprouted cereals and grains in this milled form. These cereals and grains are rich in normal food nutrients, vitamins, minerals and specific phytochemicals.

Cereals and grains help in calcium fortification, maintaining a healthy heart and a healthy immune system. Breads of soya flour and linseed provide phytoestrogenic natural substances that mimic the structure of the hormone oestrogen.

The sprouts are known for their powerful nutritive and healing properties, they are the best "living food" because they contain high proportions of proteins and essential amino acids needed for normal growth of human beings. Many sprouted seeds such as those of alfalfa and sunflower contain all the essential amino acids in their 'free state'. Sprouting increases the vitamin content of a seed dramatically. Sprouts reduce inflammation, lower fever and tone the body.

VI. VITAMIN SUPPLEMENTS

Vitamins are complex substances of organic origin which in small quantities are necessary for the maintenance of human and animal life. Some of the important water soluble and water insoluble vitamins are discussed below.

A. Vitamin B-Complex

Specific vitamin B are recommended for daily requirement to combat high levels of homocysteine, a known risk factor for heart diseases. Homocysteine accumulates in the blood secondary to protein intake, specially from meat. Vitamin B extra is generally recommended to those who use caffeine, alcohol, excessive sugar or oral birth pills in their diet, since B vitamins are water soluble and easily excreted from the body.

Vitamin B1 or thiamin deficiency is mostly observed with individuals using white rice. Riboflavin-5-phosphate is a cofactor for vitamin B2 which is beneficial in people who lack the enzyme to convert vitamin B2 because of nutritional factors or disease condition. Niacinamide deficiency may cause neurological and skin problems. The major sources are the peanut, ragi, pulses, soyabean etc. The body can also synthesis niacin from tryptophan panthothenic acid. The panthothenic acid deficiency affect adrenal gland, immune and cardiovascular system.

Vit. B6 is crucial for glucose production, hormone modulation and neuro transmitter synthesis. Pyrodoxal 5-phosphate is considered as an active form of vitamin B6. Vitamin B12 deficiency may be observed in vegetarian people as plant has no appreciable vitamin B12. Folic acid is a B-complex vitamins which contribute to healthy bone formation.

Among the other vitamins, vitamin C is the body's main water soluble anti oxidant. Anti oxidants are nutrients that block some of the damage caused by free radicals, which are by-products that result when our bodies transform food into energy. Vitamin E and beta-carotene are two other well known antioxidants. Vitamin C is required for the growth and repair of tissues in all parts of our body. It is necessary to form collagen. Vitamin C is essential for the healing of wound and for the repair and maintain cartilage, bones and teeth. Vitamin C deficiency can lead to dry and splitting hair, gingivitis, rough, dry, scaly skin, wound-healing rate, scurvy, easy bruising, nose bleeds, swollen and painful joints, anemia etc.

The body does not manufacture vitamin C on its own nor does it store it. It is therefore important to include plenty of vitamin C containing foods in your daily diet. Citrus fruits are rich in vitamin C.

VII. PROBIOTICS

Probiotics (for life) can be described as a living micro organism which when ingested with or without food improves the intestinal microbial balance and consequently the health and functioning of large intestine. The major sources are the cultured dairy products such as natural cheese, yogurt, kefir and butter milk lactobacillus also in green foods such as wheat grain, spirulina and chlorella.

There are over 400 different bacteria living in the human GI tract, of all these lactobacillus acidophilus is one of the major component of the probiotic fighter. It enhances the immune system. Lactobacillus acidophilus can reduce the incidence of vaginal infections including thrush and bacterial vaginosis. Bifidobacteria and Streptococcus thermophilus both found in yoghurt can prevent young children suffering from diarrhoea also in treating travelers diarrhoea and rotavirus infection. Probiotics only have a transient effect and regular daily in take is needed to bring about health benefits. Probiotics gaining importance against helicobacter pylori infection, colonic cancer, irritable bowel syndrome, pancreatitis, antibiotic induced diarrhea, Crohn's disease and pouchitis. Probiotics are food components that escape digestion by the normal human digestive enzymes and safety in intact form, reach the colon after passage through the stomach and small intestine where they selectively promote the growth of probiotics.

