ABSTRACT
Spinach is a commonly consumed leafy vegetable packed with micronutrients and phytochemicals. It has functional ingredients such as lutein, betaine, flavonoids, neoxanthin, galactolipids. Each of these has its own physiological significance. Studies have indicated that regular consumption of spinach substantially lowers the risk of age related macular degeneration, one of the leading causes of irreversible blindness among adults. There is an inverse association between spinach consumption and cataract risk. Spinach leaf protein concentrate has a strong cholesterol lowering effect in rats. Age related changes on brain function can be delayed by long term consumption of spinach. It has high anti proliferative activity on cancer cells. However, people prone to oxalic acid stones are to reduce consumption of spinach. So quantity and frequency of spinach consumption can be increased.

Keywords: functional ingredients, antioxidant activity; age related macular degeneration; aging; cancer

INTRODUCTION
Spinach (Spinaciaoleracea) is a commonly consumed green leafy vegetable. Although it is native to central and southwestern Asia, now it is produced and consumed throughout the world. Green leafy vegetables such as spinach form an important part of a balanced diet. Their micronutrient content, along with a combination of low calorie content, low glycemic index, dietary fiber make it an appealing healthy choice. The contribution of dark green leafy vegetables to total micro nutrient intake of 2-5 year old children in rural South Africa is quite significant. For children consuming dark green leafy vegetables, imifina (collective term for various dark green leaves that are eaten as a vegetable) and Spinach contributed significantly to the dietary intake of calcium (21 to 39%), iron (19-39%), vitamin A (42 to 68%) and riboflavin (9 to 22%). This contribution can be increased if these vegetables are consumed more frequently and by a larger proportion of children.

COMPOSITION OF SPINACH
Spinach is a rich and affordable source of micronutrients and phytochemicals. It contains vitamin C (28.1 mg), vitamin K (482.9), folate (194 mcg), calcium (99 mg), iron (2.71 mg), magnesium, manganese, zinc etc per 100 g of edible portion. Xanthophyll is a class of carotenoids with oxygenated carotenes. The molecular formula of xanthophyll is C40H56O2. They are typical yellow pigments of leaves. Spinach contains a variety of xanthophylls. Rangaswamy L et al. (2005) have determined these by HPLC method. Table 1 gives the xanthophyll composition of spinach.

Bioactive Components
Studies indicate that spinach is a source of bioactive components such as lutein, betaine, flavonoids, neoxanthins and galactolipids. Table 2 gives the quantity of each of these components.
The general structure, physiological role and nutritional significance of each of these is discussed below.

**Lutein:** It is one of the natural carotenoids synthesized only by plants. It has the following structure (Fig 1). Lutein and zeaxanthin are the only carotenoids found in the retina. They are mainly present in the macula where they act as absorbers of blue light. By preventing a substantial amount of blue light from reaching the underlying structures, they protect against oxidative damage induced by light. This oxidative damage is believed to play a role in the causation of age-related macular degeneration (AMD).

**Betaine:** Betaine is a chemical compound alternatively called trimethylglycine, glycine betaine, lycine, and oxyneurine. It is a zwitterionic quaternary ammonium compound. The chemical structure is shown below (Fig 2). Betaine performs the following physiological functions. (Stuart A. S. C. 2004).4

1. It is an osmolyte. So it protects the cells, proteins and enzymes from environmental stress such as lack of water, unfavourable temperature, or salinity
2. It is a source of methyl groups. Therefore it participates in methionine cycle in kidney and liver.
3. Betaine can reduce the serum homocysteine concentration in case of mild or severe hyper homocystinuria. Via Methionine cycle.
4. It may have a role in epigenetics and athletic performance.

Deficiency of methyl groups results in hypomethylation which has the following consequences. Increase in plasma homocysteine concentration, decrease in s-denosyl methionine concentration, impaired hepatic fat metabolism resulting in fat accumulation (steatosis). This leads to dyslipidemia. Coronary, cerebral, hepatic and vascular diseases may result due to faulty liver metabolism.

