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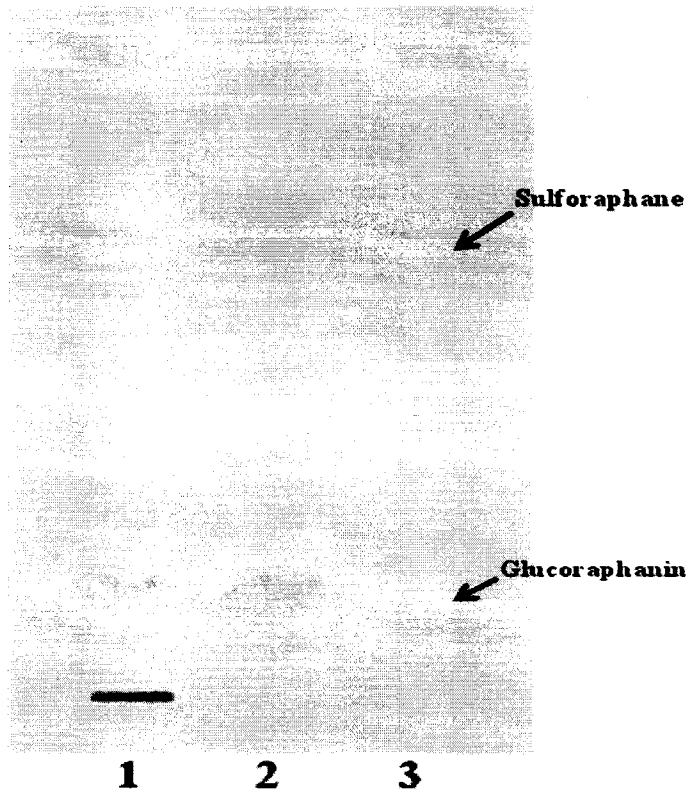


FIGURE 1

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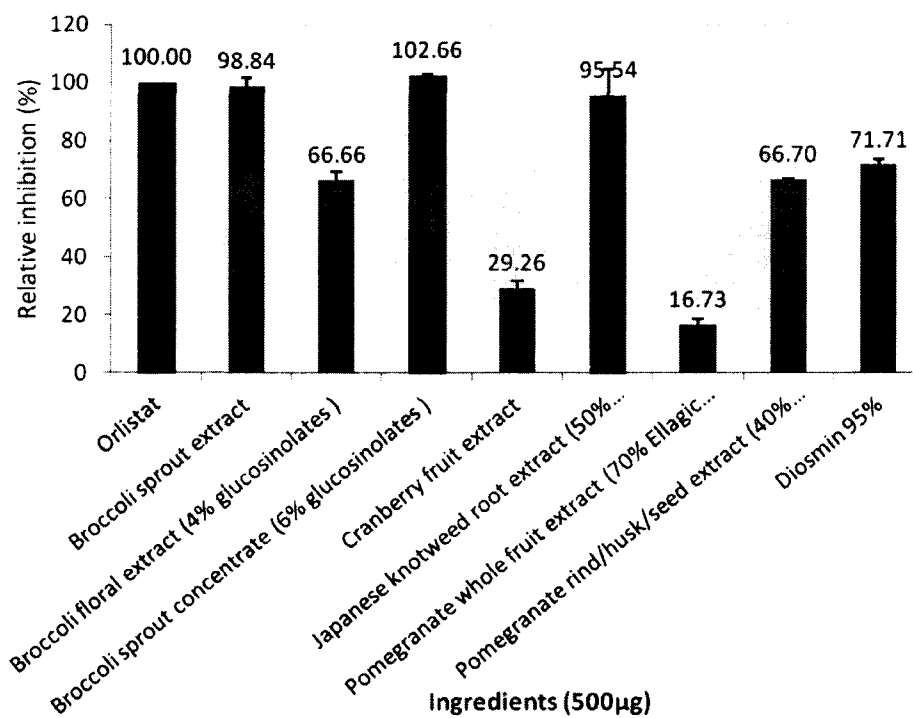
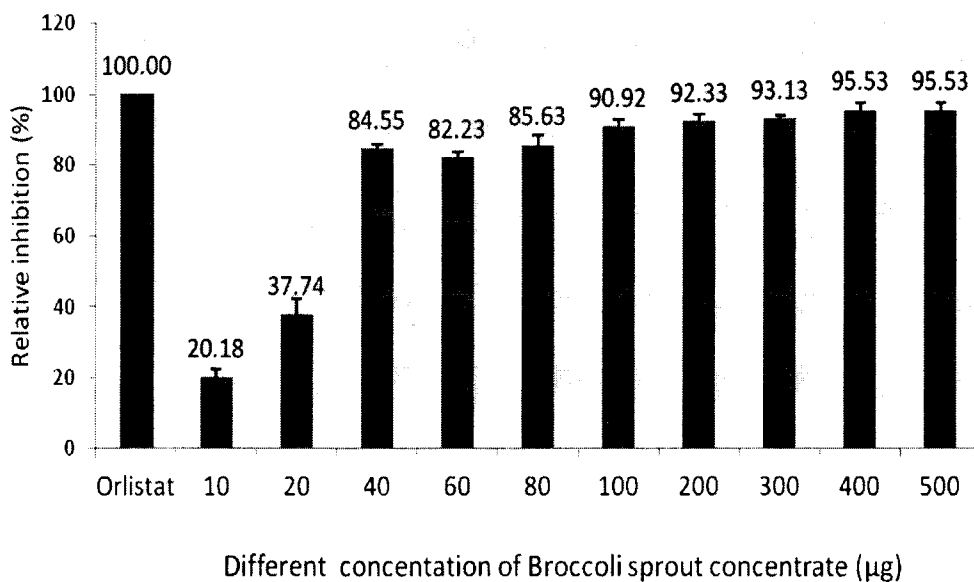


