Spinach (*Spinacia oleracea* L.)

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Abstract

Recent insights about food and nutrition signify that the consumption of fresh produce is defensive to general acute and chronic disorders. Dark green leafy vegetables are acknowledged to ameliorate these symptoms as they possess abundant nutrients and biological properties. *Spinacia oleracea* (spinach) is widely available and accepted traditional green, leafy vegetables in the world: it is an excellent choice for micronutrients and phytonutrients. Thus, the consumption of this vegetable is recommended on a regular dietary regimen. The phytochemicals and bioactives that are derived from spinach (raw and cooked) are capable of a) scavenging singlet oxygen species and inhibit oxidative stress, b) alter gene expressions that are associated with metabolic activities, tumors, acute and chronic inflammation, and antioxidant system, and c) diminish diet intake by altering hormones involved in obesity. These biological activities are associated with various metabolic disorders. Hence, regular consumption of spinach would provide a qualitative well-being against cancer, obesity, hyperglycemia, and hyperlipidemia. This chapter provides insights about the functional

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and biological properties of spinach involved in different investigations. The mechanistic process of spinach and spinach-derived phytochemicals imparting health benefits is explored.

Keywords

Spinach · Phytochemicals · Metabolic activities · Oxidative stress · Health benefits

8.1 Introduction

Botanical Name, common name and scientific classification.

Spinacia oleracea is generally known as Spinach (English). The other native forms of the names are as follows:

Sanskrit: Chhurika.
Hindi, Gujarati, Marathi: Palak.
Kashmiri: Palakh.
Bangla: Palang.
Tamil: Pasalai.
Telugu: Mathubucchali (Guha and Das 2008).

The ayurvedic name of spinach is "Paalankikaa." It is known as "Paalak" and "Vasaiyila-keerai" in Unani and Siddha, respectively (Khare 2007). The plant profile and the scientific classification is provided in the Table 8.1.

Spinach is originally a leafy vegetable which is dark green in color belonging to the Amaranthaceae family, which includes beets and chard (Morelock and Correll 2008). It is commonly annual and rarely biennial plant. Generally, it reaches 30 cm height and can survive even low temperatures in temperate regions. Spinach is broadly categorized corresponding to their leaf texture. This contains three variants:

Table 8.1 Scientific classification of *Spinacia oleracea* L.

Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub class	Caryophyllidae
Order	Caryophyllales
Family	Chenopodiaceae
Genus	Spinacia L.
Species	*Spinacia oleracea L.*

Source: Natural Resources Conservation Service www.plants.usda.gov smooth, savoy, and semi-savoy. Smooth spinach leaves are recommended for salads and processing, meanwhile, the other two are widely used for cooking. Among the three variants, savoy is dark green in color with wrinkled and wavy leaves. On the other hand, semi-savoy is a crossbreed variant and has marginally wrinkled leaves and is considerably simpler to process than traditional savoy. As the name says, smooth-leaved spinach is observed to have exceptionally smooth leaves. Spinach is adaptable with all culinary process with health-benefitting bioactive nutrients. Therefore, it is highly desired among all populations (Nishihara et al. 2001). It is available and can be cooked even during the winter periods or can be processed as canned or frozen product. Despite low-calorie content, spinach provides greater amount of phytonutrients and antioxidants. Also, they have abundant levels of iron and calcium. Additionally, spinach is a great resource of flavonoids. They exhibit biological properties against reactive oxygen species, cancer, inflammation, gamma radiation, and weight gain. Spinach is similarly employed in the prevention and treatment of bone loss linked with ageing and osteoporosis and aims at reducing the pain and inflammation in arthritis. Spinach is an absolute choice for energy boosting and has beneficiary effects on the heart and circulatory system. The other potential health benefits of spinach consumption include improved blood glucose control, lowered risk of cancer, and improved bone health (Metha and Belemkar 2014). Spinach is largely comprised of water (91.4%), protein (2.9%), carbohydrate (3.6%), and fat (0.4%). The lipid portion is primarily comprised of mono- and poly-unsaturated fatty acids with trace amounts of saturated fatty acids. Serving of 100-g spinach includes higher concentrations of magnesium, potassium, and iron and meets 20%, 16%, and 15% individually of their recommended dietary allowance (Elvira-Torales et al. 2019). The micronutrient composition is significantly diverse compared to other generally used green leafy vegetables.