Spinach is one of the food items with high betaine content (600-645 mg/100g).5 Betaine-aldehyde dehydrogenase was purified from spinach leaves and characterized by Keita A et al (1987).6

**Flavonoids:** They are a class of secondary plant metabolites widely distributed in plants. They function as plant pigments and are responsible for yellow/red/blue color. In vitro studies have shown that some of these flavonoids show anti-allergic, anti-inflammatory, anti-oxidant, anti-microbial and anti-cancer activities. There is very limited in vivo or clinical research to prove or disprove this claim. Rudolf Edenharder et al isolated 13 flavonoid compounds from spinach and some of these were found to be potent antimitagens.

**Neoxanthin:** It is a type of xanthophyll found in green leafy vegetables such as spinach (Table 1). It is an intermediate in the biosynthesis of plant hormone abscisic acid and is synthesized from violaxanthin. It has the following structure (fig 3). A study by Luca Dall’Osto et al (2007)8 has shown that neoxanthin acts as an antioxidant within the photosystem II super complex and neoxanthin protects membrane lipids from reactive oxygen species and superoxide anions. Eiichi Kotake-Nara et al (2001)9 have shown that neoxanthin can reduce the risk of prostate cancer and the details are discussed under spinach and cancer.

**Galactolipids:** They are glycolipids with galactose as sugar group widely found in plant kingdom. They are present in cell membrane lipids. Monogalactosyldiacylglycerol (MGDG) and Diagalactosyldiacylglycerol (DGDG) are present in higher amounts in chloroplast membranes. They are believed to play a role in photosynthesis. The chemical structure of a common galactolipid is shown below (Fig 4). Wang R (2002)10 has estimated that spinach contains 3300-3880 (mg/kg) of MGDG (Monogalactosyldiacylglycerol), a galactolipid. Lars P. Christensen (2009)11 has reported that different studies have estimated MGDG (mg/kg) content of spinach on fresh weight basis as 546,
850 and 3300-38, 800 and DGDG (mg/kg) content of spinach on fresh weight basis as 563.
A study has shown that extraction of dry spinach leaves resulted in a glycolipid enriched fraction. This contained 3 types of glycolipids-MGDGs, DGDGs and sulphoquinovosyldiacyl glycerol at a high ratio. These play a role in DNA synthetase inhibitory activity, inhibition of cancer cell growth and antitumor activity (Mizushima et al 2005).

Bioavailability of Nutrients
Bioavailability, defined as the proportion of an ingested trace element in food that is absorbed and utilized for normal metabolic and physiological functions or storage (Jackson, 1997)\(^\text{13}\), is an important factor in deciding the nutritional status. The chemical form in which a nutrient is present in food, nutrient interactions within the food, the presence of anti nutritional factors and processing of food all influence the bioavailability of a nutrient. Spinach contains anti nutritional factors like oxalic acid which reduce the absorption of calcium and iron by forming insoluble complexes with them. Oxalic acid may lead to oxalate stones in the urinary tract of some people. Therefore people who have oxalate stones in their urinary tract are advised to reduce eating vegetables like spinach. Dietary fiber may also interfere with absorption of nutrients.

Studies have shown that bioavailability of nutrients in spinach depends on the form in which it is consumed. β carotene present in raw spinach has less bioavailability compared to cooked and pureed spinach (Cherry Rock et al 1998).\(^\text{13}\) The plasma βcarotene levels were three times higher after daily consumption of carrots and spinach for 4 weeks in processed form than when they were consumed in raw form. Bioavailability of β carotene is increased by the disruption of cell wall structure and loss of cellular integrity of spinach leaves (Jacqueline et al).\(^\text{14}\) The table 3 shows bioavailability of spinach in different forms. Bioavailability of lutein is higher than that of beta carotene.

