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Broccoli: An Insight into Formulation and Patentability Aspects

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Abstract

Broccoli is an edible plant which has many medicinal advantages. Chemical constituent of broccoli include sulforaphane, glucoiberin, glucoraphanin, carotenoids, myrosinase and vitamins, Sulforaphane being the most useful one. Sulforaphane is an isothiocyanate which is formed due to the activity of enzyme myrosinase on glucoraphanin. Broccoli can be used for treatment of cancer, skin disease, arthritis, diabetes, kidney disease, cholesterol, etc. This article highlights the global scenario of nutraceuticals and the present available formulations made from broccoli. This article also gives a brief knowledge about formulations of broccoli that have been researched. Research has been done but presently there are very less formulations that are available to the people. With the current growing populations and health trends, need for nutraceuticals like broccoli based products is increasing.

Keywords: Broccoli; Sulforaphane; Myrosinase; Formulations; Broccoli sprouts; Cancer

Introduction

Broccoli is an edible plant which is classified under the italic cultivar group of species *Brassica oleracea*. It is a type of cruciferous vegetable and belongs to family Brassicaceae. It was originated in Italy about 2000 years ago. The word broccoli comes from the Italian plural of broccolo, which means "the flowering crest of a cabbage" and is the diminutive form of brocco, meaning "small nail" or "sprout". Broccoli has large green flower head which is arranged like a tree like structure branching out from a thick edible stalk. Broccoli resembles a cauliflower and has been considered a uniquely valuable food among Italians [1].

Its common name include: Chou broccoli, common broccoli, calabrese, cruciferous vegetable, indole-3-carbinol (13c) and di-indolylmethane (DIM) [2]. There are three types of broccoli. The most familiar is Calabrese broccoli, often referred as "broccoli". It has thick stalks and green heads. Sprouting broccoli consists of large number of heads with many stalks. Purple cauliflower has head shape with tiny flowering buds. Broccoli is a cool-weather crop which grows best at an average daily temperature between 18°and 23° [3].

Vegetables are good sources of natural antioxidants and biologically active components. They help in supplying certain constituents that are deficient in other foods. Epidemiological studies indicate that a diet rich in cruciferous vegetables, such as broccoli, kale, kai choi, cabbage, Brussels sprouts and watercress, can reduce the risk from cancers [4-8]. Chemical constituents of broccoli include glucoraphanin, glucoiberin, sulforaphane, carotenoids, vitamins and myrosinase [9]. Broccoli has high vitamin C and dietary fiber. It contains multiple nutrients like diindolylmethane (DIM) and small amount of selenium which has anticancer properties [10]. Broccoli contains glucoraphanin which can be processed into anticancer compound sulforaphane.

Brassicaceous vegetables like broccoli contain high concentration of vitamins, minerals and a special group of phytochemicals sulfur-containing glucosides called glucosinolates [11]. On hydrolysis by the endogenous enzyme myrosinase (thioglucoside glucohydrolase), these compounds yield a variety of bioactive products. Depending on the chemical structure of glucosinolates and other factors like pH and temperature during enzmymic cleavage, these bioactive products like thiocyanates, nitriles, sulfate, isothiocyanate, D-glucose and oxazolidine-2-thiones are formed [12-14].

Sulforaphane [l-isothiocyanato-4-(methylsulfinyl)-butane] is one of the constituents which has being identified in broccoli as a product of enzymatic- or acid hydrolysis of the corresponding co-(methylsulfinyl)-alkyl-glucosinolate (glucoraphanin). This sulforaphane has been a great deal of interest [15,16]. Glucoraphanin (GRA), the inert glucosinolate precursor of sulforaphane (SFN), is hydrolysed by an enzyme called Myrosinase into biologically active isothiocyanate. This enzyme is present in fresh broccoli and its sprouts. The process of conversion starts soon as the fresh vegetable is chewed or otherwise any causing process that could damage the cells. Consequently, there is partial conversion even before the compound reaches the stomach. Myrosinase is found to be present in the microbial flora of the lower intestine of animals and humans, hence by the time GRA fully passes the gastrointestinal system, a significant fraction of GRA is expected to be hydrolyzed and become bioavailable as SFN [17].

Sulforaphane (SFN) (4-methylsulfinylbutyl isothiocyanate) is a naturally occurring isothiocyanate, which was first identified in broccoli extracts. It was identified as the principal inducer of quinone reductase and glutathione S-transferases activity. It also protect against carcinogens and other toxic electrophiles [15,18,19]. Like other anticarcinogenic isothiocynates, sulforaphane is a monofuctional inducer. It is the most potent inducer and the potency is enhanced due to the presence of oxygen on sulfur [20]. It was noted in many studies that, sulforaphane can reduce the incidence of a number of forms of tumor [21-24]. Sulforaphane is a promising cancer chemo protective agent and hence has attracted many researchers' interest [25].

Broccoli has many medical uses. It has a main role in the prevention and treatment of various cancers. Broccoli is generally consumed raw or after cooking. It has higher healing effects when consumed raw.

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Emily Ho, an associate professor and researcher at the Linus Pauling Institute at Oregon State University, tells The Salt, cooking broccoli for too long can destroy the enzyme that breaks down the chemicals called glucosinolates into cancer-fighting agents [26].

Traditional cooking methods such as steaming, oven cooking or microwaving affect the nutritional values as well as its texture. This nutrient loss occurs due to tissue damage and subsequent loss of glucosionolates [27-30]. Due to disruption of the vegetable tissue, glucosinolates come in contact with myrosinase from within the intra- and inter-cellular vacuoles and initiate hydrolysis. These cooking methods may partially or completely denature myrosinase. Therefore activity and cofactors of myrosinase, the temperature and acidity of medium, and concentrations of residual glucosinolates may affect the proportions of metabolites of glucosinolates produced during cooking and ingestion of cooked food [31,32].

Reported Activity of Broccoli in Various Ailments

Cancer

Epidemiological evidence has suggested that consumption of a diet rich in cruciferous vegetables reduce the risk of several types of cancers. It was also suggested that it can reduce the risk of chronic degenerative disease. Foods such as blueberry and broccoli contain high levels of antioxidants. These antioxidants are the chemicals that can reduce the cell damage which are caused by molecules called free radicals. They have been found to reduce the growth of cancer cells under laboratory conditions in a test tube [33].

Egner et al. proposed the use of broccoli sprout beverage in the rapid and sustainable detoxication of airborne pollutants. He suggested that broccoli sprouts are rich source of glucosinolate, glucoraphanin which generates a chemopreventive agent called sulforphane. Sulforaphane is an inducer of glutathione S-tranferases (GST) and other cytoprotective enzymes. 12 week randomized clinical trials were done on two hundred and ninety one study participants. These participants were exposed to substantial levels of airborne pollutants. Urinary excretion of mercapturic acids of the pollutants, acrolein, benzene and crotonaldehyde were measured before and after the intervention of broccoli diet. Significant increase in the levels of excretion of the glutathione-derived conjugates of benzene, acrolein but not crotonaldehyde was found in those participants who received broccoli rich diet. Thus intervention with broccoli sprouts enhances the detoxication of air borne pollutants [34].