8.1.1 History

Spinach, being consumed by human race through various cultures throughout history, is notably employed in the Mediterranean, Middle Eastern, and southeast Asian cuisines. The origin of spinach was believed to be from Persia (Iran). Apparently, spinach got its attention across other portions of Asia and Europe via the trading process. (Hu et al. 2007) The ancient documents of spinach are recorded by the Chinese in 647 A.D., and it was specified as the herb of Persia. (Morelock and Correll 2008). The spinach was immensely popular in the twelfth century and reached Spain. Spinach was accepted in England and France during the fourteenth century from Spain. Germany had the knowledge regarding the thorny-seeded variant spinach around the thirteenth century while the smooth seeded spinach variant arrived in the sixteenth century. It became remarkably familiar there since the production escalated in spring, while there was a scarcity for all other vegetables in that cycle of history (Adamson 2004). “The Forme of Cury” is the earliest English cookbook, to mention cookery recipes involving raw and processed spinach. Catherine de’ Medici, the queen of France in 1533, consumed spinach on a regular diet regimen, and spinach acquired wider acceptance during her era. In the course of the World War I, French soldiers who were hurt from hemorrhage were offered wine blended with extracts from spinach. Throughout those periods, more effective variations of spinach were planted widely (Pegge 2006).

8.1.2 Production (India, World)

Spinach is produced in cool season, so the plant is mostly grown during winter and early spring. Spinach is harvested before the seed stalk develops. The spinach is cut off about an inch above the soil surface. It can be cultivated on every kind of topsoil getting decent drainage capability. It provides better yield while grown on a combination of sandy and alluvial soil, and the optimum pH of the soil for spinach growth should extend from 6 to 7. The optimal temperature for growth ranges between 15 and 30 °C with the rainfall of 80–120 cm. While harvesting, well-grown succulent and tender leaves will be trimmed. On an average, the crop can undergo 4–6 trims. The entire yield of spinach can range between 80 and 100 quintals per hectare land (Simko et al. 2014). In India, Telangana, Kerala, Tamil Nadu state, Karnataka, Maharashtra province, and Gujarat are the leading producers of spinach (Singh et al. 2018a, b).

8.1.3 Botanical Description

The botanical description of this plant includes stem, leaves, and flowers. The spinach plant is generally observed with simple leaves that stems from the axis. This usually measures around 2–30 cm long and 1–15 cm wide. The shoots develop in a rosette and are commonly wrinkled or flat. The crop creates small creamy-green blooms that are 3–4 mm thick. The blooms generate small fruit bundles that will cover the seeds within. The stem portion is usually vertical with 30 cm tall, rounded, soft, and succulent(Zikalala 2014). Spinach leaves are complementary in structure, with long petiole, with sections of a sharp three-sided structure and are soft mutually on the perimeters. The male flowers are observed in the axial region with short structures. The calyx is generally 4-parted and twinned anthers are present. Female flowers are axillary, sessile, and congested. Calyx are 2-tipped along with a projecting point on both the sides, developing into spikes during the ripening of seeds (Kirtikar and Basu 2005).

8.2 Characterization of the Chemical Compounds Responsible for Antioxidant Proprieties and the Pathways Involved in the Biological Activities

Spinach is an excellent source of micronutrients of not only vitamins but also of flavonoids, phenols, and carotenoids. The list of chemical compounds abundant in spinach is given below.

8.2.1 Flavonoids

Spinach is immensely rich in the flavonoids. Numerous flavonoids including querecetin, myricetin, spinacetin, luteolin, jaceidin, patuletin, lutein epoxide, neoxanthin, glucuronic acid 3,5,7,3′,4’pentahydroxi-6-methoxiflavone, pheophytin b, neoluteinare documented to be detected in spinach (Kaur et al. 2016).

