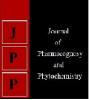


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Nutraceuticals in vegetables: New breeding approaches for nutrition, food and health: A review

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Abstract

Vegetables are major source of biologically active nutraceuticals and playing an important role in human diet. It has been explored recently as sustainable alternatives for the control and prevention of many diseases. They have received considerable attention in diet because are safe, efficacious, have potential nutritional value as well as therapeutic effects. Among natural dietary supplements, vegetables being low in calories are enriched with vitamins, minerals, antioxidants and phytochemicals could be explored at maximum level to ensure healthy environment. Some of the common phyto-nutraceuticals such as lycopene from tomato, carotenoids from carrot etc. play vital role in disease prevention/reduce disease risk factors through antioxidant activities. Functional attributes of many traditional vegetables are being discovered, while new food products are being developed with additional nutraceutic components. Therefore the plant substances which are important in human nutrition must be clearly identified and should be intend to breed cultivars with improved nutritional attributes through conventional and molecular breeding approaches.

Keywords: Approaches, nutrition, food and health

1. Introduction

Nutraceuticals are any substance that is a food or a part of a food and provides medical or health benefits, including the prevention and treatment of diseases. The term "Nutraceutical" was coined by combining the terms "Nutrition" and "Pharmaceutical" in 1989 by Dr Stephen De Felice, Chairman of the Foundation for Innovation in Medicine. (Faruque *et al.*, 2013). A product isolated or purified from foods that is generally sold in medicinal forms not usually associated with food. A nutraceutical is demonstrated to have a physiological benefit or provide protection against chronic disease (Gupta *et al.*, 2013; Sarin *et al.*, 2012) ^[16, 37].

Vegetables are functional foods nutraceuticals because they provide minerals and nutrients which are health promoting. They play an important part in the human diet and are a major source of biologically active nutraceuticals. It have been explored recently as sustainable alternatives for the control and prevention of large number of diseases. They have received considerable attention because they are safe, efficacious and have potential nutritional value as well as therapeutic effects. Among natural dietary supplements, vegetables being low in calories are packed with vitamins, minerals, antioxidants and phytochemicals.

Some popular phyto-nutraceuticals include lycopene from tomato, carotenoids from carrot etc. Such compounds play a role in disease prevention/reduce disease risk factors through antioxidant activity. Functional attributes of many traditional vegetables are being discovered, while new food products are being developed with additional nutraceutic components. Therefore the plant substances important to human nutrition must be clearly identified and should be intend to breed cultivars with improved nutritional attributes through conventional and molecular breeding approaches (Rai *et al.*, 2012) ^[33].

2. Phytonutrients and Nutraceuticals from Vegetables

The phytonutrients can be classified into various groups on the basis of alike protective functions as well as individual physical and chemical characteristics of the molecules. It is important to note that all classes of phytonutrients are required to be consumed for a sound and healthy body. Focusing neutraceuticals, there is greater potential for opportunities that are new, novel as well as exciting. The following is a list of important phytonutrients and their useful medicinal values.

Anthocyanidins: Anthocyanidins are a type of flavonoids, also known as flavonals, which provide cross-links that hook up and strengthen the intertwined collagen protein strands found

in tissues, tendons, ligaments and bone matrix. These also act as free radical scavenger in tissue fluids (Jeong *et al.*, 2013) ^[19]. In human beings, risk of myocardial infarction is reduced by taking high amount of anthocyanins (Cassidy *et al.*, 2013; Al-sane and Perata, 2011) ^[6, 1].

Carotenoids

The carotenoid family consists of carotenes andxantophylls. There are more than 600 naturally occurring carotenoids. The subclass terpenes comprises of bright red, orange and yellow pigments present in various vegetables *viz.*, tomatoes, spinach, oranges, pink grapefruit and red palm oil. Carotenes are chemically classified as 40-carbon tetraterpenes, missing the hydroxyl or keto groups (beta carotene), while xanthophylls include carotenoid alcohols and keto-carotenoids cryotpxanthin, canthaxantin, zeaxanthin and astazanthin.