Munday et al. studied the inhibition of urinary bladder carcinogenesis by broccoli sprouts. He suggested that isothiocyanates are cancer chemoprotective agents and broccoli is a rich source of isothiocyanate. In their experiment they induced bladder cancer in rats by N-butyl-N-(4-hydroxybutyl) nitrosamine. On administration of aqueous extract of broccoli sprouts, they observed that there was a significant and dose-dependent inhibition of bladder cancer development. They also observed that the extract inhibited the incidence, multiplicity, size and progression of bladder cancer. The extract caused the induction of glutathione S-tranferase and NAD(P)H:quinine oxidoreductase 1 in the bladder which are protectants against oxidants and carcinogens. High bioavailability and rapid urinary excretion of isothiocyanates led to its selective deliverance to the bladder epithelium where it prevented bladder cancer [35].

Further Pappa et al. studied the proliferation of human colon cancer cells *in vitro* by quantitative combination effects between sulforphane and 3,3'-diindolylmethane. They proposed that isothiocyanates (ITCs) and indoles which are derived from cruciferous vegetables possess growth-inhibiting and apoptosis-inducing activities in cancer lines *in vitro*. In their experiment they studied the impacts of defined combinations of SFN and DIM (ratio 1:4, 1:2, 1:1, 2:1, 4:1) on cell cycle progression, cell proliferation and apoptosis induction in cultured colon carcinoma cells. Calculations were based on the method of Chou et al. They concluded that at lower concentration, antagonistic effect was observed in terms of cell growth inhibition.With increasing concentration, the antagonistic effect changed into synergistic interaction. They also concluded that SFN in combination with DIM resulted in strong G2/M cell-cycle arrest, which was not observed with either compound when given alone [36].

Formation of DIM: Figures 1 and 2 and Table 1.

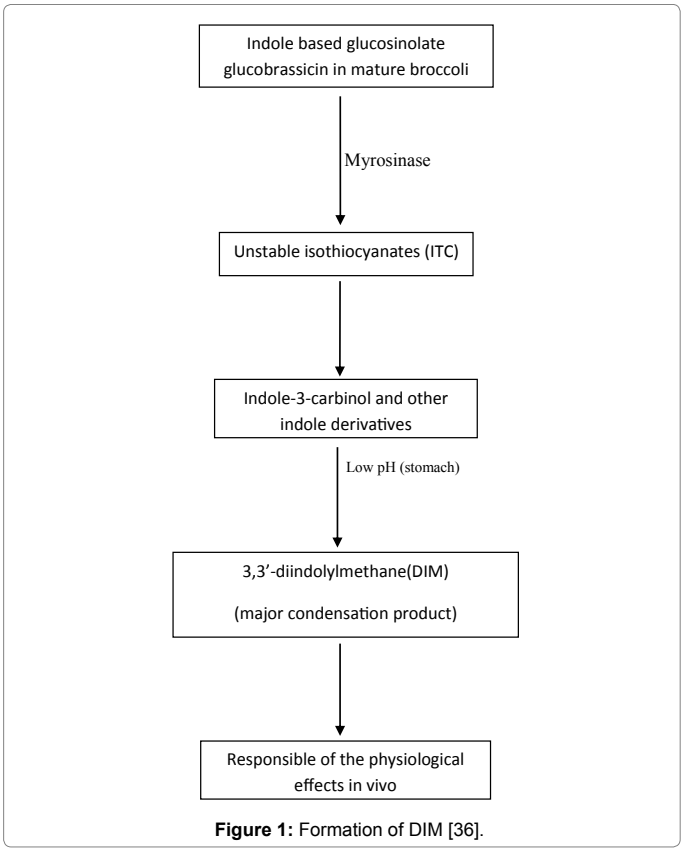

Figure 1: Formation of DIM [36].

Chemical Constituent in Broccoli	Useful in treatment of
Sulforaphane	1. Head and neck cancer
	2. Prostate Cancer
	3. Acute Lymphoblastic Leukemia (ALL)
	4. Breast Cancer stem cells
	5. Pancreatic Cancer
	6. Squamous Cell carcinoma
	7. Colon cancer
Indole-3-carbinol	1. breast cancer
	2. Cervical cancer
	3. Skin Cancer
	4. Prostate Cancer
	5. Ovarian Cancer
Diindolylmethane	1. Breast Cancer
	2. Prostate Cancer
	3. Ovarian Cancer

Table 1: Chemical constituent in Broccoli.

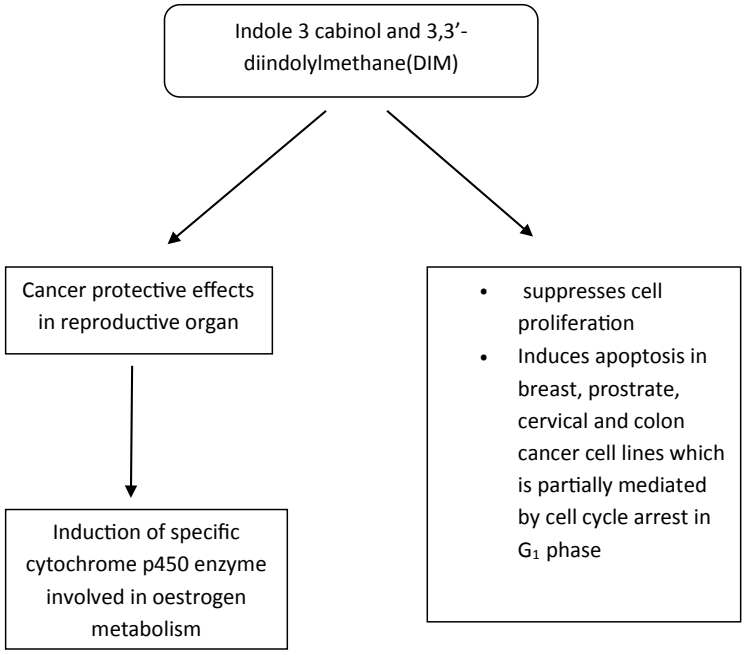

Figure 2: Action of DIM [36].

Arthritis

Broccoli is said to help in curing arthritis. Osteoarthritis is a joint disorder. It is caused by the breakdown of cartilage and bones in joints. The parts of body that are mostly affected are hands, spine, hips, knees, feet. Its symptom includes stiffness and pain [37].

In a research, it was found that the compound sulforphane that is obtained from cruciferous vegetables like Brussels sprout, red cabbage, broccoli slows down the destruction of cartilage in joints hence can be used for the treatment of arthritis. Researchers did a study on this and found that sulforphane blocks the enzyme that causes joint destruction. This study has been published in a journal "arthritis and rheumatism". The researchers found that mice fed with sulforphane rich diet suffered significantly less cartilage damage as compared to the mice that was not fed with this compound.

According to IAN CLARK, professor of musculoskeletal biology at UEA, the results from the study of sulforphane in influencing joint health are very promising. They have worked on three laboratory models, - cartilage cells, tissue, and mice. The team is planning a small trial on 40 osteoarthritis patients which will be undergoing joint replacement surgery. In this trial, 2 weeks before their surgery, half patients will be eating "superbroccoli" which will be specially bred and will contain high amounts of sulforphane. The other half will not be given "superbroccoli". After surgery the team will compare these two categories of people and will look whether the compound can be detected in replace joints or not and whether it altered joint metabolism [38,39].