**Antioxidant Constituents:**
Different antioxidant constituents present in a vegetable such as alpha tocopherol, beta carotene, vitamin C, selenium or phenolic compounds etc. contribute to the total antioxidant capacity of a vegetable. Amin Ismail et al (2004)\(^\text{15}\) have shown that the high antioxidant activity of spinach is due to high alpha tocopherol, beta carotene and ferulic acid. The total antioxidant activity of spinach by different methods is given in Table 4.

The active fractions from aqueous spinach extracts have been chemically identified (Margalit Bergman et al (2001)).\(^\text{16}\) There are 4 hydrophobic fractions (glucuronic acid derivatives of flavonoids), 3 fractions of trans and cis isomers of p-coumaric acid derivatives and others are meso-tartarate derivatives of p-coumaric acid. There is a variation in the flavonoid content among different genotypes of spinach (Mi Jin Cho et al (2008)).\(^\text{17}\) 18 flavonoids representing glucuronides and acylated diand triglycosides of methylated and methylene dioxide derivatives of 6 oxygenated flavonols were identified (patuletin, spinacetin, spinatoside and jaceidin). There was a 2.0 fold variation in the total flavonoids (1805-3703 mg/Kg) and 1.7 fold variation in the total antioxidant capacity among genotypes. The correlation between antioxidant capacity and total flavonoid content was found to be high (0.96).

The medicinal effects may be enhanced in diseases such as cancer by using antioxidant cocktails (Ravit Hait-Darshan et al 2009).\(^\text{18}\) There was a synergistic antioxidant activity between commercial antioxidants and natural antioxidant NAO ((NAO is a unique, powerful antioxidant isolated from spinach leaves). The commercial antioxidants used were 3 polyphenols - ferulic acid, caffeic acid and epigallocatechin-3-gallate (RGCG).

**Effect of Spinach consumption on serum antioxidant status**
Epidemiological studies have clearly established that fruit and vegetable consumption is associated with better health. Antioxidants present in fruits
and vegetables are believed to be responsible for this. However the overall antioxidant status in humans as affected by fruit and vegetable consumption is not very clear.

There was a 7-25% increase in serum antioxidant capacity during 4-h period after the consumption of spinach, strawberries, red wine or vitamin C (Cao et al. 1998). The absorption of phenolic compounds in these foods might have resulted in the increase in antioxidant capacity.

Organic Spinach

Organic farming does not use chemicals during food production, processing or storage. So the pest pressure may put greater stress on the synthesis of a plant’s chemical defense mechanism (Carl and Davis 2006). So, more secondary plant metabolites are expected to be produced in organic plants. These secondary plant metabolites, which are mostly antioxidants, may also benefit human health. Some studies have shown that organic foods contain more antioxidants, although there is no conclusive evidence to prove or disprove this claim.

The Environmental Working Group has reported that spinach is one of the dozen most heavily pesticide-contaminated produce (EWGs 2011 Shoppers Guide to Pesticides in Produce). Permethrin, dimethoate, and DDT are common pesticides found on spinach. It will be interesting to know if these pesticides have any influence on the nutritional composition or bioavailability of nutrients present in spinach.

Ren et al (2001) have compared the antioxidant activity of five organically cultivated and generally cultivated vegetables (welsh onion, quiang-gen-cal, spinach, chinese cabbage, green pepper). The results reveal that organically grown spinach has 120% higher antioxidant activity compared to generally cultivated spinach. At least two flavonoid contents were more than double (significant at 95% level in t test) in organically grown welsh onion, quiang-gen-cal and spinach as revealed by LC/MS quantitative analysis compared to generally cultivated vegetables. A comparative study by Eunmi Koh et al (2012) has shown that the mean levels of ascorbic acid and flavonoids were significantly (P<0.001) higher in organically grown spinach where as nitrate content was significantly (p<0.001) higher in conventional spinach and there was no significant difference in oxalate content in organic and conventional spinach.

Influence of spinach consumption on some patho and physiological conditions: A number of studies have indicated that daily consumption of spinach has a positive influence on various organs of human body. These have been categorized under the following headings.