Fructo-oligo saccharides (FOS) are increasingly used as food supplements. FOS have more long-lasting effect as they encourage the growth of Bifido bacteria already present in the gut. At least 10gm FOS is needed daily.

VIII. SINGLE CELL PROTEIN (SCP)

The mass production of bioprotein from the single-cell organism like bacteria or fungi termed as microbial biomass or single cell protein (SCP). Sacchromycescerevisiae were first established for the production of single cell proteins. The biomass were utilized in the forms of soups and sausages. Single cell proteins has more nutritive value than the normal living cells. An ideal biomass consist of the components such as carbohydrates, proteins, vitamins, lipids and trace amount of mineral and salts.

A. Spirulina

Spirulina plankton is a blue green vegetable micro algae is a good example of single cell protein. In India, research work is in progress at Central food Technology Research Institute, Mysore on spirulina to develop some single cell proteins as a supplement to food. Spirulina, which is the only natural source providing the highest amount of protein known to man that is, 71%, and it is three times that of soyabean and five times that of meat. It contain proteinous nitrogen (11.36%), total organic nitrogen (13.35%), nitrogen from nucleic acid (1.9%), lipid content is (5-6%), having more essential fatty acid (vitamin F) composed of oleic, linoleic, gamma linoleic, palmitic, palmitoleic, heptadecanoic acids. About 40% of the fats include glycolipids including sulpholipids (2-5%) which have significant anti-HIV activity. Spirulina containsthe carbohydrate in the form of glycogen and rhamnose.

Because of the presence of b-carotene with 9-Cis-carotenoid isomer, it has more antioxidant activity. The mineral content (3-6%) mainly includes iron which has better absorption than natural iron. The better absorption is due to its soluble complexes with phycocyanin which is protein derived from algae having the linear tetra pyrrole viz phycocyanobilin and resembles haemoglobin. Phycocyanin enhances general immunity and useful in lymphocytic activity against cancer.

The enzyme content in spirulina is in the form of super oxide dismutase and it is known for its free radical scavenging effects and plays a vital role in pathophysiological conditions like atherosclerosis, arthritis, cataract, diabetes and also in emotional stress and aging process. Gamma linolenic acid of spirulina helps to reduce cholesterol levels. It has appetite suppressing activity.

Water extract of spirulina inhibits HIV-1 replication in human derived T cell lines and in human peripheral blood mononuclear cells. Like all other microbial cells, spirulina contains all natural vitamins, B complex, minerals and other growth factors. Vitamin B12 can be utilized only from vegetable source.

The World Health Organization has found spirulina has an excellent food for human consumption. Spirulina is FDA approved food supplement and can be marketed in USA as a natural food.

B. *Spirulina capsule Tablets*

Athletes and joggers take spirulina for quick energy synthesis. To a particular extent indicates immense progress in anemia, diabetes, healing of wounds and lowering of cholesterol. It acts as a protein supplement in diet for malnourished children and adults.

IX. HEALTH DRINKS

Drinks are the fast developing area of Nutraceuticals. Some of these health drinks are fortified with the anti-oxidants, vitamin A, C, E. The fruits and vegetable juices have also been shown to produce the health benefits. A Tropicana fruit juice fortified with calcium provides about 365mg calcium per 250 ml glass. An ideal health drink increases physical endurance, improves and increases concentration and reaction speed.

A. *Almond Sharbat (almond soft drink) Ingredients*

Sugar, Almond, Rose water, Cardamom.

B. *Nutrition*

Prepared of the choicest almonds and blended cardamom, saffron, and rose water. With a matchless taste, and it is full of values both for brain and body. Regular use enriches body with protein, iron, calcium, phosphorus and other proteins. It can be utilized with either hot water or cold milk.

C. *Nutritional value per glass*

Iron	-	0.69mg
Protein	-	2.80mg
Carbohydrate	-	28.52g
Energy	-	206.32cal
Vitamin	-	139.631

D. *Saffron Sharbat*

Kesar is used in the preparation of kesar sharbat (saffron soft drink).

E. *Ingredients*

Sugar, Saffron, Cardamom

F. *Nutrition*

It is beneficial in the treatment of several digestive disorders, specially flatulent colic. Kesar is useful in treating skin disorders, blood purifying qualities and is also a great anti-oxidant it is a great drink for physical fitness.