Broccoli can be used for the treatment of arthritis but patients need to eat several pounds of this vegetable daily to derive its significant benefits. Sulforphane cannot be converted into a medicine because its natural form is too unstable. UK drug company EVGEN PHARMA has developed a synthetic version of chemical which is stable and offers a potential of pill treatment. Drug is called sulforadex or SFX-01. Its single dose is equivalent of eating 5.5 lb of broccoli in one day. This drug has been tested on mice and it was seen that the drug sulforadex significantly improved gait balance, bone architecture and movement. There is effective treatment for this disease other than joint replacement or pain relief hence the potential for SFX-01 is massive. Results from these studies were presented at 4th joint meeting of European calcified tissue society (ECTS) and international bone and mineral society (IBMS) in Rotterdam [40,41].

In university of Pittsburg, it was found that it could prevent throat cancer. In laboratory it was tested on mice and it was seen that broccoli extracts gave mice protection against throat cancer [40,41].

Diabetes

Diabetes is a chronic disease that is characterized by high sugar levels. It can lead to cardiovascular diseases, loss of vision, damage to retina and kidney disease [42].

Hendy et al. carried out experimentation on male rats to determine the effect of broccoli on the antioxidant enzyme of kidney and liver. Fifty experimental rats were divided in five groups. The first group was kept as control. The remaining four groups were injected with Streptozotocin to induce diabetes and were then kept on a standard diet of 0, 10, 20, 30% of broccoli, respectively, for two months. The results showed significant increase in the concentration of malondialdehyde (MAD) in the liver and kidney tissues of the rats getting 10, 20 and 30% of broccoli respectively. Also the concentration of enzyme glutathione S-transferase (GST) decreased in the liver tissues of these rats [43].

Xue et al. suggested the role of activation of NF-E2 related factor-2 (nrf2) in the protection of endothelial cells by sulforaphane in hyperglycemia. In the experiment carried out on the human microvascular endothelial cells, they proposed sulforaphane induced nuclear translocation of nrf2 and increased ARE-linked gene expression in the activation of nrf2. Activation of nrf2, which regulates the transcription of protective and metabolic enzymes, also increases the expression of transketolase and glutathione reductase. This

protective mechanism reverses hyperglycemia induced biochemical dysfunctioning of the endothelial cells [44].

Skin disease

According to Talalay, topical application of broccoli extract may provide long-lasting protection against UV damage and its effect even continues after the extract itself has disappeared from the system [45]. In his study, he concluded that the topical application of sulforaphane rich extract provides protection against carcinogen in humans and this protection is catalytic as well as long lasting [46].

Bird in her article wrote that the sulforaphane has the ability to upregulate the cells own defense against UV damage. This defense system of cells includes a network of cytoprotective proteins which helps to protect against the damage that is caused by toxins and carcinogens. Sulforaphane causes inhibition of activation of procarcinogens. Procarcinogens are inactive carcinogens which become activated when present in organism. Sulforaphane also causes disposal of damaged and potentially neoplastic cells by cell cycle arrest and programmed cell death. It also suppresses the inflammatory response. The team did the experiment on humans having erythema. Erythema is reddening of the skin which is caused by UV radiation. They measured erythema as a marker to evaluate the damage caused by UV. For three consecutive days, the broccoli extract containing sulforaphane was applied to the defined areas of subject's skin prior to UV exposure. The protective effect of sulforaphane was calculated from the fractional decrease in erythema. The team concluded that reduction in erythema by broccoli extract was 37.7%. They also concluded that sulforaphane doesn't remain in tissues for more than few hours but treatment with broccoli extract has long lasting effect. Sulforaphane upregulate the longer lasting proteins of cell's innate protective system and this continues to work long after the extract has been removed from system, hence helps in curing erythema [46,47].

Kidney disorders

The use of broccoli for prevention of chronic kidney disease can be summarized in the following points:

- Certain immune system disorders damage the kidneys. Broccoli being an excellent source of vitamin C keeps the immune system in shape. It can thus play a pivotal role in the prevention of kidney diseases in people suffering from immune system disorders.
- Kidney diseases cause an imbalance in the phosphorous levels in the body. Renal diet may require foods with high or low phosphorous levels. Therefore, broccoli being a low source of phosphorous can be given to patients with high phosphorous levels in blood.
- Diabetes is a leading cause of kidney disease. Broccoli is a smart carb and is high in fiber and chromium. It also regulates insulin levels. Therefore the maintenance of low blood sugar level prevents the occurrence of diabetes induced kidney diseases [48].

Formulations of Broccoli

Due to its wide role in treating enumerable diseases and conditions, broccoli based formulations have been widely researched. The need for formulating the drug rather than taking it in its natural form arose due to many reasons, which are:

- Cooking of broccoli degrades the enzyme myrosinase which is responsible for the conversion of glucosinolates into cancer fighting agents.
- Preference to eat raw broccoli (to prevent enzyme degradation) is less, especially in children who avoid green vegetables.
- Also, myrosinase enzyme can convert glucosinolates into sulforaphane-nitriles which are non-nutrients and are contraindicated for health. Hence it calls for the use of formulations containing high levels of sulforaphane but low levels of sulforaphane-nitrile.

Marketed formulations

Source naturals broccoli sprouts tablets (Figure 3):

Supplement Facts

Serving Size 2 Tablets

Servings Per Container 30

	Amount Per Serving	%DV
Calories	5	
Calcium	354 mg	35%
Broccoli Sprouts Standardized Extract	500 mg	†
Yielding 2,000 mcg Sulforaphane		

†Daily Value not established. [49]

Other ingredients: dibasic calcium phosphate, stearic acid, hydroxypropyl cellulose, microcrystalline cellulose, silica, and modified cellulose gum.

Figure 3: Natural broccoli sprout tablet [49].

- Sulphoraphane, an isothiocyanate, is believed to stimulate enzymes in the body.
- Freshly germinated broccoli sprouts contain from 30 to 50 times the concentration of isothiocyanates as mature broccoli.
- Provides 2,000 mcg of sulforaphane daily, equivalent to eating more than a pound of fresh broccoli [49].

Sulforaphane (from broccoli) capsules (Figure 4):

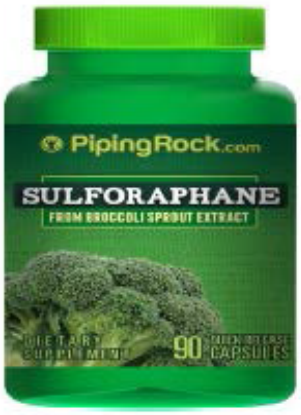

Figure 4: Sulforaphane capsule (Contains 400 mcg per capsule) [50].

- Helps fight cell-damaging free radicals.

Ingredients: Sulforaphane (from broccoli sprout concentrate standardized to 0.1% sulforaphane). Other Ingredients: Gelatin Capsule, Rice Powder, Vegetable Magnesium Stearate, Silica.

Directions: For Adults, take one (1) quick release capsule per day with water. Made in United States [50].