FIGURE 2

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**FIGURE 3**

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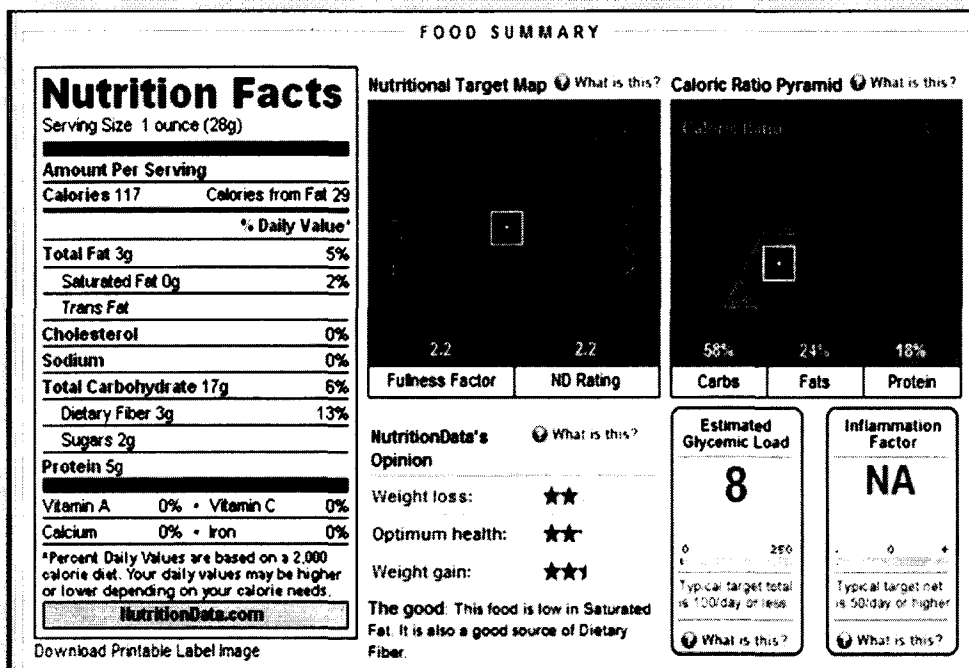


FIGURE 4

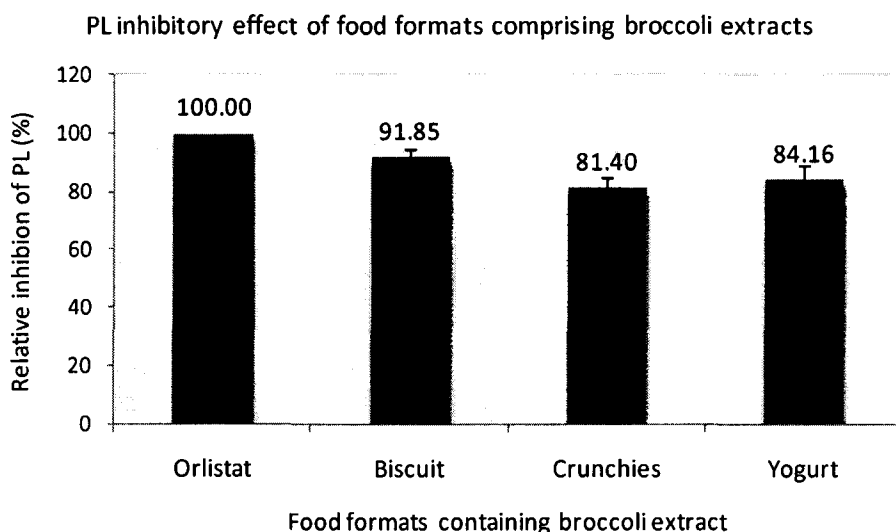


FIGURE 5

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## **FIELD OF INVENTION**

The present invention relates to a food composition comprising broccoli extracts for the maintenance of cardiovascular health. It also relates to a food product comprising the food composition in the manufacture of a nutritional product, a supplement, a treat, or a functional food product for the maintenance of heart health and prevention, alleviation and/or treatment of cardiovascular diseases.