G. *Nutritional Value Per Glass*

Protein	-	0.10g
Carbohydrate	-	35.52g
Energy	-	144.64cal
Fats	-	0.24g

H. Orange sharbatIngredients

Sugar, orange juice

I. Nutrition

Orange fruit juice is prepared from natural orange juice which is rich source of vitamin A,B,C and calcium also contain sodium, potassium, magnesium, sulphur and chlorine. It is an ideal soft drink for all season. It can be used deliciously in various frozen deserts, pudding, custards etc. Vitamin C given energy to tried mind and refreshes it.

J. Nutritional Value Per Glass

Iron - 0.6mg

Protein - 0.0924g

Carbohydrate - 36.16g

Energy - 145cal

Vitamin C - 158.18mg

X. POLYUNSATURATED FATTY ACIDS (PUFA)

Human body is capable of synthesizing most of the fatty acids it needs except the two major polyunsaturated fatty acids, i.e., omega-3-fatty acid and omega-6-fatty acids. These fatty acids are required to be supplemented from the diet. The polyunsaturated fatty acids are the known precursors for arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA). These fatty acids have been found to regulate blood pressure, heart rate, blood clotting and immune response. Omega-3-fatty acids have been reported to be important fatty acids in the prevention of heart diseases and also in the treatment of arthritis. Omega-3 fatty acids are mostly found in cold water fishes such as tuna, salmon and mackerel. It is also present in dark green leafy vegetables, flaxseed oil and in certain vegetable oils. The fatty acids such as AA and DHA are essential for the development of the foetus and also during the first six months after birth. The deficiency of these fatty acids may result in poor development of foetus and may also cause a variety of problems such as premature birth to underweight babies. Breast milk is a very rich source of DHA. Most of the infant formulas which are used as a substitute of breast milk should be supplemented with DHA, as per the recommendation by World Health Organization.

XI. HERBS AS FUNCTIONAL FOOD

A great attention has nowadays been given to discover the link between dietary nutrients and disease prevention. Large numbers of herbs which had been in use since unknown time have been shown to play a crucial role in the prevention of disease. In addition to the macro and micro nutrients such as proteins, fats, carbohydrates, vitamins or minerals necessary for normal metabolism, a plant based diet contains numerous nonnutritive phytoconstituents which may also play an important role in health enhancement. A brief overview of the role of various herbs in disease prevention, with a focus on bioactive components from flaxseeds, garlic, citrus, fruits, soybean, ginkgo biloba has been given in this part of the nutraceuticals.

A. Flaxseeds

Flaxseeds are the dried ripe seeds of *Linum usitatissimum*, family Linaceae. The components are of great interest as functional food. Flaxseed incorporation into the diet is particularly attractive from the perspective of specific health benefit. Flaxseed has been recorded as one of the six plant materials as cancer preventive foods. Alpha linolenic acid (ALA) has a broad spectrum of health advantages. It inhibits the production of eicosanoids, alters the production of several prostanoids, reduces blood pressure in hypertensive patients and lowers triglycerides and cholesterol. Dietary ALA may retard tumour growth and plays an important role in metastasis. It has been suggested that ALA is dietary essential for optimal neurological development of humans especially during fetal and early postnatal life.

Dietary fibres of flaxseeds contain about 6% mucilage which has nutritional value. It appears to play a role in reducing diabetes and coronary heart disease risk, preventing colon and rectal cancer and reduces the incidence of obesity.

B. Ginkgo Biloba

Ginkgo biloba, family Ginkgoaceae, known as fossil tree is an important drug used in traditional Chinese medicine since more than 2800 years. Mainly leaves and edible seeds are used as drugs. Ginkgolides A, B, C and bilobalide are also the therapeutically active constituents. Leaf contains 6-hydroxykynurenic acid, a metabolite of tryptophan.

The leaves are recommended as being beneficial to the heart and lungs. Ginkgolides present in the leaves are able to alleviate the adverse effects of platelet-activating factor in a number of tissues and organs both in animals and in humans. It is also effective in the treatment of arterial insufficiency in the limbs and in the brain.