Spinach and age related macular degeneration: The leading cause of irreversible blindness among adults is age-related maculardegeneration (AMD). Epidemiological studies have revealed an inverse association between AMD and consumption of carotenoid rich foods. Johana et al (1994) have shown that higher frequency of spinach or collard greens intake substantially lowers the risk for AMD (P for trend <.001). But the intake of preformed vitamin A (retinol) or vitamin E or vitamin C did not reduce the risk for AMD.

Spinach and cataract: Consumption of spinach and other greens at least 5 times/week resulted in 47% lower risk for cataract extraction compared to less than once month consumption (Hankinson et al. 1992). A retrospective case control study in Northern Italy by Tavani et al (1996) has also given similar results. They found an inverse association between spinach and cruciferae consumption and cataract risk in 207 cataract extraction patients and 706 controls. There was 40% less likelihood of cataract extraction in individuals who consumed spinach at least occasionally compared to those who never ate spinach. Suzan et al (2000) have shown that dietary sources of lutein, particularly spinach and dark green leafy vegetables were most consistently associated with protection against cataract.

The causes for cataract and age-related macular degeneration include both environmental and
genetic factors and studies do indicate that dietary factors, particularly antioxidant vitamins and xanthophylls may reduce the risk of these degenerative eye diseases.

**Spinach and CVD**

Spinach is a rich source of fibre. So it has cholesterol lowering effect. HPLC analysis of pepsin–pancreatin digest of spinach Rubisco showed the presence of four new inhibitory peptides for angiotensin I-converting enzyme (ACE) [Yang et al (2003)]. They are MRWRD, MRW, LRIPVA, and IAYKPAG. The antihypertensive effect of these inhibitory peptides on rats is shown below (table 5).

Satoh et al (1995) have studied the effect of spinach leaf protein concentrate (SPCC) on serum cholesterol and amino acid concentration in rats fed a cholesterol-free diet. At lower dietary fat level (2%), SPCC had a strong cholesterol lowering effect. This is due to inhibition of cholesterol and bile acid absorption by the intestine and increase in the concentration of some serum amino acids. Spinach is one of rich sources of betaine which has a role in protecting internal organs, improve vascular risk factors and enhance performance (Stuart Craig(2004). Olthof and Verhoef (2005) have shown that rise in homocystein levels after meals can be reduced by foods rich in betaine. Increase in homocysteine levels can result in the development of CVDs. Therefore betaine rich diet may reduce the risk of CVD but it also adversely affects serum lipid concentration, which increases the risk for CVDs. So its exact role is not yet clear.

**Spinach and Gout**

Intake of high protein foods and purine rich foods are considered to be risk factors for gout. A 12 year period prospective study (Hyon K Choi et al 2004) on 47,150 men for a period of 12 years, has shown that there is no increase in the risk of gout by consuming purine-rich vegetables like spinach in moderate amounts.

Spinach and Kidney Stones

A study has shown that eight foods —spinach, rhubarb, beets, nuts, chocolate, tea, wheat bran, and strawberries— significantly increase the risk of calcium oxalate kidney stones. (Linda K Massey et al 1993). Although for healthy people endogenous metabolic synthesis is responsible for most of the oxalic acid excreted in the urine, kidney stone formers are advised to reduce the consumption of foods with high oxalic acid content.

**Spinach and Brain Function**

The volume of infarction in the cerebral cortex reduced significantly and post-stroke locomotor activity increased significantly in rats which received blueberry, spinach, or spirulina enriched diets (Yun Wang et al 2005). This effect was not directly mediated through changes in physiological functions as no difference in blood biochemistry, blood CO2, and electrolyte levels were found. Authors found that there was significantly lower caspase-3 activity in the ischemic hemisphere in rats receiving blueberry, spinach, or spirulina enriched diet. So they suggest that ischemia/reperfusion-induced apoptosis and cerebral infarction can be reduced by chronic treatment with blueberry, spinach, or spirulina enriched diets.