Vitamin A activity is only present in alpha, beta and epsilon carotene, out of these the beta carotene is the most active (Yoon et al., 2012) [41]. The antioxidant activity of alpha carotene and epsilon carotene is 50-54 and 42-50%, respectively of the antioxidant activity of beta carotene. The alpha, beta, gamma, epsilon, lycopene and lutein carotenes have been found to be provide protection against tumors of the lung, colorectal, breast, uterine and prostate (Yoon et al., 2012) ^[41]. The overall protective effects of carotenes will be additive if taken together, since these are tissue-specific. The carotenes also augment immune response and can protect skin cells against UV radiation (Mahima et al., 2014)^[31]. These also help liver in safely eliminating pollutants and toxins from the body. Xanthophylls help in the protection of vitamin A, E and various other carotenoids from oxidation. Xanthophylls especially canthaxantin migrates to the skin and protects it from sunlight. The cryptoxanthin has been shown to protect vaginal, uterine and cervical tissues.

Catechins: The chemical structure of catechins is slightly different from other flavonoids but has the same chemo protective activities. Green tea (*Camellia sinensis*) is rich in catechins (Scoparo *et al.*, 2012) ^[36].

Flavonoids: Flavonoids constitute a subclass of phenols that improve the effects of ascorbate-vitamin C. The hesperidin found in citrus fruits, quercetin in grapefruit and rutin in buckwheat are some of the important flavonoids. These are beneficial in allergic conditions, inflammation, liver disorders, platelet aggregation, pathogens (bacteria and viruses), cancer and ulcers and acts as antioxidant. These inhibit a number of specific enzymes thereby help in preventing various diseases and maintenance of a healthy body. The flavonoids block the Angiotensin-converting Enzyme (ACE) that is responsible for raising blood pressure. The platelet stickiness and aggregation is prevented by blocking the cyclooxygenase enzyme that breaks down prostaglandins. Flavonoids are also helpful in protection of the vascular system (Dam et al., 2013) ^[10]. The risk of estrogen-induced cancers in females can be reduced by flavonoids due to blocking of certain enzymes producing estrogen. Aldose-reductase can convert the galactose sugar into the potentially harmful form of galacticol. Flavonoids may retard development of cataracts in individuals with inborn errors in sugar metabolism such as diabetes by blocking aldose-reductase (Dam et al., 2013)^[10].

Glucosinolates: These are frequently present in the vegetables of *Cruciferae* family and set in motion the

detoxification enzymes in liver, white blood cells and cytokines thereby helps in boosting immunity. The isothiocyanates, dithiolthiones and sulforaphane are the bio-transformation products of glucosinolates that are involved in blocking enzymes which are responsible for tumourous growth in liver, lung, breast and gastrointestinal tracts (Baskar *et al.*, 2012)^[4].

Indoles: Indoles include phytonutrients that interact with vitamin C and their complexes bind with chemical carcinogens. These also help in activating the detoxification enzymes. The acid in stomach helps in the formation of bio-transformation products of indoles like the ascorbigen.

Isoflavones: This is a subclass of phenol found in beans and other legumes and its function is similar to flavonoids in effectively block enzymes promoting tumor growth, the important ones include genistein and daidzein which are found in soy products and the herb, *Pueraria Lobata* (Kaufman *et al.*, 1997) ^[23]. The incidence of breast, uterine and prostate cancers is rare in people who consume traditional diets rich in soy foods.

Isoprenoids: The isoprenoids neutralize free radicals by grabbing any free radicals attempting to attach lipid membranes passing them off to other antioxidants.

Limonoids: This is a subclass of terpenes that is found in citrus fruit, a peel that is specifically directed to protect lung tissue and prevent breast cancer that responses to oestrogen (Kim *et al.*, 2012; Sun *et al.*, 2013) ^[24, 38]. The chemotherapeutic activity of limonoids may be due to induction of both Phase I and Phase II detoxification enzymes in the liver.

Lipoic acid and ubiquinone: Lipoic acid and ubiquinone (coenzyme Q) are antioxidants which can efficiently quench the hydroxyl radicals and are active on both lipids and tissue fluids and scavenges peroxyl, ascorbyl and chromanoxyl radicals. It can protect both vitamin E and vitamin C, as can function in both lipid and water phases. They can also protect catalase and glutathione, thus helpful in liver detoxification activities.