## **BACKGROUND OF THE INVENTION**

Cardiovascular diseases (CVDs) remain the biggest cause of deaths worldwide. The percentage of premature deaths from CVDs ranges from 4% in high-income countries to 42% in low-income countries, leading to growing inequalities in the occurrence and outcome of CVDs between countries and populations. There is a considerable body of evidence regarding the nutritional background of atherosclerosis in general and coronary heart disease in particular. High dietary intakes of saturated fat, trans-fat cholesterol and salt combined with low intake of fruits, vegetables and fish are linked to cardiovascular risks. Cardiovascular risk factor is closely linked to diet and physical inactivity which results in obesity when there is an imbalance between energy intake in the diet and energy expenditure. Frequent consumption of high-energy foods such as processed foods high in fats and sugars promotes CVD and obesity compared to low-energy foods. A healthy diet can contribute to a healthy lipid profile and a desirable cardiovascular health (CVH).

In recent years, dietary approaches have become more popular in the management of CVD which include consuming diets lower in saturated and trans fatty acids and higher in potentially helpful heart-protecting bioactive food components, such as monounsaturated fatty acids, plant sterols, omega-3 fatty acids, dietary fiber, dietary antioxidants, folic acid and vitamin B12. There is a growing awareness amongst people about the role of food on cardiovascular health of adults and children. Consumers seek credible information regarding factors important for their well-being and cardiovascular health.

The association between triglycerides and the risk of heart disease has not been as clear as it is for cholesterol. In recent years several studies have established that people with elevated levels of triglycerides are indeed at an increased risk of cardiovascular health. More interestingly, Asian Indians have the highest rates of heart disease in the world, three times higher than in the United States. In spite of a fairly healthy lifestyle, many Asian Indians have very high levels of serum triglyceride and low levels of High Density Lipoproteins (HDL) or the good cholesterol. This abnormality in triglyceride regulation has been attributed to genetic pre-disposition in Asian Indians (Miller *et al.*, 2001, *American Journal of Cardiology*; 87: 220-1).

Triglyceride absorption inhibitors form one of the most important strategies in the management of CVH through nutrition. Pancreatic lipase (PL) or triacylglycerol acyl hydrolase (EC 3.1.1.3), the principal lipolytic enzyme synthesized and secreted by the pancreas, plays a key role in the efficient metabolism of triglycerides in the human gut. PL is responsible for the hydrolysis of 50% to 70% of the total dietary fats and removes fatty acids from the  $\alpha$  and  $\alpha'$  positions of dietary triglycerides, yielding  $\beta$ -monoglycerides and long chain saturated and polyunsaturated fatty acids as the lipolytic products or free fatty acids (FFA). FFA are absorbed by the enterocyte to synthesize new triglyceride molecules, which are transported to the different organs via lipoproteins, especially chylomicrons, after a meal.

Recently, newer CV approaches for the treatment of obesity have involved inhibition of dietary triglyceride absorption via inhibition of pancreatic lipase (PL) as this is the major source of excess calories. In recent years, Orlistat is the most widely used PL inhibitor. Orlistat blocks absorption of about 25 per cent of fat in the meal. However, Orlistat is notorious for its gastrointestinal side effects which include oily and loose stools with excessive flatus due to unabsorbed fats reaching the large intestine and frequent or urgent bowel movements. Hence, the success of any pancreatic lipase inhibitors which will be used for the management of triglyceride depends on the identification of new PL inhibitors that lack these unpleasant side effects. At present,

the potential of any new natural ingredient for the management of cardiovascular health through inhibition of pancreatic lipase is still largely unexplored.

Natural products provide a vast pool of PL inhibitors that can possibly be developed into clinical products. Lipase inhibitors have been reported in cereals, wheat bran, wheat germ, soybean, Chinese medicines and various herbs. Inhibition of PL has been described in the presence of unfermented (green tea) and semi-fermented (oolong tea) extracts and numerous polyphenols such as flavones, flavanols, saponins, tannins, and chalcones (Inoue *et al.*, 2000, *International Journal of Obesity*; 24: 758-764; Suzuki T *et al.*, 1992, *Biosci. Biotechnol. Biochem*; 56: 1478-1479; Jianping Ye *et al.*, 2008, *Endocr. Metab. Immune Disord. Drug Targets*; 8(2): 99–111).

Designing a new food product with custom fortification to obtain a desired health-benefit is not an easy task. Numerous factors have to be considered since the stability of nutrients and their retention in foods are affected by temperature, moisture, pH, oxygen, bioavailability, and component interactions. Therefore, it would be desirable to produce a food composition comprising ingredients which results in improving the overall nutritional benefit of the product translating into health benefits for the consumers without any prevalent side effects.