8.2.2 Phenolic Compounds

Spinach is a better source of phenolic compounds and carotenoids. The polyphenols detected from the spinach and spinach derived extracts are para-coumaric acid, ortho-coumaric, and ferulic acid. Spinach also illustrates the existence of various carotenoids like lutein, 9′-(Z)-neoxanhin, violaxanthin and β-carotene, (Hedges and Lister 2007).

8.2.3 Vitamins and Minerals

Spinach comprises of high-level of vitamin A, C, E, and K. Also; it is similarly rich in folic acid. Along with these health-promising compounds, a variety of minerals are available in spinach and spinach-derived extracts including calcium, manganese, magnesium, iron, copper, zinc, potassium, and phosphorus (Joseph et al. 1998).

8.3 Antioxidant Properties and Mechanisms Involved

Spinach is renowned for its antioxidant beneficial mechanisms. The antioxidant mechanism of spinach isolate was demonstrated in vitro observed on eradicating ABTS (2,2′- Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical, superoxides ($O2^{-}$), $Fe2^{+}$, peroxynitrite, peroxyl, and hydroxyl radical. Derivatives from spinach demonstrated elevated overall free-radical-scavenging capacity. Spinach extracts used in vitro studies demonstrated that they aim at preventing (~35%) the peroxide production and malondialdehyde (MDA) generation due to oxidative stress (Singh and Rajini 2004). Spinach and spinach-derived extracts also include derivatives of the antioxidant flavonoids like glucuronide, patuletin, neoxanthin, and spinacetin.

Specifically, acylated flavonol glycosides from spinach comprising of patuletin and its derivates and the glucuronated flavonoid established the maximum antioxidant ability in vitro (Bergman et al. 2003). The antioxidant mechanism of spinach and spinach-derived extracts was analyzed in laboratory rodents. Spinach powder that is freeze-dried displayed considerably lowered lipid peroxidation levels in liver and DNA damage of the leukocytes (Ko et al. 2014).

In another experiment, 0.64% of spinach-derived extract was supplemented for Fischer rats over 8 months and was observed that there was substantial reduction in 2′,7′-dichlorofluorescin generation, which is a notable indicator of free radicals in the striatum and cerebellum (Lomnitski 2000a). In a separate experiment, spinach-derived extract (1% suspension) was superficially applied and exhibited better effects than 5% vitamin E in suspended form against malonaldehyde generation and reducing lipo-oxygenase activity. This was observed in the epidermal layer of UV-exposed experimental mice. Plasma lipid peroxidation and superoxide (H2O2) levels in these animals were considerable reduced (−15%) than the control ones. Along with the above-mentioned evidence, spinach and spinach-derived extracts contain higher levels of glycosylated derivatives of para-coumaric acid and flavonoids (Wang 2018). Clinical studies have also offered a few data about the antioxidant mechanism of spinach.

An exclusive study on elderly women was conducted (age = 66.9 year and n = 7–8). This showed that inclusion of spinach in every meal (294 g) had greatly impacted the ORAC (oxygen radical absorbance capacity), TEAC (Trolox equivalent antioxidant capacity), and FRAP (ferric reducing capacity of plasma) (+25%), (+24%), (+21%) respectively post 4 h than the control population without spinach consumption (Heim et al. 2002). Another 2-week study on males with the regular intake of spinach powder (10 g suspended in milk or water) had reduced DNA damage and repaired strand breakage in lymphocytes. In this research, plasma lutein levels were observed to be hiked by 2-folds post consumption of spinach powder. Also, spinach scavenges superoxide anions and hydroxyl radicals, increases serum lutein, and induces antioxidant enzymes comprising of catalase, superoxide dismutase, glutathione reductase, and glutathione. In an exclusive study 48 healthy male and female subjects were subjected to consume raw or cooked 20 g spinach/day for 3 weeks. The results showed improved plasma lutein levels and erythrocyte glutathione reductase levels. Apparently, all these clinical investigations support the perception that spinach, and its derived extracts, enhances oxidative defense mechanism involving the upregulation of antioxidant enzymes and its representative genes (Roberts and Moreau 2016).