Bulk supplements pure broccoli extract powder (1 Kg): Broccoli extract powder is a yellowish powder which is derived directly from the essence of broccoli flowers and stems. It contains sulforaphane which gives the powder its yellow hue. This compound can also be found in all kinds of vegetables such as broccoli, cauliflower, bok choy, cabbage, mustard, turnips, etc. [51] (Figure 5).

Broccoli liquid extract, organic broccoli (*Brassica oleracea*) sprouting seed tincture herbal supplement (4 oz) (Figure 6):

- Highest Strength, Super concentrated Broccoli liquid extract.
- Super concentrated Broccoli extract: dry material/ menstruum ratio 1:3.

Ingredients: Cerified Organic Broccoli (*Brassica Oleracea*) Sprouting Seed.

Directions: Shake well before use. 20-30 drops, three times a day or as directed by your practitioner. The best way to use liquid herbal tinctures is to put the suggested amount in a glass of water, tea or juice and drink the entire contents. Tinctures can also be administered directly into the mouth without the assistance of water however some

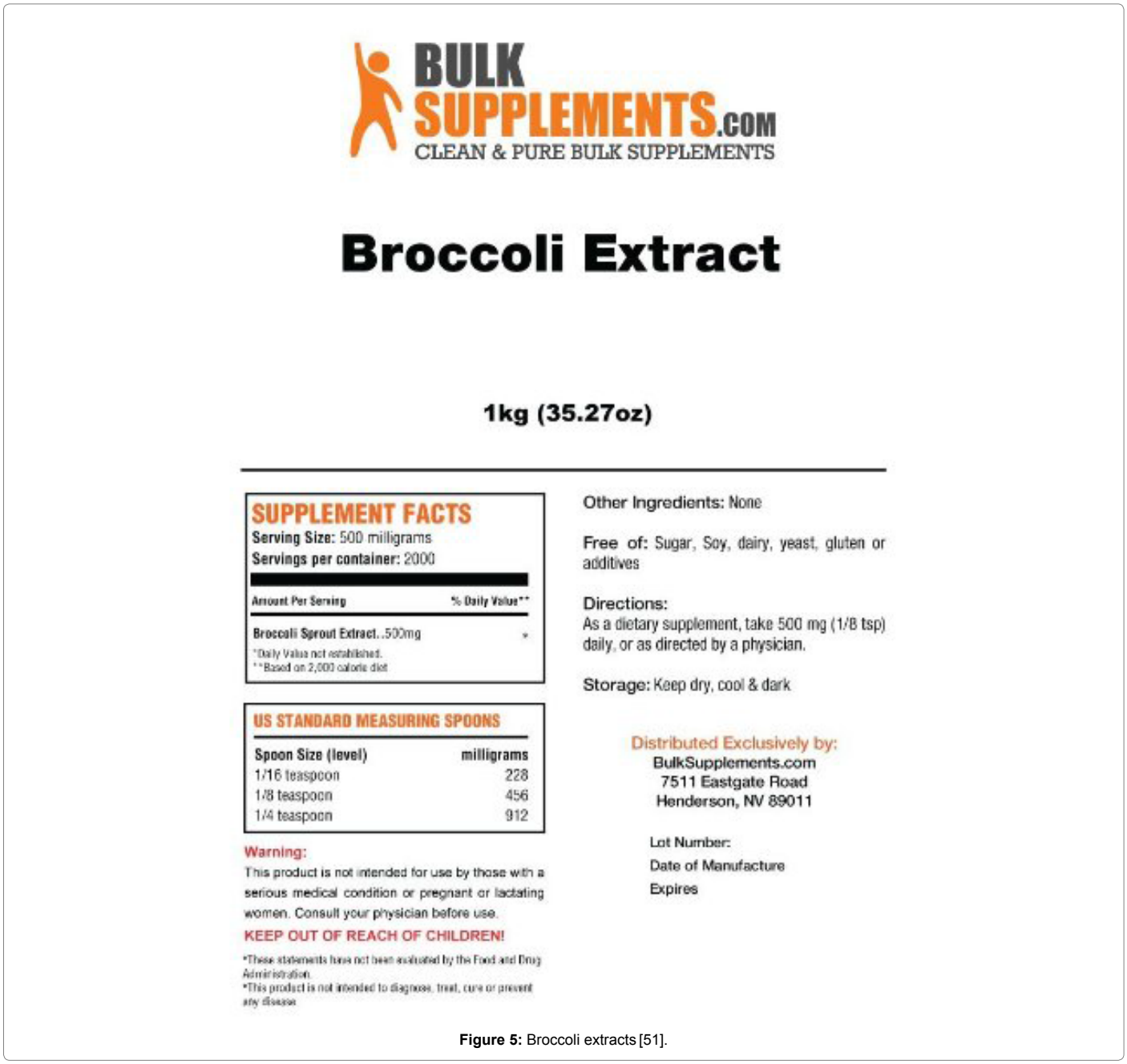

Figure 5: Broccoli extracts [51].

Figure 6: Broccoli liquid extract [52].

tinctures are unpalatable which is why most people prefer to dilute them into a beverage [52].

Formulations on which research has been done

Gold nanoparticles: The present scenario is related to the use of gold nanoparticles as effective drug delivery system. Gold nanoparticles are non-cytotoxic, have tunable physical and chemical properties and large surface area which provide a ready surface for targeting molecules and specific biomarkers. Thus gold nanoparticles have a huge scope in selective targeting and improved therapeutic effect of anti-cancer drugs. They also play pivotal role in enhancing the stability and hence the effect of the otherwise labile drugs.

Khoobchandani et al. coated gold nanoparticles with the phytochemicals of broccoli and studied it's *in vitro* anticancer activities against prostrate (PC-3), breast (triple negative MDA-MB-231, T47D and SkBr3) and multiple myeloma (U266) cancer cell lines. When compared with broccoli cell extract, B-AuNPs (Broccoli gold nanoparticles) showed maximum cell growth inhibition against cancer cell lines. Being highly porous and metabolic in nature, cancer cells internalize solutes rapidly in comparison to normal cells via enhanced permeability and enhanced permeability retention time (EPR). B-AuNPs are thus rapidly and selectively delivered into the cellular matrix of cancer cells, where they inhibit growth and show strong antiproliferative action [53].

Thus the use of gold nanoparticles for the delivery of sulforaphane and other phytoconstitiuents of broccoli helps in improving their stability and the bioavailability.

Magnetic gold nanoparticles: The use of magnetic nanoparticles in biomedicine for targeted drug delivery, diagnosis and as contrasting agent holds great potential. Cancer therapy using targeted drug delivery systems helps to specifically destroy cancer cells without any effect on normal cells. This helps in prevention of undue side effects related to cancer therapy. Also, sulforaphane being instable and sensitive to oxygen, heat and alkaline condition, is difficult to distribute in food and pharmaceutical industry.