#### **BRIEF DESCRIPTION OF ACCOMPANYING DRAWINGS**

Figure 1 shows the High performance thin layer chromatography [HPTLC] finger print of Broccoli sprouts extracts. Lane 1: Aqueous extract of Broccoli sprouts, Lane 2: Methanol extract of Broccoli sprouts, Lane 3: Mixture of 6-glucosinolate and sulphoraphane standards.

Figure 2 shows the performance of various plant extracts on pancreatic lipase enzyme activity.

Figure 3 shows the dose response of broccoli sprouts on human pancreatic lipase enzyme activity.

Figure 4 provides the nutritional information of a food product comprising broccoli sprout extracts.

Figure 5 shows the inhibition of human pancreatic lipase by different food products comprising broccoli extract composition.

## **DETAILED DESCRIPTION OF THE INVENTION**

Those skilled in the art will be aware that the invention described herein is subject to variations and modifications other than those specifically described. It is to be understood that the invention described herein includes all such variations and modifications. The invention also includes all such steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

### Definitions

For convenience, before further description of the present invention, certain terms employed in the specification, examples and appended claims are collected here. These definitions should be read in light of the remainder of the disclosure and understood as by a person of skill in the art. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art.

The articles "a", "an" and "the" are used to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article.

The terms "comprise" and "comprising" are used in the inclusive, open sense, meaning that additional elements may be included. The terms will be understood to imply the inclusion of a stated element or step or group of element or steps but not the exclusion of any other element or step or group of element or steps. It is not intended to be construed as "consists of only."

The term “food grade” refers to being regarded as safe for human and/or animal consumption by the relevant regulatory authorities. The term also refers to components which can be safely ingested by humans or animals.

The term “food” or “food product” refers to liquid, semi-solid and/or solid food products such as nutritional compositions suitable for human and/or animal consumption.

The term “food product ingredient” or “food supplement ingredient” refers to a product which is suitable for being added to a final food product, or a food supplement, or during the production process of a food product, or a food supplement.

The present invention discloses the beneficiary role of broccoli extract in the management of dietary triglyceride through inhibition of pancreatic lipase activity. Broccoli sprout concentrates with glucosinolates have beneficial effect on general health. The broccoli extracts can be used as fortifying components and in the development of health products, health supplements and compositions for general well-being and management of cardiovascular health.

Broccoli is a nutrient source which contains many bioactive components including glucosinolates, flavanoids, minerals, and antioxidants. Glucoraphanin, gluconasturtiin, and glucobrassicin are 3 glucosinolate phytonutrients found in a special combination in broccoli. Broccoli is high in vitamin C, dietary fiber, carotenoids, especially lutein and beta-carotene and multiple nutrients with potent anti-cancer properties, such as sulphoraphane, diindolylmethane and small amounts of selenium. The 3, 3'-diindolylmethane in broccoli is a potent modulator of the innate immune response system with anti-viral, anti-bacterial and anti-cancer activity. Broccoli is also an excellent source of indole-3-carbinol, a chemical which boosts DNA repair in cells and appears to block the growth of cancer cells.

The inventors found surprising results when they found that extracts of broccoli showed potent pancreatic lipase inhibition activity and might be an excellent alternative strategy

for the development of safe and effective functional foods for the management of cardiovascular health.

Accordingly, it is the objective of the present invention to provide a composition comprising the novel natural ingredient, broccoli extracts, for use in the preparation of healthy food products for human and pet consumption. Another object of the present invention is to provide orally ingestible food or pet food composition which provides effective inhibition of the pancreatic lipase activity. The use of the composition in maintaining cardiovascular health by administering an effective amount of the composition is also disclosed.

Broccoli is classified in the Italica cultivar group of the species *Brassica oleracea*. Broccoli most closely resembles cauliflower, which is a different cultivar group of the same species. Broccoli has large flower heads, usually green in color, arranged in a tree-like fashion on branches sprouting from a thick, edible stalk. The mass of flower heads is surrounded by leaves. The broccoli extracts are obtained from the broccoli plant part selected from a group consisting of roots, stalk, flower, florets, sprouts, and seeds. The broccoli sprout extracts demonstrate excellent pancreatic lipase inhibitor activity. Inhibition of the lipase enzyme promotes lesser absorption of the dietary triglycerides leading to health benefits in terms of good cardiovascular health.

The present invention discloses food compositions fortified with 1% broccoli extract with the glucosinolate present in the effective range of 0.01% to 20%, most preferably in the range of 0.05% to 6% glucosinolates, which provide effective inhibition of pancreatic lipase activity.