C. *Garlic Organosulfur Compounds*

Garlic consists of the fresh or dried bulbs of *Allium sativum*, family Liliaceae. It is a perennial erect bulbous herb indigenous to Asia but commercially cultivated in most countries. Garlic is used as an adjunct to dietic management in the treatment of hyperlipidaemia and in the prevention of atherosclerotic (age dependent) vascular changes. Fresh garlic juice, aged garlic extract or the volatile oil, all lowers cholesterol and plasma lipids, lipid metabolism, and atherogenesis both in vitro and in vivo. The mechanism of garlic's antihypercholesterolaemic and antihyperlipidaemic activity appears to involve the inhibition of hepatic HMG-CoA reductase and remodeling of the plasma lipoprotein and cell membrane. The overall activity of garlic is mainly due to the presence of sulfur compounds such as allin, allicin, ajoene and others.

Garlic has been reported to reduce the risk of colon cancer and lung carcinoma. Consumption of one or more servings of fresh or powdered garlic per week resulted in a 50% lower risk of cancer of the distal colon and a 35% lower risk of cancers anywhere in the colon.

D. *Citrus Limonoids*

Citrus fruit consumption has been shown to protect against a variety of human cancers. The citrus fruits such as oranges, lemons, limes and grapefruits are the principal source of important nutrients like vitamins C, folate, fibres and vitamins E, but the other monoterpene compounds known as limonoids are reported to be responsible for the anticancer activity. d-limonene, a predominant monocyclic monoterpene found in essential oil of citrus fruits has been reported to be a cancer chemopreventive agent.

The mechanism of antitumour activity of limonoids include the induction of hepatic detoxification enzyme, glutathione S-transferase and uridine diphosphoglucuronosyl transferase. Limonene has little or no toxicity in humans and has been suggested as a good candidate for human clinical chemoprevention.

E. *Soya Products*

Soyabean, Glycinmax, family Leguminosae has clearly been a plant food in the spotlight in the 1990s. It has been recognized as an excellent source of protein, equivalent to quality to animal protein. Soya has been extensively investigated for its ability to treat and prevent a variety of chronic diseases including cancer. Soyabean meals, concentrates and isolates are used as meat substitute and have many healthful benefits. Soyabean is also a major source of lecithins which yields liposomes used to formulate stable emulsions and finds major use in food technology.

The primary isoflavones in soya, genistein and daidzein are structurally similar to the estrogenic steroids and have been reported to have estrogenic and antiestrogenic activities. Due to their weaker activity, isoflavones may act as antiestrogens by competing with the more potent naturally occurring estrogens for binding to the estrogen receptor. Due to this, soya consumption may reduce the risk for estrogen-dependent cancers.

South east Asian population who consume 20-80 mg of genistein per day are found to have significantly lower incidence of breast and prostate cancer. Genistein has been reported to be a potent and specific inhibitor of protein tyrosine kinase. Genistein also inhibits DNA topoisomerase II activity, alters cell cycle specific events, induce apoptosis and inhibits angiogenic process which is essential for tumour growth.

F. *Tomato Lycopenes*

Lycopene is a carotenoid principle present in lycopersicon family Solanaceae known throughout the world as tomato. Clinical studies have indicated that lycopene significantly lowered the risk of prostate cancer. The candidates that consumed processed tomato products about 10 times per week had less than one half the risk of developing prostate cancer.

Lycopene activity is likely to be related to its antioxidant function because lycopene has been reported to be the most efficient quencher of singlet oxygen in biological system. Lycopene has also been shown to reduce risk of other types of cancers of digestive tract, pancreas, cervix, bladder and skin. Recently it has been proved that low plasma lycopene levels may be an independent risk factor for lung cancers especially in smokers.

XII. MILK BIOLOGICALLY ACTIVE COMPONENTS AS NUTRACEUTICALS

Milk contains components that provide critical nutritive elements, immunological protection and biologically active substances to neonates. Milk proteins are currently the main source of a range of biologically active peptides. Concentrates and these peptides are potential health enhancing nutraceuticals for food and pharmaceutical applications.