Phytosterols: Phytosterols are present in green and yellow vegetables and their seeds. These can effectively compete with dietary cholesterol absorption through intestines and thus make easy the excretion of cholesterol from the body and therefore are helpful in alleviating the risk of cardiovascular diseases (Jones *et al.*, 1997; Awaisheh *et al.*, 2013) ^[20, 2]. These have also been reported to be helpful in blocking the development of cancer in various organs especially colon, breast and prostate glands.

Phenols: Phenols are a large group of phytonutrients protecting the humans from various kinds of oxidative damages. Phenol gives the blue, blue-red and violet colorations to berries, grapes and purple eggplant. The red colour in bilberry (*Vaccinium myrtillus* L.) is due to the presence of high phenolic anthocyanidins. The phenols block specific enzymes that cause inflammation and protect platelets from clumping most likely by modification of the prostaglandin pathways.

Terpenes: These form the largest classes of phytonutrients and are commonly present in green foods, soy products and

grains. Carotenoid i.e., beta carotene is one of the most studied terpenes. The antioxidants property of the terpenes protects lipids, blood and other body fluids from attack by free radical oxygen.

Thiols: Thiols comprises of sulfur-containing phytonutrients present in garlic and cruciferous vegetables (cabbage, turnips and members of the mustard family). Allylic Sulfides subclass is abundantly found in garlic, onions, leeks, shallots and chives (Hofgen *et al.*, 2001) ^[18] and are released when the plants are cut or smashed. These possess antimutagenic and anticarcinogenic properties as well as immune enhancing and cardiovascular protective properties (Vazquezand Miatello, 2010) ^[40]. Garlic and onions activate liver detoxification enzyme systems and are also effective against tumors, bacteria, fungi, viruses, parasites, cholesterol and platelet/leukocyte adhesion factors.

Tocotrienols and tocopherols: Tocotrienols and tocopherols

naturally occur in grains and palm oil. Tocotrienols can suppress growth of breast cancer cells growth while the tocopherols have cholesterol lowering effects.

3. Enhancement of Nutraceuticals In Vegetables

Nutraceuticals isolated from major vegetable crops are enlisted in table1. The fresh fruit and vegetable industries are an important contributor to the world economy. The global market for nutraceuticals and functional foods is expanding at an exponential rate. Many nutraceuticals (vitamin C, E, carotenoids etc.) are antioxidants and have preventive roles against certain cancers and cardiovascular diseases (Scheerens, 2001) ^[34]. There is a need to enhance the health benefit properties of fresh produce which in turn will add value and create new opportunities for producers and processors by reaching the health-oriented markets. This goal can be achieved by providing technologies that can ensure the delivery of high quality products with high quantities of the desired nutraceuticals.

Table 1: Nutraceuticals isolated from vegetables	
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Chemical compound	Plant source	Properties	
Allicin	Garlic, onion, parsnip	Antifungal; antibacterial; antioxidant; used to treat arteriosclerosis	
Beta carotene	Carrots, pumpkins, sweet potatoes, winter squash, Watermelon.	Anti-aging; anti-cancerous; improve lung function; reduce complications associated withdiabetes	
Tocopherol	Broccoli, carrot, celery, onion	It is a fat-soluble antioxidant that stops the production of reactive oxygen species formed when fat undergoes oxidation	
Betaine	Green leafy vegetables	Reduces toxic buildup of homocystine	
Plant Glucosamine	Lettuce, peas, cabbage	Chondroitin and glucosamine are part of normal cartilage and act as a cushion between the joints	
Glutathione	Cruciferous vegetables	A tripeptide, which provides antioxidant properties thereby protecting the cells against damage by free radicals	
Hesperitin	Green vegetables	Anti-inflammtory	
Saponin	beans, other legumes	Reduces blood cholesterol levels and the risk of cancer	
Quercitin	Onion, broccoli, cabbage, lettuce, tomato	Useful in the treatment of progressive Alzheimer's disease; used in cancer and heart disease	
Lignan	broccoli	Antioxidants; reduce ill effects in the body as cellular destruction, aging.	
Luteoline	Cauliflower, celery, sweetpepper	A carotenoid which shows eye benefits	
Ferulic acid	Turnip	Have anti-oxidizing properties that can moisturize skin, help with light and weather damage	
Sulphoraphane	Broccoli	Used against breast cancer	
Proanthocyanin	Red cabbage, egg plant	Help in urinary tract infections by inhibiting adhesion of microorganisms like <i>E. coli</i> to the urinary tract wall	

Basically there are two methods to enhance nutraceuticals in vegetables*viz*, Controlled abiotic stress and breeding approaches.