Manjili et al. combined sulforaphane with PEGylated gold coated Fe_3O_4 magnetic nanoparticles. They then studied the effect of the conjugated drug system in the breast cancer cell line MCF-7 and compared it with the effect of sulforaphane alone and bare nanoparticles. The results showed that the loading of sulforaphane with PEGylated gold coated Fe_3O_4 magnetic nanoparticles led to augmentation in the cytotoxic effect of the drug. This effect was more enhanced than the controls (sulforaphane alone and bare nanoparticle). Furthermore, determination of the loading efficiency and *in vitro* of sulforaphane showed that its releasing rate was much higher in acidic condition rather than normal pH. This showed that this nanoparticle system would be more effective in the acidic environment of cancer cells. The nanoparticle system also enhanced the therapeutic effect of sulforaphane by promoting drug mediated conversion of BAX, Bcl-2 and MMP-9 gene expression to induce apoptosis and suppress metastasis.

Manjili et al. then concluded that the use of magnetic gold nanoparticle based DDS helped overcome the instability and short half-life of sulforaphane along with increased the cytotoxic effect of the drug on breast cancer cells [54] (Figure 7).

Microsphere encapsulation: It is a non-toxic, particulate drug delivery system that offers slow and sustained release of drugs which are otherwise unstable and are metabolized at faster rates.

Sulforaphane is unstable and has short half-life (<2 h). It is also rapidly metabolized through mercapturic acid pathway to first form a glutathione-sulforaphane conjugate and then N-acetylcysteine-sulforaphane conjugate.

Do et al. encapsulated sulforaphane with albumin microspheres by spray drying and carried out *in vivo* studies in melanoma tumor-bearing C57BL/6 mice. The results showed an increase in therapeutic efficacy and decrease in side-effects. This can be rooted to the sustained release of the drug in the cancer cells. Initially, the inhibitory effect of sulforaphane solution was more than the microspheres. This can be attributed to ready availability of the drug from the solution to the cancer cells. But after 4 week post treatment time, microspheres showed 15% more inhibitory effect on the tumor growth due to sustained and prolonged release of the drug into the cancer cells [55].

Soy-tomato-broccoli sprout beverage: Riddle et al. did his experiment on maximising sulforaphane delivery and sensory acceptability of a novel SOY-TOMATO-BROCCOLI SPROUT beverage. His group first developed a functional beverage of tomato juice and soy isoflavone. This beverage contains lycopene and isoflavones which has anticancer properties. He then incorporated broccoli sprouts which contain high levels of isothiocyanates into soy-tomato beverage to enhance the anticancer properties. The goal of this project was to develop a formulation for this beverage that maximizes potential cancer preventative effects while retaining sensory acceptability [56].

In 2006, Matusheski et al. discovered that epithiospecifier protein (ESP) is present in broccoli, which can decrease the sulforaphane formation by converting its precursor glucophanin into sulforphane nitrile which doesn't have anticancer properties. Heating broccoli can inactivate ESP as well as myrosinase enzyme [57] (Figure 8).

Matusheski et al. discovered that myrosinase is thermally more

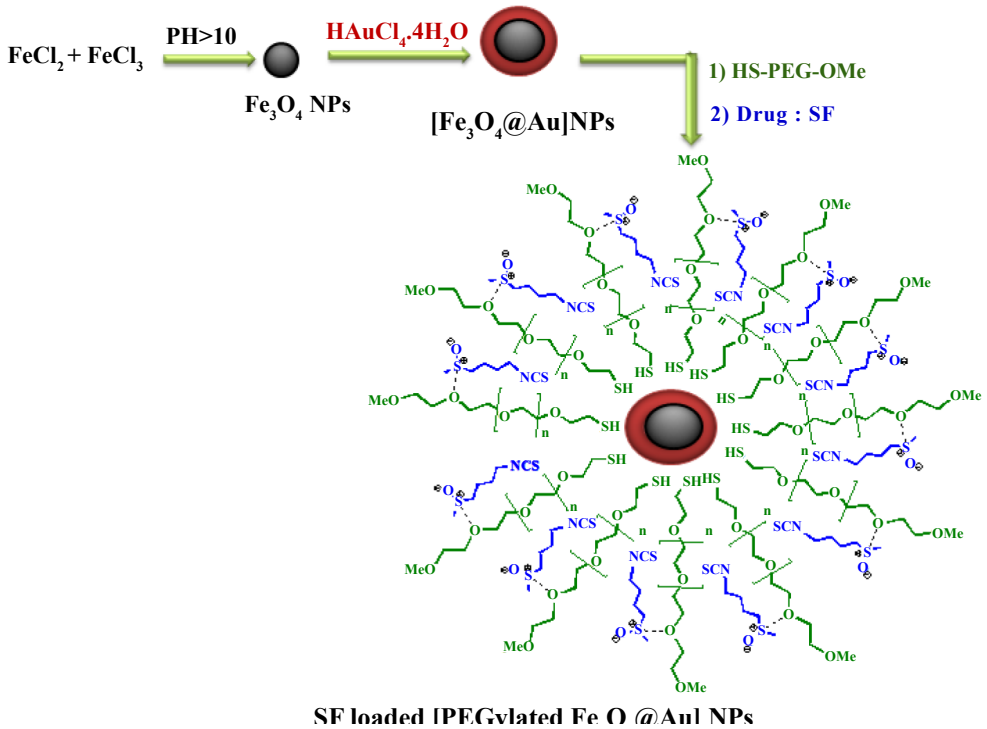

Figure 7: Representation of SF loaded [PEGylated Fe_3O_4@Au] Nps preparation [54].

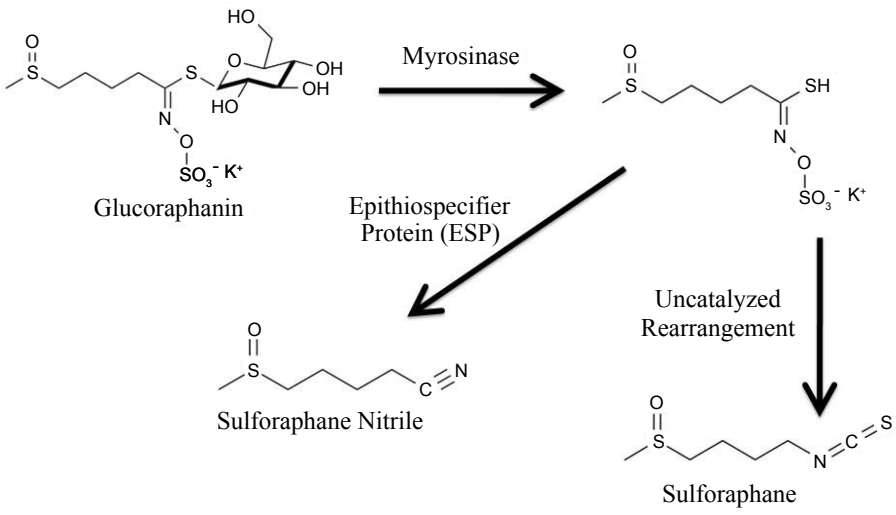

Figure 8: Formation of sulforaphane [56].

stable than ESP and heating broccoli at 60°C for 10 minutes can inactivate ESP without inactivating myrosinase hence increasing the amount of sulforaphane formed *in vitro* [56,57].