The food composition according to the invention may preferably be used as a physiologically functional food product. Preferably, the consumption of the food composition is effective in causing the consumer one or more, preferably two or more, and most preferably all three of the following effects: a) inhibition of pancreatic lipase activity; b) lowering of triglyceride levels; c) reduction in level of low density lipoproteins (LDL).

The health food composition comprising broccoli extracts can be formulated with one or more high satiety index products. The high satiety index components prevent overeating and thus help in calorie control and calorie consumption.

The broccoli extract may be formulated as crystalline, microcrystalline, particles, granules, powder, spray-dried, lyophilized, and the like, forms that will admix with a foodstuff composition, and dissolve or undergo disintegration or dissolution in the presence of fluids in the mouth, stomach and/or intestine.

Preferably, the food composition is in the form of a liquid, semi-solid, granular, powder and/or a solid. The composition may be used as functional foods, food products and/or food supplement. The composition can be formulated into a solid product, a semi solid product, particulate mixture, a granulate product, a liquid concentrate, a ready-to-drink beverage, an infant formula, a powder, a tablet, a capsule, a nutritional formula, a food supplement, pet food or combinations thereof. The food product may preferably be a biscuit, a yogurt, a baked snack, a protein shake powder or a ready-to eat snack.

The food composition can include one or more high satiety index products, carbohydrates, proteins, fats, minerals, nutrients, vitamins, trace elements, gums, resins, flavour enhancers, emulsifiers, preservatives, binders and sweeteners.

Carbohydrates with low glycemic index include dietary fibers, complex carbohydrates, soluble fibres, galactomanans, glucomanans, gums, resistant starch, resistant dextrins, inulin, lignans, pectins, beta-glucans, and oligosaccharides such as fructo-oligosaccharide and manno-oligosaccharide.

Sources for the dietary fibers include but are not limited to whole grains, germinated grains, and high fibre cereals like wheat, barley, bulgur, rice, oat, buckwheat, and psyllium. Carbohydrates with low glycemic index have cholesterol lowering benefits, reduce the total cholesterol and low density lipoprotein (LDL) levels and increase satiety levels.

Proteins like soy proteins, nuts, flaxseeds, legumes, pulses, beans such as soy beans, kidney beans, lima beans *etc.*, protein hydrolysates; defatted protein flour, whole grains, berries, fruits and vegetables are rich sources of dietary phytosterols, which are beneficiary health providing ingredients. Dietary phytosterols are known to lower LDL cholesterol levels and triglyceride levels. The triglyceride lowering effect of phytosterols is due to a reduction in triglyceride rich very low density lipoprotein (VLDL) particles produced by the liver. The triglyceride lowering effects of phytosterols may be more pronounced in individuals with elevated triglycerides.

Unsaturated fats like monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA) and omega-3 fatty acids prove to be beneficial in health food compositions. Some important sources of unsaturated fats rich in MUFA, PUFA and omega-3 fatty acids are nuts and seeds like flax, walnuts, sunflower, peanut, soy, sesame *etc.*, high fat fruits like olives and avocados, oils from macadamia, grapeseed, peanut, avocados, olives, safflower, almond, sunflower, tea, sesame and corn, whole grain wheat, cereal, oatmeal, oily fish like salmon, sardines and tuna, algal oil, fish oil and seafood.

The food composition can further include atleast one ingredient selected from a group consisting of minerals, nutrients, vitamins, trace elements, gums, resins, flavor enhancer, emulsifiers, preservatives, binders and sweeteners.

Slow release sugars and/or sugar replacers selected from a group of dextrose, glucose, fructose, sucrose, high-fructose corn syrup, stevia, aspartame, sucralose and saccharin, are included in the food composition.

The composition can be prepared by dry mixing low glycemic index carbohydrates, phytosterols containing proteins and unsaturated fats with broccoli extracts, additives, preservatives, dietary fibers *etc.* The components of the composition can be admixed as is known in the art and formulated according to standard procedures. Preferably, where applicable, each of the components of the composition is GRAS approved.

The composition can be provided as an article of manufacture comprising a unit amount of the composition contained within the packaging material, wherein the composition

comprises an effective amount of broccoli extracts comprising glucosinolates in the range of 0.6 - 30  $\mu$ g to provide cardiovascular health benefits. The beneficiary features of the food composition comprising broccoli extract are inhibition of the pancreatic lipase enzyme by more than 50%, contain high calories, shows medium glycemic index, is high in protein content, and is enriched with beneficiary unsaturated fats and dietary fibers.

The liquid food composition according to the invention may comprise common food ingredients like polyphenols, flavanoids, water, flavour, sugar, fruits, minerals, vitamins, stabilizers, thickeners, starches *etc.*, in appropriate amounts. Preferably the liquid food compositions are soy-based products or fruit juice based products.