Controlled Abiotic stress: Certain abiotic stress treatments, like temperature, UV light, wounding, phytohormones, altered gas composition, heat shock and water stress etc., will influence the secondary metabolism of fresh produce and augment the synthesis of phytochemicals with nutraceutical activity or lessen the synthesis of undesirable compounds. Abiotic stresses affect the biosynthesis of the three principal groups of secondary metabolites viz., terpenes, phenolics and nitrogen-containing compounds. Phytochemical accumulation or losses also gets affected by inducing an increase or reduction in key enzyme activities of secondary metabolic pathways (Dixon and Paiva, 1995) ^[11]. The fresh produce industry must explore the usefulness of controlled stresses to enhance the health benefit properties of fresh-cut or whole fresh produce. Similarly, the food processing and dietary supplement industries can also utilize it to obtain healthier processed products or boost nutraceutical yields. Pre-harvest

abiotic stresses have been used to enhance the quality and yield of products in the field (Kalt et al., 2001)^[21]. Exposure to higher light intensity or those with less frequent irrigation has been found to augment vitamin C production (Lee and Kader, 2000) ^[27]. An increase in pungency levels can be observed in water-stressed pepper fruits (Estrada et al., 1999) ^[12]. Postharvest abiotic stresses may affect the levels of secondary metabolites in plant tissues. The stage of ripening and temperature affects the anthocyanin accumulation and its content in certain fruits (blackberries, apples and strawberries) and also in colored potatoes during cold storage (Lewis et al., 1999) ^[28]. Wounding helped in increasing the anthocyanins and phenolic acids in red pigmented lettuce (Ferreres et al., 1997) ^[14] and phenolic compounds in carrots (Babic et al., 1993) ^[3]. Phytohormones, such as ethylene induce phenolic compounds in carrots (Lafuente et al., 1996) ^[25]. Light stimulate the accumulation of chlorogenic acid in potato tubers (Li et al., 2003) ^[29], anthocyanin in red cabbage (Craker and Wetherbee, 1972)^[9].

The genetic potential of vegetables and fruits can be boosted to yield products with potent health benefit properties by using stresses to induce accumulation of targeted phytochemicals. Controlled postharvest abiotic stresses have useful applications in terms of increasing nutraceutical levels fruits and vegetables. Post-harvest abiotic stresses such as methyl jasmonate increased the anthocyanin content in purple fleshed potatoes (Reyes *et al.*, 2001) ^[32] and differentially potentiated phenolic compounds, avoiding the accumulation of isocoumarin phytoalexins in purple carrots (Heredia *et al.*, 2001) ^[17]. Antioxidant property of lettuce was found to be increased by wounding (Kang and Saltveit, 2002) ^[22].

The abiotic stresses are not always beneficial as phytochemical loss or production of undesirable compounds has been reported after some stress treatments. Chlorophyll loss in broccoli due to temperature has been well documented. Heat treatments may reduce phenolic synthesis in lettuce (Velarde and Saltveit, 2001) ^[39]. Water stress may induce ascorbic acid loss in leafy greens (Lazan *et al.*, 1987) ^[26]. Induction of the bitter compounds, isocoumarin, in carrots (Lafuente *et al.*, 1996) ^[25] and xauthotoxin in parsnips (Shattuck *et al.*, 1988) ^[35] by the action of ethylene has been observed.

Breeding Approaches: There are different types of breeding approaches are applicable for the enhancement of nutraceuticals. In conventional breeding system, screening and selection of superior genotypes, back cross and mutation breeding can be incorporated whereas in molecular breeding approaches, marker assisted selection and introduction of transgenic crops could be a potential tool to exploit the nutraceutical properties of respected crops. The genes responsible for nutrient enrichment in vegetables are listed in Table 2.