Shikita et al. [58], Shapiro et al. [59], Matusheski and Jeffery [60] proposed that daikon radish sprouts are a natural abundant source of myrosinase, hence when be combined with broccoli will contribute natural myrosinase. Based on the observations they developed four prototypes of freeze-dried broccoli sprout powder that can be mixed with soy-tomato juice.

1. Raw broccoli sprouts (RP),
2. Steamed broccoli sprouts (SP),
3. 60°C pretreated broccoli sprouts (60P), 4) 90% steamed broccoli sprouts with 10% raw daikon radish sprouts (SDP).

They then performed a sensory experiment to determine which of these powders would provide the beverage the best compromise between sensory acceptability and cancer preventative potential. They also found the concentration of broccoli sprout powder that is to added to juice before the sensory acceptability is significantly hampered. They concluded that the extent and rate of sulforaphane formation in soy-tomato juice depends on the way the broccoli sprouts are heated.

Mixture of broccoli sprouts and raw daikon radish sprouts (SDP) showed a delayed release of sulforaphane indicating that this beverage may provide the healthful benefits of active myrosinase without providing the pungency as in raw broccoli sprouts of broccoli sprouts which was preheated at 60°C. They further concluded that 5% SDP to soy-tomato juice represents the best prototype that provides the best compromise between maximising ITC delivery and providing sensory acceptability [56] (Figure 9).

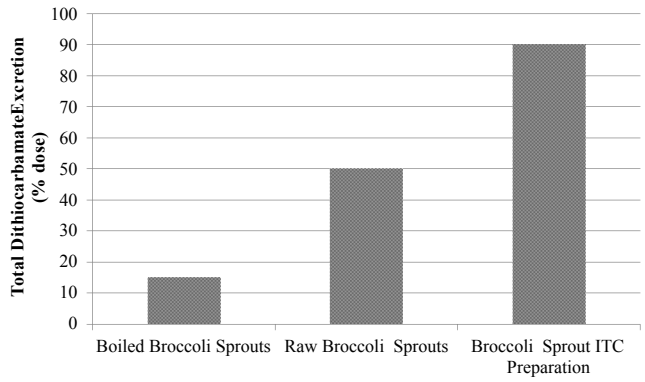

Figure 9: Bioavailability of isothiocyanates and glucosinolates in cooked and raw broccoli. Adapted from Shapiro et al. [56].

Complex of sulforaphane with hydroxypropyl-β-cyclodextrin: Wu et al. prepared a complex of sulforaphane with hydroxypropyl-β-cyclodextrin in order to enhance the stability of sulforaphane. The complex was prepared by co-precipitation method and inclusion ratio was found to be 1:1. He concluded that stability of the inclusion complex against heat alkaline conditions, oxygen was greatly enhanced and this method was effective in maintaining the anticancer and antioxidant functions of sulforaphane. Further he proved the formation of the inclusion complex by FTIR, ^{1}H NMR and UV/visible spectroscopy [61].

Combinations of aspirin, curcumin and sulforaphane: Yang et al. combined the chemopreventive agents in nanoparticles for cancer prevention. He incorporated aspirin and curcumin in nanoparticle and administered orally, in combination with sulforaphane [62]. Aspirin inhibits cyclooxygenase and has anti-inflammatory activity whereas Curcumin is known to inhibit NF-κB [63,64]. Sulforaphane causes induction of phase II xenobiotic-metabolizing enzymes, antioxidant enzymes, and other detoxification enzymes [65].

Grandhi et al. did their study on combinatorial nanotechnology based oral delivery of chemopreventive agents in suppression of pancreatic carcinogensis. The experiment was done on hamsters and cancer was induced by *N*-nitroso-bis(2-oxopropyl)amine. They used a combination of aspirin, curcumin and sulforaphane and found that these agents significantly reduced tumor multiplicity, tumor incidence and severe histological lesions. They concluded that when aspirin and curcumin were delivered in solid-liquid nanoparticle in combination with sulforaphane solution to hamsters, the effective inhibitory dosage were reduced by a factor of 10 as compared with the sulforaphane mixture in free form [66] (Figure 10).

Patents of Broccoli So Far

Broccoli based nutritional supplements [67]

Patent Pub. No.: US 2013/0323225 A1; Inventor: Kean Ashurst, Taylorsville, KY (US).

Two broccoli based components are combined to provide nutritional supplements. When ingested, they provide sulforaphane by reacting in the intestines. These two broccoli components are treated separately to remove oils using supercritical fluid extraction. The temperature and pressure of the process are controlled so that the broccoli components have the desired properties for nutritional supplements. Process for producing broccoli components is as follows:

The first material is produced from broccoli seeds or sprouts and it comprises of glucoraphanin rich meal. The second material consists of broccoli seed which have been treated to deactivate the ESP while maintaining the activity of myrosinase. Both the broccoli materials are combined in an acid resistant or enteric coated capsule to avoid the release of contents in stomach. When ingested, glucoraphanin in the first material is hydrolyzed by myrosinase in the second material to release sulforphane. Ascorbate may also be added to the nutritional supplement to enhance the conversion. The present invention also relates to an improved process. It provides a source of myrosinase for nutritional supplements. In this process the broccoli seeds are first prepared for extraction of oils by flaking and rolling techniques. The extractions of oils were done by these two techniques. These techniques stretch the cells and allow access of carbon dioxide to the cells.

The first quantity of flake rolled broccoli is deoiled by SCFE to remove a large quantity of the oil in the seed by using techniques described in U.S. Patent Application No. 2009/0081138. This process produces a seed meal having 1-2% or less oil. The second quantity flake rolled broccoli is partially deoiled by SCFE to deactivate the ESP while preserving myrosinase. The deoiled broccoli seed may be combined with glucoraphanin rich meal to provide a source of sulforaphane when ingested.

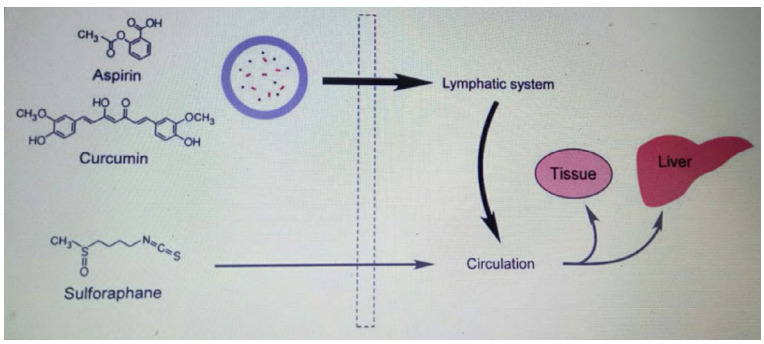

Figure 10: Absorption of aspirin and curcumin in solid–lipid nanoparticles in combination with sulforaphane [62].

Broccoli line M7009 [68]

Patent Pub. No.: US 7351884 B2; Inventor: Robert Barham, Gilroy, CA (US); David Joynt, Hollister, CA (US).