**Spinach and Aging**

Studies have shown that CNS functional declines in aging and age related neurodegenerative diseases are due to increased vulnerability to oxidative stress. Antioxidants are believed to play a role in reducing this stress. Onset of age related neural signal-transduction and cognitive behavioral deficits in rats can be retarded by long term dietary strawberry, spinach or vitamin E supplementation (Joseph et al (1998)). In this study following parameters were examined. 1) Oxotremorine-enhanced striatal dopamine release 2) Cerebellar betareceptor augmentation of GABA responding 3) Striatal synaptosomal Ca2+ Clearance 4) Carbachol-stimulated GTPase activity 5) Morris Water Maze performance. There
was a greatest reduction of age related effects on all above parameters except parameter 4 in spinach fed rats. Strawberry had the maximum reduction in parameter 4. The authors opine that age related changes on brain function can be prevented and sometimes even reversed by nutritional intervention with fruits and vegetables.

Spinach and Cancer: Among the common vegetables, spinach has the highest in vitro anti-proliferative activity on Hep G2 human liver cancer cells. The bioactivity index BI, calculated as $BI = \frac{1}{2} \left( \text{Total antioxidant activity score} + \text{anti-proliferative activity score} \right)$ for dietary cancer prevention was highest for spinach. The authors opine this is not solely due to the phenolic contents. It might be due to unique phytochemicals present in spinach (Yi-Fang Chu et al 2002). Longnecker et al (1997) have shown that the risk of breast cancer can be reduced by regular intake of carrots, spinach and supplements containing Vitamin A. They further found that eating carrots or spinach more than twice weekly compared to no intakes was associated with an odds ratio of 0.56 (95% confidence interval 0.34-0.91). The data shows a protective association between intake of carrot and spinach and risk of breast cancer although they do not distinguish among several potential explanations for the relation.

Thirteen compounds which acted as antimutagens were purified by Edenharder et al (2001) by preparative and micropreparative HPLC from a methanol/water (70:30, v/v) extract of dry spinach. These compounds acted as antimutagens against the dietary carcinogen 2-amino-3-methylimidazo[4,5-f]quinoline in Salmonella typhimurium TA 98. Eiichi Kotake-Nara et al (2001) investigated whether various carotenoids present in foodstuffs were potentially involved in cancer preventing action on human prostate cancer. The effect of neoxanthin from spinach on reduction in cell viability is shown in Table 6. Neoxanthin was found to reduce cell viability through apoptosis induction in the human prostate cancer cells. The results indicate that the risk of prostate cancer can be reduced by ingestion of spinach which is rich in neoxanthin. Monogalactosyldiacylglycerols (MGDG) 1 and 5, have been isolated from fresh spinach leaves by bioassay-guided fractionation as a galactolipid with possible cancer preventive effects. MGDG has inhibitory effects on tumor promoter-induced Epstein-Barr virus (EBV) activation (Lars P. Christensen, 2009).

Abraham Nyska et al (2001) showed that skin papilloma counts in female hemizygous Tg AC mice could be reduced significantly ($P<0.01$) when treated topically with a Natural Antioxidant from spinach (NAO).

CONCLUSION
Spinach is a rich source of micronutrients, phytochemicals and functional ingredients such as lutein, betaine, flavonoids, neoxanthin, galactolipids. There is enough scientific literature to show that regular consumption of spinach substantially lowers the risk of age-related macular degeneration, one of the leading causes of irreversible blindness among adults. There is an inverse association between spinach consumption and cataract risk. Spinach leaf protein concentrate has a strong cholesterol lowering effect in rats. Age related changes on brain function can be delayed by long term consumption of spinach. It has high anti proliferative activity on cancer cells. However, people prone to oxalic acid stones are to reduce consumption of spinach. So quantity and frequency of spinach consumption can be increased.