The liquid food composition of the invention can advantageously be used as a dietary component especially for consumers with a wish to decrease cardiovascular risk, such as high triglyceride levels and high cholesterol levels. Especially advantageously the food composition is for use to lower triglyceride levels.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purposes of exemplification only. Functionally-equivalent products, compositions, and methods are clearly within the scope of the invention, as described herein.

One embodiment of the present invention provides a composition comprising a low glycemic index carbohydrate, a protein, an unsaturated fatty acid and extracts of broccoli, wherein the extract of broccoli comprises at least 0.01% to 20% glucosinolates.

Another embodiment of the present invention provides a composition comprising a low glycemic index carbohydrate, a protein, an unsaturated fatty acid and extracts of broccoli, wherein the extract of broccoli comprises at least 0.05% to 6% glucosinolates.

In another embodiment of the present invention, there is provided a composition comprising 50% to 70% by weight of low glycemic index carbohydrates, 10% to 25%

by weight of protein, 1% to 15% by weight of total fatty acids and 0.3% to 4% by weight of broccoli extracts based on the total weight of the composition, wherein the broccoli extracts comprise atleast 0.01% to 20% glucosinolates.

In another embodiment of the present invention, there is provided a composition comprising a low glycemic index carbohydrate, a protein, an unsaturated fatty acid and extracts of broccoli, wherein the extract of broccoli comprises atleast 0.01% to 20% glucosinolates, wherein the carbohydrate, protein, fat and broccoli extract is present in the ratio of 60: 11: 19: 1.

In still another embodiment of the present invention, there is provided a composition, comprising a low glycemic index carbohydrate, a protein, an unsaturated fatty acid and extracts of broccoli, wherein the extract of broccoli comprises atleast 0.01% to 20% glucosinolates, wherein the broccoli extracts are obtained from the broccoli plant part selected from a group consisting of roots, stalk, flower, florets, sprouts and seeds.

In another embodiment of the present invention, there is provided a composition, comprising a low glycemic index carbohydrate, a protein, an unsaturated fatty acid and extracts of broccoli, wherein the extract of broccoli comprises atleast 0.01% to 20% glucosinolates. wherein the carbohydrates with low glycemic index are selected from a group consisting of dietary fibers, complex carbohydrates, soluble fibres, beta-glucans, galactomanans, glucomanans, gums, resistant starch, resistant dextrins, inulin, lignans, pectins, oligosaccharides, whole grains, germinated grains, wheat, barley, bulgur, rice, oat, buckwheat and psyllium.

In yet another embodiment of the present invention there is provided a composition, comprising a low glycemic index carbohydrate, a protein, an unsaturated fatty acid and extracts of broccoli, wherein the extract of broccoli comprises atleast 0.01% to 20% glucosinolates, wherein the protein is selected from a group consisting of milk powder, skim milk powder, milk proteins, legume proteins, cereal protein, protein hydrolysates, whey protein, caseinates, casein glycomacropetides, whole grains, pulses, fruits, nuts and seeds.

In still another embodiment of the present invention, there is provided a composition, comprising a low glycemic index carbohydrate, a protein, an unsaturated fatty acid and extracts of broccoli, wherein the extract of broccoli comprises at least 0.01% to 20% glucosinolates, wherein the unsaturated fatty acid is selected from a group consisting of soy beans, oils of walnut, flax, sunflower, peanut, sesame, olives, avocados, macadamia, grapeseed, peanut, safflower, almond, tea, corn; algal oil, fish oil, fish, seafood, whole grain wheat, cereal and oatmeal.

In another embodiment of the present invention, there is provided a composition comprising a low glycemic index carbohydrate, a protein, an unsaturated fatty acid and extracts of broccoli, wherein the extract of broccoli comprises at least 0.01% to 20% glucosinolates, optionally comprising at least one ingredient selected from a group consisting of minerals, nutrients, vitamins, trace elements, gums, resins, flavor enhancer, emulsifiers, preservatives, binders and sweeteners.

Another embodiment of the present invention provides a process of producing a food composition, wherein said process comprises a) providing a pre-mix composition comprising a low glycemic index carbohydrate, a protein, an unsaturated fatty acid and broccoli extracts, wherein the broccoli extracts comprise at least 0.01% to 20% glucosinolates; b) blending the pre-mix composition with a liquid to form a food blend c) heating the food blend at a temperature not greater than 175°C for about 10 to 15 minutes.

In another embodiment of the present invention, there is provided a process of producing a composition comprising a) providing a pre-mix composition comprising a low glycemic index carbohydrate, a protein, an unsaturated fatty acid and broccoli extracts, wherein the broccoli extracts comprise at least 0.01% to 20% glucosinolates; b) blending the pre-mix composition with a liquid to form a food blend c) heating the food blend at a temperature not greater than 175°C for about 10 to 15 minutes, wherein the process optionally comprises the step of pasteurizing the food blend prior to heating the food blend.