Several bioactive peptides may be used as nutraceuticals, for example, in the treatment of diarrhea, hypertension, thrombosis, dental diseases as well as mineral malabsorption and immunodeficiency. Minor whey proteins such as lactoferrin, lactoperoxidase, lysozyme and immunoglobulins are considered as antimicrobial proteins. Milk also contains some natural bioactive substances. These include oligosaccharides, fucosylated oligosaccharides, hormones, growth factors, mucin, gangliosides and endogenous peptides which are present in milk at secretion.⁴

Bioactive Proteins/Peptides as Natural Ingredients of Milk

- 1) Thyrotropin-releasing hormone (TRH)
- 2) Luteinizing hormone-releasing hormone (LHRH)
- 3) Somatostatin (SIH)
- 4) Gastrin-releasing peptide (GRP)
- 5) Calcitonin
- 6) Adrenocorticotropic hormone (ACTH)
- 7) Insulin
- 8) Growth factors
- 9) Relaxin
- 10) Prolactin
- 11) Thyroid stimulating hormone (TSH)
- 12) Lysozyme
- 13) Lactoperoxidase
- 14) Lactoferrin
- 15) Transferrin
- 16) Immunoglobulins (IgA, IgM, IgG)
- 17) Enzymes (eg. Plasmin)

XIII. MELATONIN CONTENT IN GRAPE

The discovery of melatonin in plants has opened up a new field of knowledge in the food and nutritional sciences. It has been found in edible plants, medicinal herbs and seeds. Its role in plants varies from regulation of seasonal and circadian rhythms to antioxidant defence against environmental stresses. Particularly in germ tissues of seeds and flowers and also in growth and development regulation, the same as the plant hormone auxin (indole-3-acetic acid) and indolic compounds.

Moreover, auxins and their derivatives such as indole-3-methanol (or indolcarbinol), in addition to this role as plant hormones, exert antioxidant, anticarcinogenic and antimutagenic properties, thus enhancing the chemopreventive potential of plant-derived food stuffs. From the nutraceutical point of view, it has significant synergistic antioxidant effect with polyphenols.

Melatonin, which is also present in grape extract of some *Vitis vinifera* cultivars, particularly Nebbiolo, Croatina, Cabernet Sauvignon, Sangiovese and Merlot. Its concentration determined with a modified high-performance liquid chromatographic (HPLC) method.

A. Melatonin Extraction for HPLC Analysis

Kolar et al. method proved to be the most reliable in detecting melatonin. It was further improved with the following modifications. Frozen grape skin (5g) was crushed in a mortar with liquid nitrogen, placed in 50ml polypropylene tubes with 10ml of extraction buffer [1mol L⁻¹ Tris-Hydrochloric Acid buffer, pH 8.4, 0.4 mol L⁻¹ perchloric acid plus 0.1% EDTA, 0.05% sodium thiosulphate and 10mol L⁻¹ ascorbic acid]. Extraction was achieved by incubation on an orbital shaker for 1 hour room temperature after 15min of sonication. After centrifugation (10000g, 10min, 40C) and filtration through nylon cloth, a system of two C18 Sep-Pak cartridges connected in series was utilized to purify and concentrate melatonin, which was finally eluted with methanol. Samples were stored in a freezer at -200C until use. All the above extraction steps were performed in disposable vessels, which were rinsed in concentrated nitric acid and autoclaved at 1200C for 15 min before use, to avoid contamination.

B. Determination of Melatonin by HPLC with Fluorimetric Detection

Fluorescence detection (FD) was suitable for melatonin detection in grape skin extracts owing to the characteristic fluorescence of indolic compounds at specific excitation and emission wavelength.

HPLC analysis was performed on an LC-10A Dvp, SIL-10A Dvp HPLC system with a SPD-10AVP and Rf-10 A¹ detectors.

The HPLC pumps, auto sampler and detectors were controlled by class VP 3.4 software. A Phenomenex Luna RP C18 column (250 × 4.6mm i.d, particle size 5mm) provided with a guard column obtained from Chemtek Analytica, was employed for all the analyses at room temperature (24°C). The mobile phase consisted of a mixture of sodium acetate (10m mol L⁻¹), adjusted to PH 4.5 with concentrated acetic acid, Na₂ EDTA (0.01mmol L⁻¹) and 200 mL L⁻¹ acetonitrile, delivered iso cratically at a flow-rate of 1mL min⁻¹.

A Volume of 20ml of each sample solution was injected. Fluorimetric detection was recorded at $\lambda_{ex} = 280\text{nm}$ and $\lambda_{em} = 345\text{nm}$ and UV detection at a wave length of 310 nm. Under these chromatographic conditions, melatonin was eluted from the column in a range of 18 - 20min.