Vegetable crops	Gene	Nutrient Enrichment
Potato	Or	β-Carotene
Cauliflower	Or	β- Carotene
Potato	AmA1	Protein
Potato	Crt B	β- carotene
Tomato	В	β- carotene
Sweet potato	asp-1	High protein
Tomato	chi-a	High flavanoids
Tomato	LC and C1	Kaempferol
Tomato	Aft, Abg	Anthocyanin
Cucumber	Ore	β- carotene
Tomato	Phytoene synthase (Psy-1)	Carotene
Red cabbage	MYB	Anthocyanin
Purple cauliflower	Pr	Anthocyanin
Sweet potato	IbMYB1	Anthocyanin
Tomato	cry-2	Lutein
Tomato	ySAMdc; spe-2	Lycopene
Potato	Dxs	Phytoene
Tomato	GCH1	Folate
Lettuce	Gch1	Folate
Lettuce	Pfe	Iron
Lettuce	Gul oxidase	Ascorbate
Tomato	hmgr-1	Tocopherols

4. Achievements through Breeding

Orange Cauliflower (Or gene): The cauliflower (*Brassica oleracea* L. var. *botrytis*) *Or*gene is a semi-dominant, single-locus mutation that induces the accumulation of high levels of β -carotene in various tissues of the plant, turning them orange. Plants that are homozygous for the *Or* gene possess an intense orange coloration in these tissues and produce small curds, whereas the heterozygous plants are less

pigmented and have normal-sized curds that provide commercial potential. The *Or*gene appears not to exert its effect via the direct regulation of carotenogenic gene expression. Instead, it may induce carotenoid accumulation by initiating the synthesis of a carotenoid deposition sink. Therefore, the *Or*gene represents a novel carotenoid gene mutation (Li and Garvin, 2003) ^[29]. The nutratical rich varieties of different vegetable crops are included in Table 3.

Orange Cucumber (Ore gene): The metabolic precursor of vitamin A, β -carotene, is essential for human health. The gene(s) controlling β -carotene quantity (Q β C) has been introgressed from Xishuangbanna gourd (XIS, possessing β - carotene; *Cucumis sativus* L. var. *Xishuangbanna nesis* Qi et Yuan; 2n = 2x = 14) into cultivated cucumber (no β -carotene; *Cucumis sativus* L.). Its mature fruit possesses an orange-colored endocarp/mesocarp, in which the quantity of β - carotene (Q β C) can reach nearly 700 µg b-carotene per 100 g flesh weight. The XIS gourd is cross-compatible with commercial cucumber, and thus should be considered a source germplasm for increasing its β -carotene (provitamin A) content for improved nutritional quality (Bo, 2012) ^[5].

Purple Cauliflower (Pr gene): Anthocyanins are responsible for the color of many flowers, fruits, and vegetables. An interesting and unique Purple (Pr) gene mutation in cauliflower (Brassica oleracea var botrytis) confers an abnormal pattern of anthocyanin accumulation, giving the striking mutant phenotype of intense purple color in curds and a few other tissues. Pr encoded a R2R3 MYB transcription factor that exhibited tissue-specific expression, consistent with an abnormal anthocyanin accumulation pattern in the mutant. Transgenic and cauliflower plants expressing the Pr-D allele recapitulated the mutant phenotype, confirming the isolation of the Pr gene. Up-regulation of Pr specifically activated a basic helix-loop-helix transcription factor and a subset of anthocyanin structural genes encoding flavonoid 3'hydroxylase, dihydroflavonol 4-reductase, and Leucoanthocyanidin dioxygenase confer to ectopic accumulation of pigments in the purple cauliflower (Chiu et al., 2010)^[8].