In another embodiment of the present invention, there is provided a composition, comprising a low glycemic index carbohydrate, a protein, an unsaturated fatty acid and extracts of broccoli, wherein the extract of broccoli comprises atleast 0.01% to 20% glucosinolates, wherein the composition is formulated as a solid product, a semi solid product, particulate mixture, a granulate product, a liquid concentrate, a powder, a tablet, a capsule, and combinations thereof.

Another embodiment of the present invention provides a food product comprising the composition comprising a low glycemic index carbohydrate, a protein, an unsaturated fatty acid and extracts of broccoli, wherein the extract of broccoli comprises atleast 0.01% to 20% glucosinolates.

In another embodiment of the present invention, there is provided a food product comprising the composition comprising a low glycemic index carbohydrate, a protein, an unsaturated fatty acid and extracts of broccoli, wherein the extract of broccoli comprises atleast 0.01% to 20% glucosinolates, wherein the food product is a ready-to-drink beverage, a snack, a ready-to-cook formula, a ready-to eat product, an infant formula, a nutritional formula, a food supplement, a seasoning, or a pet food.

Although the subject matter has been described in considerable detail with reference to certain preferred embodiments thereof, other embodiments are possible. As such, the spirit and scope of the invention should not be limited to the description of the preferred embodiment contained therein.

#### **Preparation of Broccoli extracts**

Stock solution of broccoli extracts was prepared by using commercially available broccoli powder. 50 mg of broccoli extract was dissolved in 1ml of solvent, preferably dimethyl sulfoxide, and vortexed for 30 seconds. The solution was then centrifuged at 3000 rpm for 3 minutes. The pellet was discarded and supernatant used as the stock solution. The final concentration of the stock solution was 50 mg/ml. This stock solution was further used for the preparation of the working solutions as per the requirement of the experiments.

### High performance thin layer chromatography [HPTLC] finger print of Broccoli extracts

The broccoli sprout extracts were analyzed through high performance thin layer chromatography [HPTLC] for quality control and to maintain the consistency of the broccoli extracts of various batches when purchased from a commercial vendor.

Broccoli sprouts of 1g were suspended separately in 10 ml each of water, methanol and dichloromethane solutions. The suspensions were then sonicated for 30 minutes at room temperature. After sonication, the samples were centrifuged at 10000 rpm for 20 minutes; the supernatants were collected and filtered using 0.45 micron syringe filters. The filtered samples were used for HPTLC analysis.

The chromatographic plates were pre-loaded with 25 $\mu$ L of the filtered broccoli sample and developed using n-Butanol:n-Propanol:Glacial acetic acid:Water [30:10:10:10 v/v] solvent system. The developed chromatograms were derivatized using 5% sulphuric acid-methanol followed by heating the plate at 110°C for 15 minutes. The post-derivatized plates were visualized under short (254 nm) and long (366 nm) UV illumination as shown in Figure 1. Lane 1: Aqueous extract of Broccoli sprouts, Lane 2: Methanol extract of Broccoli sprouts and Lane 3: Mixture of 6-glucosinolate and sulphoraphane standards. The active ingredients of the broccoli extracts identified in the HPTLC were sulphoraphane and glucoraphanin, as seen in Figure 1.

### **Screening of potential plant extracts for pancreatic lipase inhibitor activity**

#### Preparation of assay solution

Plant extracts of Cranberry fruit, Japanese knotweed root (50% Resveratrol), Pomegranate whole fruit (70% Ellagic acid), Pomegranate rind/husk/seed (40% Punicalagins), Broccoli sprout, Broccoli florets (4% glucosinolates), and Broccoli sprout concentrate (6% glucosinolates) were selected for the pancreatic lipase inhibition assay. Orlistat 5 $\mu$ M was used as a positive control.

0.1M Tris-HCl buffer at a pH of 7.5 is used as the assay buffer as well as the solvent for enzyme and emulsifier solutions.

The substrate, para-nitrophenol tagged laurate molecule was prepared in isopropanol to a final concentration of 200 $\mu$ M.

Human pancreatic lipase (Sigma Aldrich, India) was diluted to 200 $\mu$ L with assay buffer so as to obtain a final concentration of 50U/200 $\mu$ L. The diluted enzyme was stored at 4°C until further use. Diosmin 95%, a modified form of Hesperidin which is a natural flavanoid, is used as a potential inhibitor.

#### Photometric estimation of pancreatic lipase inhibitor activity

The pancreatic lipase inhibitor activity assay was carried out in a total reaction volume of 200 $\mu$ L in 96-microwell plate comprising assay buffer 0.1M Tris-HCl pH 7.5, emulsifier 0.1%, 200  $\mu$ M substrate, 5 $\mu$ M inhibitor and 1U pancreatic lipase enzyme. The reaction was started by adding the enzyme at 37°C. The colorimetric measurements of the absorbance was recorded at 410 nm measured every minute for 1 hour. Each assay was performed in duplicate. The blank comprises the assay buffer, emulsifier, substrate, and either inhibitor or ingredient.