C. Spectrophotometric Determination of Melatonin

As melatonin and its derivatives are able to generate chemiluminescence in acidic potassium permanganate solution and formaldehyde. UV spectra were detected after the addition of grape skin extracts of (a) 1 mol L⁻¹ sulfuric acid, (b) 6 × 10⁻⁴ mol L⁻¹ potassium permanganate, 30% formaldehyde and 1 mol L⁻¹ sulfuric acid; and (c) 1.2 × 10⁻³ mol L⁻¹ potassium permanganate, 30% formaldehyde and 1 mol L⁻¹ sulfuric acid.

XIV. RESULTS

HPLC analysis of grape skin extracts obtained with the modified Kolar et al method and using a calibration curve with six different standard melatonin concentrations (10, 50, 100, 200, 500 and 1000 pgmL⁻¹) allowed the identification of a characteristic peak, very likely corresponding to melatonin. Its identification was obtained by an overlay of standard and sample chromatograms (Fig. 2(a)) and also of samples with or without standard enrichment (Fig. 2(b)).

Chemiluminescence detection confirmed the presence of melatonin in grape skin samples (Fig. 3) two characteristic absorption bands, at 225 and 281 nm, were observed in presence of 1mol L⁻¹ sulfuric acid (curve 'a' in Fig.3) further more, the addition of potassium permanganate and formaldehyde oxidative milieu considerably modified the absorption band at 281nm, as expected (curve 'c' in Fig. 3).

XV. MARKET INTEREST OF NUTRACEUTICALS

The nutraceuticals industry is still in its formative period, and at present there is no universal agreement or legal definitions of the terms and designations used by this industry sector. According to the widely accepted definition, "A nutraceutical is any substance that is a food or a part of a food and provides medical or health benefits including the prevention and treatment of disease." Products include isolated nutrients, dietary supplements and processed foods such as cereals, soups, soyfood, and beverages. The nutraceuticals market comprises two principal segments: Functional Foods and Dietary Supplements.

Functional foods are similar in appearance to a conventional food or beverage, are consumed as part of a normal diet, and have been demonstrated to have physiological benefits or to reduce the risk of chronic disease beyond basic nutritional functions. Functional foods can also promote growth and development and enhance performance, and can take many forms. Some may be conventional foods with bioactive components that can now be identified and linked to positive health outcomes (e.g., soy protein, oat fiber, cranberries, tomatoes and carrot juice). Some may be fortified to enhance foods or specifically created to reduce disease risk (e.g., vitamin- and mineral-fortified cereal, folate-fortified flour and grain products, calcium-enriched orange juice or milk, phytosterol-fortified spreads). Dietary supplements are foodstuffs that are intended to supplement the normal diet and that are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in pharmaceutical dose form and administered orally. Dose forms include capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities. Dietary supplements contain all products that can be purchased by the consumer without a prescription. The nutraceutical market is becoming more competitive with the entry of pharmaceutical and major food companies into the nutraceutical arena. Also, many food companies have established their nutraceutical divisions with a view toward a diversified product line. Pharmaceutical companies have also joined the race by acquiring dietary supplement producers. Recent years have marked the entry of major food and pharmaceutical companies into the nutraceutical marketplace, including Kellogg, Heinz, M&M, Quaker Oats, Unilever, Cargill, Hormel, Glaxo-SmithKline, Warner-Lambert, Johnson & Johnson and Wyeth.



The 2004 global nutraceuticals market at the retail level is estimated at approximately \$106 billion and is poised to grow at a compounded annual growth rate of 6.0% during 2004–2009 to exceed \$140 billion in 2009. Functional foods represent the largest and fastest growing segment worldwide, with sales estimated at \$77 billion for 2004 and projected to reach \$103 billion by 2009. The functional food segment is expected to retain its leading position to 2009.

A. Marketed Products

ABC & Tea	Aloe C	Vitrin
Testron	Symbiotropin	vivaxl

XVI. CONCLUSION

Nutraceuticals are food supplements and have nutritional value. The present junk foods will not provide any nutritional value, rather it adversely effect the body. Hence it is concluded that nutraceuticals can be recommended as a regular part of the diet.

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