Broccoli is a cool weather crop. If exposed to warm temperatures (>75° F.) for extended periods of time causes the broccoli head to become rough with uneven flower bud sizes and thus commercially unacceptable. As a result, it can only be grown in areas having cool weather conditions. Barham et al. produced heat tolerant broccoli plants. These plants are capable of producing a commercially acceptable broccoli head under heat stress growth conditions. He exposed the plant to a maximum temperature of 90°F for at least 5 consecutive days during the growth cycle. For at least one day during the growth cycle, plants are exposed to a maximum temperature of at least 95°F. For at least 15 days during the growth cycle, when the plants are exposed to a maximum temperature of 85° F. For at least 25 days during the growth cycle, when the plants are exposed to a maximum temperature of at least 75°F. The plants are exposed to a maximum temperature of at least 80°C for at least 20 days during the growth cycle and other heat stress growth conditions. This invention is further directed to broccoli plants having all of phenotypic characteristics of the plants produced from heat tolerant broccoli seeds. It is further directed to biological material that is isolated from the plants. These materials include the gene involved in heat transfer. It is further directed to the plants and seeds produced from crossing other broccoli lines with the plants grown from the heat tolerant broccoli seeds. This invention further directs the method of breeding heat tolerant broccoli lines (Figure 11).

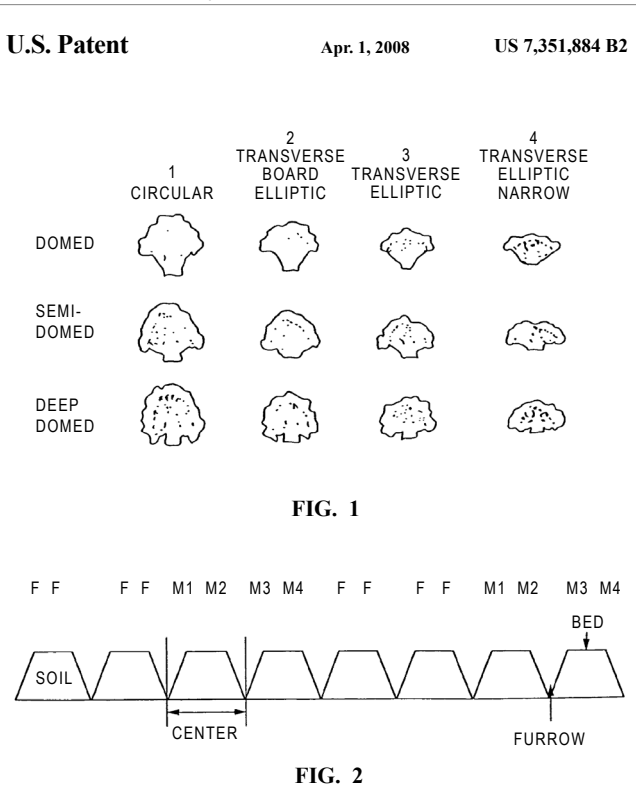

Figure 11: It shows various broccoli head shapes. Broccoli heads are referred to as domed, semi-domed and deep-domed. The shapes of the various domes are (1) circular; (2) transverse broad elliptic; (3) transverse elliptic and (4) transverse elliptic narrow. It shows a cross section of eight 40-inch beds utilized for field production of the hybrid seed of this invention. In this diagram: F=female line seed-line; M1=first male planting seed-line; M2=second male planting seed-line; M3=third male planting seed-line and M4=fourth male planting seed-line [68].

Broccoli trimming machine [69]

Patent Pub. No.: WO 1989 001300 A1; Inventor: Wylie John V; Lewis David K.

Broccoli trimming machine is used for cutting or scalping broccoli heads. It is used in such a manner that it yields separated broccoli fleurettes of substantially uniform size and shape (Figure 12).

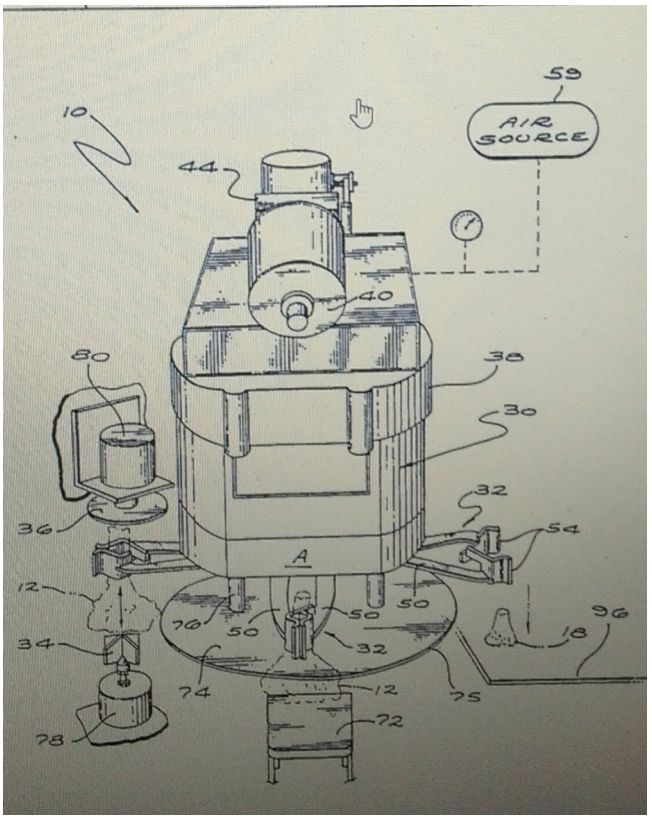

Figure 12: Broccoli trimming machine [69].

Broccoli plants tolerant to hollow/split stem disorder [70]

Patent No.: US 8,030,550 B2; Inventor: Van Den Bosch Franciscus; Boon Meinardus. Van den Bosch et al. invented seeds and plants of high yield and quality with tolerance to hallow/split stem disorder. Their invention further provides seed and plants of the broccoli lines designated BRM53-3915 and BRM53-3916 and progeny produced with at least one of these plants as a parent. His invention also relates to the seeds, plants and tissue cultures of hybrid broccoli variety RX05951180.

Broccoli hybrid RS05151611 and parents thereof [71]

Patent No.: US 8,759,621 B2; Inventor: Carl E. Mero, Arroyo Grande, CA (US).

Mero et al. invention provided seeds and plants of broccoli hybrid RS05151611 and the parent lines thereof. They also provided methods for producing a broccoli plant by crossing these plants with themselves or with another broccoli plant (of another genotype). The invention further relates to plants and seeds produced by such crossing and also to the parts of such plants. Methods and compositions relating to plants, seeds and derivatives of broccoli hybrid RS05151611 and/or broccoli lines BRE51-1160SC and BRE-51-22 SI are provided in this invention. The parent lines BRE51-1160SC and BRE-51-22 SI were crossed and

hybrid RS05151611 was produced. The parent lines show uniformity and stability within the limits of environmental influence. Uniform plants of hybrid RS05151611 are obtained by crossing the parent lines.

Broccoli type having curds with detached florets [72]

Patent No.: US 8,026,416 B2

Inventor: Franciscus van den Bosch, Kesteren (NL); Meinardus Petrus Boon, Scharwoude (NL).

Van den Bosch et al. produced broccoli plants having curds with detached florets. This invention includes methods for producing these types of plants. Many varieties of broccoli grow best on well-drained soils that hold water. To maintain proper main curd in sandy soil, irrigation is important. Curds develop relative to ambient temperatures. In the heat of summer, broccoli curds maturing in July may produce seeds and flowers more quickly (four to six days) than those maturing in the fall periods and cooler spring.