Percentage inhibition was calculated as:

$$\% \text{ Inhibition} = (\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}) / \text{Absorbance}_{\text{Control}} \times 100$$

It was observed that the 500  $\mu$ g broccoli sprout concentrate comprising 3% glucosinolate in the final volume shows pancreatic lipase inhibitory activity similar to that of the positive control, Orlistat (Figure 2).

In Figure 2, the final concentration of glucosinolates present in the reaction volume for broccoli florets is 20  $\mu$ g and for broccoli sprouts is 30  $\mu$ g.

#### **Dose response of broccoli sprout extracts on human pancreatic lipase activity**

Varying concentrations of the broccoli sprout concentrate as given in Table 1 were evaluated for their response on human pancreatic lipase activity using the colorimetric

assay provided above. The final concentration of glucosinolates present in 10-500 µg of Broccoli sprout concentrate shown in Figure 3 ranges from 6 to 30 µg as shown in Table 1.

It was observed that 100-500 µg of broccoli extract showed >90% inhibition of pancreatic lipase activity with respect to the positive control, Orlistat (Figure 3).

#### **Edible composition comprising broccoli extract**

An edible food composition disclosed in the present invention having the composition as set forth in Table 2 was prepared as follows. The components, multigrain wheat flour, defatted soya flour, oats, citrus fiber, sucralose, skimmed milk powder, ammonium bicarbonate and broccoli extract as listed in Table 2 below, were mixed together to obtain a dry pre-mix.

To the dry pre-mix prepared above, olive oil was added and folded well to prepare dough. The dough was then rolled into sheets, cut into desired shape and baked for duration of 15 minutes at an oven set in the temperature of 150°C to obtain biscuits.

The edible composition prepared by the above mentioned process was further evaluated for its nutritional profile using the 'Nutritional Target Map' and 'Caloric Ratio Pyramid' generating software on [www.nutritiondata.self.com](http://www.nutritiondata.self.com). The software gives 0 to 5 stars in each of three categories: weight loss, weight gain and optimum health features, based on their nutrient density (ND Rating) and their satiating effect (Fullness Factor™). Foods that are both nutritious and filling are considered better choices for weight loss. Foods that are nutritious without being filling are considered better choices for healthy weight gain. Foods that have more essential nutrients per calorie are considered better choices for optimum health. Lower ratings do not necessarily mean that the food should be avoided but that it might be best consumed in moderation. Likewise, high ratings do not guarantee a perfect food but indicate a food that is more likely to be a positive addition to the diet.

Figure 4 provides the nutritional information of the food composition prepared by the above mentioned process. The composition has a medium glycemic index with a GI of 57.14, has a high calorie content of 18%, and is rich in dietary fibres and MUFA and low in unsaturated fats. The concentration of glucosinolates present in 1 kg of this food composition is 600 mg. The composition shows absence of inflammatory factor and an estimated glycemic load of 8%.

Figure 5 provides the relative inhibition of human pancreatic lipase by the broccoli extract composition present in different food product: the biscuits, crunchies and yogurt, measured in respect to the inhibition shown by the inhibitor, Orlistat. The biscuit composition provides a high relative inhibition of 91.85% followed by yogurt and crunchies, when compared with inhibition by Orlistat.

#### LIST OF TABLES

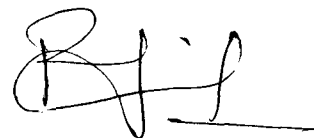
Table1: Broccoli sprout concentrates with their corresponding levels of glucosinolates.

Broccoli sprout concentrate ( $\mu\text{g}$ )	% Glucosinolates present	Concentration of Glucosinolates present( $\mu\text{g}$ )
10	0.06	0.6
20	0.12	1.2
40	0.24	2.4
60	0.36	3.6
80	0.48	4.8
100	0.6	6
200	1.2	12
300	1.8	18
400	2.4	24
500	3	30

Table 2: Ingredients present in the composition comprising broccoli extract.

<b>Raw Materials</b>	<b>g/Kg</b>
Multigrain wheat	520
Defatted soya	250
Oats	20
Citrus fiber	10
Olive oil	100
Sucralose	10
Skimmed Milk Powder	90
Ammonium bicarbonate	5
Broccoli extract	10

Dated this 19 day of July, 2012



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To,  
The Controller of Patents,  
The Patent Office, at Chennai

## **ABSTRACT**

### **FOOD COMPOSITION COMPRISING BROCCOLI EXTRACTS**

The present invention relates to novel food composition comprising broccoli extracts aimed at the management of cardiovascular health. The present invention also provides a process of production of these heart-healthy food compositions and food products comprising broccoli extracts which inhibit human pancreatic lipase activity.