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37. Edenharder, R., Keller, G., Platt, K. L., Unger, K.K., Isolation and characterization of structurally novel antimutagenic flavonoids

List of Tables

Table 1: Xanthophyll composition of spinach

<table>
<thead>
<tr>
<th>Xanthophyll</th>
<th>Quantity (mg/100g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neoxanthin</td>
<td>58.00+/−4.6</td>
</tr>
<tr>
<td>Violaxanthin</td>
<td>65+/−9.3</td>
</tr>
<tr>
<td>Lutein</td>
<td>77.58+/−6.6</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>1.51+/−0.4</td>
</tr>
<tr>
<td>Total xanthophyll</td>
<td>202.09+/−20.9</td>
</tr>
</tbody>
</table>

Source: Rangaswamy L et al 2005

Table 2: Functional ingredients of spinach

<table>
<thead>
<tr>
<th>Functional ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lutein</td>
<td>586.9µg/Kg@ .76mg/kg#</td>
</tr>
<tr>
<td>Betaine</td>
<td>60-64.5 mg/Kg**</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>1805-3705 mg/Kg*</td>
</tr>
<tr>
<td>Neoxanthin</td>
<td>25.4 mg/kg#</td>
</tr>
<tr>
<td>Galactolipids</td>
<td>3300-3880(mg/kg)***</td>
</tr>
</tbody>
</table>

Table 3: Estimated relative bioavailability of β carotene and lutein from spinach in different forms

<table>
<thead>
<tr>
<th>Spinach form</th>
<th>β carotene (%)*</th>
<th>Lutein (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole leaf</td>
<td>5.1</td>
<td>45</td>
</tr>
<tr>
<td>Minced leaf</td>
<td>6.4</td>
<td>52</td>
</tr>
<tr>
<td>Liquefied spinach leaf</td>
<td>9.5</td>
<td>55</td>
</tr>
<tr>
<td>Liquefied Spinach + Dietary fiber</td>
<td>9.3</td>
<td>54</td>
</tr>
</tbody>
</table>

*compared to carotenoid supplement
Source: Jacqueline et al 1999

Table 4: Total antioxidant activity of spinach by various methods

<table>
<thead>
<tr>
<th>Method used for analysis</th>
<th>Total antioxidant activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORAC</td>
<td>1.94 m mol TE/kg</td>
<td>a</td>
</tr>
<tr>
<td>FRAP</td>
<td>9.8 m mol/kg, 26.94 m mol Fe²⁺/l, 43-94 m mol TE/kg (dry weight)</td>
<td>b, c, e</td>
</tr>
<tr>
<td>ATBS</td>
<td>8.49 m mol TE/kg</td>
<td>a</td>
</tr>
<tr>
<td>TRAP</td>
<td>5.79 m mol TE/kg</td>
<td>c</td>
</tr>
<tr>
<td>TOSC</td>
<td>42.20 m mol TE/kg</td>
<td>d</td>
</tr>
<tr>
<td>TEAC</td>
<td>8.49 m mol TE/kg</td>
<td>c</td>
</tr>
</tbody>
</table>

TE: Troloxequivalent

Table 5: Antihypertensive effect of inhibitory peptides isolated from spinach

<table>
<thead>
<tr>
<th>Inhibitory peptide</th>
<th>Dosage (mg/kg)</th>
<th>Time for maximum reduction (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRW</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>MRWRD</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>IAYKPAG</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>LRPVA</td>
<td>100</td>
<td>No reduction</td>
</tr>
</tbody>
</table>

Table 6: Effect of neoxanthin on cancer cell viability

<table>
<thead>
<tr>
<th>Type of prostate cancer cell</th>
<th>Percent reduction in cell viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC 3</td>
<td>10.9</td>
</tr>
<tr>
<td>DU 145</td>
<td>15</td>
</tr>
<tr>
<td>LNCaP</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig 1: General structure of Lutein
Fig 2: The chemical structure of trimethylglycine

Fig 3: Chemical structure of Neoxanthin

Fig 4: General chemical structure of a monogalactosyldiacylglycerol (MGDG), a type of galactolipid