Table 3: Nutraceutical rich varieties of major vegetables and
phytochemicals

Crop	Varieties	Phytochemicals
Carrot	Pusa Asita	Anthocyanin
	Pusa Rudhira	Lycopene
	Pusa Nayanjyoti	Beta carotene
Radish	Pusa Jamuni	Anthocyanin
	Pusa Gulabi	Lycopene
Red Cabbage	Red Acre	Anthocyanin
Bitter gourd	Pusa Aushadi	Beta carotene
	Pusa Vishesh	Ca & Fe
	Pusa Hybrid -2	Ca & Fe
Tomato	Pusa Uphar, Pusa Rohini, Pusa	Vitamin C &
	Hybrid 2, Pusa Red Plum	Lycopene
Paprika	KTPL-19	Capsanthin

High glucosinolate broccoli: Consumption of broccoli (*Brassica oleracea* var. *italica*) is associated with a reduction in risk of prostate cancer, lung cancer and colorectal adenomas. The anticarcinogenic activity is most likely to be due to activity of the isothiocyanatesiberin (1-isothiocyanato-3 methylsulfinylpropane, IB) and sulforaphanederived, respectively from 3 methylsulphinylpropyl (3-MSP) and 4-

methylsulphinylbutyl (4-MSB) glucosinolates that accumulate in the florets of broccoli. Broccoli also contains glucosinolates with indolylside chains which degrade to provide indole compounds, such as indole-3-carbinol (I3C), which are metabolized to bioactive compounds such as $3,3\phi$ di-indolylmethane (DIM), that have also been associated with anticarcinogenic activity. However, the association between broccoli consumption, cancer risk and glutathione Stransferase genotype suggests that isothiocyanates may be of greater importance than indole compounds in modulating risk (Sarikamis *et al.*, 2006) ^[15]. Faulkner and colleagues (Faulkner et al. 1998) ^[13] crossed a wild relative of broccoli, B. villosa, which had high levels of 3-methylthiopropyl glucosinolate compared to broccoli, and showed that the F1 hybrids, which expressed high levels of 4-MSB, had enhanced ability to induce quinine reductasein cell cultures.

Protein rich potato(AmA1 gene): More than a billion people worldwide consume potatoes on a daily basis but unfortunately, the nutritional quality of potato tubers is greatly compromised because they contain less protein and are deficient in lysine, tyrosine, and the sulfur-containing amino acids. Nick named 'Protato' the protein packed genetically modified (GM) potato contains 60 per cent more protein than a wild-type potato and has increased levels of several amino acids. The AmA1 (Amaranth Albumin 1) gene from Grain Amaranthus, an edible plant, introgressed into normal potato. Also an increase in the essential amino acids such as lysine and sulfur amino acids, generally deficient in plant proteins, and also an increase in aspartic acid, glutamic acid, arginine, leucine, and isoleucine levels. On average, the increases in biomass ranged from 7-20 per cent. (Chakraborty et al., 2010)^[7].

Anthocyanin Rich Tomato: Anthocyanins are pigments that give flowers their characteristic red, purple, and blue. In vegetative tissues they can be synthesized in response to stressful events, such as high Irradiance or low temperatures, against which they can give protection acting both as a light screen and as scavengers for radical species. These compounds can also be important for human health, because of their antiallergic, anti-inflammatory, antiviral, and antioxidant activities. In particular, *Aft* is a dominant gene which has been introgressed into domesticated tomato plants by an Interspecific cross with *S. chilense*. The recessive gene *atroviolacea* (*ATV*), derived from the interspecific cross with *Solanum cheesmaniae*

5. Conclusion

Nutraceuticals have been explored recently as sustainable alternatives for the control and prevention of large number of diseases. They have received considerable attention because they are safe, efficacious and have potential nutritional value as well as therapeutic effects. Among natural dietary supplements, vegetables being low in calories are packed with vitamins, minerals, antioxidants and phytochemicals. They play an important part in the human diet and are a major source of biologically active nutraceuticals. Some popular phyto-nutraceuticals include lycopene from tomato, curcumin from turmeric, carotenoids from carrot etc. Majority of the vegetable originated nutraceuticals are claimed to possess multiple therapeutic benefits; though substantial evidence for their benefits as well as unwanted effects is lacking. The present review has been devoted towards better understanding of the phyto-nutraceuticals from different vegetables based on their disease specific indications and enhancing nutraceutical qualities of vegetables through biotechnological molecular approaches.

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