Broccoli curds should be closed, compact, dark green and tight (no yellow petals showing). Broccoli heads "green" according to the amount of sunshine reaching the crown of the curds. The crown is the upper surface of the broccoli curd covered by the florets. A deep green, uniform curd color is a desirable trait in broccoli. All current commercial broccoli varieties have curds with very tightly packed florets. All present commercial broccoli varieties having curds that are deeply placed within the canopy. The curds as well as stalk do not green uniformly due to lack of direct sunlight and as a result yellow discoloration occurs especially around the perimeter of the curd.

The present invention provides a broccoli plant comprising of a main curd that has elongated secondary stems. These secondary stems support the detached florets. The present invention also provides a broccoli plant having curd which comprises of uniformly green detached florets and they don't show substantial yellowing.

Global Scenario

Nutraceutical is the hybrid of 'nutrition' and 'pharmaceutical'. Nutraceuticals are food or part of food which plays a significant role in modifying and maintaining normal physiological function of human beings. The principal reasons for the growth of the nutraceutical market worldwide are the current population and the health trends.

Nutraceuticals can be classified as, probiotics, polyunsaturated fatty acids, antioxidants, dietary fibre, prebiotics and other different types of herbal/natural foods. These nutraceuticals help in combating some of the major health problems such as obesity, cholesterol, cardiovascular diseases, cancer, skin disease, osteoporosis, arthritis, diabetes, etc. In whole, 'nutraceutical' in food industry has lead to the new era of medicine and health, which has become a research oriented sector [73] (Figure 13).

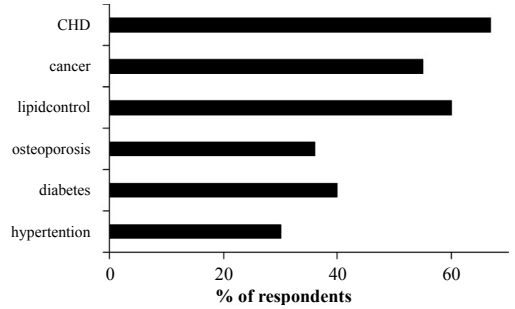

Figure 13: Therapeutic areas covered by nutraceutical products [73].

Dr. Meštrović defined Nutraceuticals as a broad umbrella term that is used to describe any product derived from food sources with extra health benefits in addition to the basic nutritional value found in foods. They can be considered non-specific biological therapies used to promote general well-being, control symptoms and prevent malignant processes [74].

With the ever growing statistics of the people suffering from diseases like cancer, arthritis, diabetes and many more, a lot of research has been done to develop treatment regimens. With the present drugs of synthetic origin given for cancer, the side effects they cause seem to outweigh their usage. This calls for the use of chemicals of natural origin for the treatment and preventive therapy. And this is where our nutraceuticals come (Figures 14 and 15).

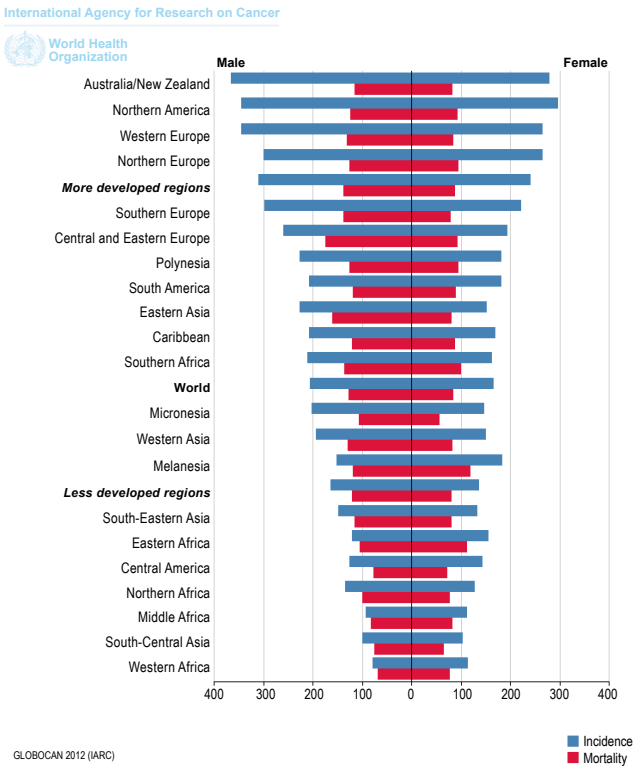

Figure 14: Incidence of cancer in more and less developed countries – Globocan estimated rates adjusted to the age structure of the world population. The differences in incidence and mortality rates provide an indication of the need for the therapy [75].

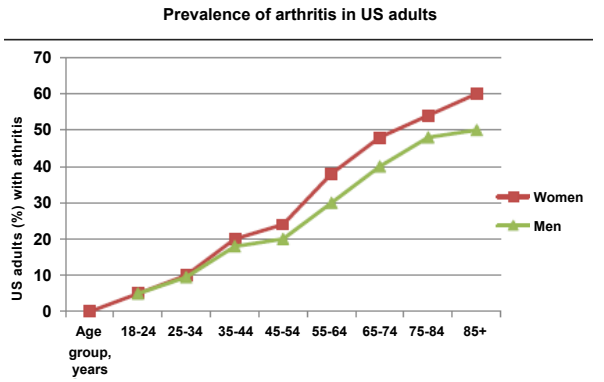

Figure 15: Prevalence of arthritis in US adults [76-81].

According to NHIS arthritis Surveillance, arthritis affects more women than men in every age group [78]. In US, arthritis and rheumatism are the leading causes of disability in US [79]. Hootman et al. projected that by 2030, 67 million in US will have HCP-diagnosed arthritis [80]. Arthritis is affecting people of all ages, thus the development of sulforaphane based formulations can be of immense help in today's scenario.

The use of nutraceutical has increased over the past few years due to increase consumer awareness and chronic diseases such as high blood pressure, diabetes, gut disorder and rickets [81].

In 2011 the global nutraceutical product market had reached $142.1 billion and according to the new market report from Transparency Market Research, Albany, NY; The nutraceutical product market is expected to reach $201.8 billion growing at a CAGR of 6.3% by 2017 [82].

Conclusion

The current lifestyle is the major cause of many diseases. Cancer is the second most fatal disease [83]. FDA in US treats Nutraceuticals as dietary supplement. Nutraceuticals helps in treatment as well as prevention of diseases by not only providing disease related nutrition but also enhancing body immunity. Besides cancer, Nutraceuticals also helps in abetment of many diseases like arthritis, kidney diseases, diabetes, and skin disorders. Broccoli is one of the vegetable commonly found and has very promising future. Various research done earlier shows that it contain sulforaphane which helps in treating many disease. Consumption of broccoli as such can be difficult and hence efforts are being made to extract the major constituents and convert it into various formulations. The broccoli extract is hygroscopic hence need special care to provide longer life to formulation. As the research further advances, extraction and isolation of the active ingredient will improve and better formulations will be developed for the benefit of the mankind.

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