8.4 Health Benefits

Every diseases and disorders are usually associated with current pharmacological therapies. Apparently, most of these are related with unfavorable and irreversible side effects including nausea, aversion, constipation, faintness, and even weight gain. Hence, functional foods are alluring substitutes in the therapy and prevention

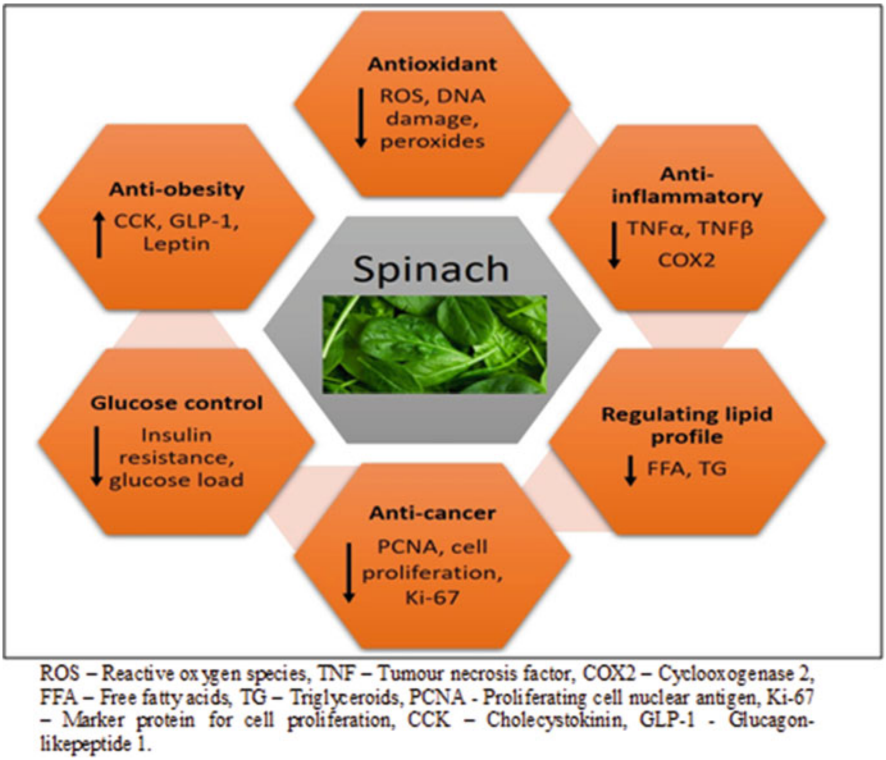

Fig. 8.1 Functional properties and health benefits of spinach

of lifestyle disorders (Frewer et al. 2003). The functional properties and the health benefits of spinach are given in Fig. 8.1.

8.4.1 Anti-inflammatory Properties

Immune responses occurring naturally to any biological infection are inflammation. Long-term inflammation can cause severe and persistent disorders including cell proliferation, cardiac disease, and type-2 diabetes mellitus (Manabe 2011). The anti-inflammatory potential of spinach was investigated in different laboratory animals. Spinach-derived extracts (10 mg/kg via i.p.) were supplemented to male Wistar rats for 8 successive days and then was administered with a dose of lipopolysaccharide (LPS-10 mg/kg via i.p.). The results exhibited reduced hepatic lacerations caused by LPS-induction and, diminished cyclooxygenase-2 (COX-2) production, which creates the initiation of inflammatory cells (e.g., monocytes, polymorphonuclear leukocytes). Correspondingly, laboratory rabbits in New Zealand receiving spinach extracts (10 mg/kg, via i.p.) were examined with LPS (10 mg/kg via i.p.) for 8 consecutive days and were found to reduce lesions in major organs caused by

LPS treatment. It is well known that LPS stimulates inflammation via toll-like receptor-4 (TLR-4) and regulating nuclear factor kappa B (NF-κB), in the cytoplasm attached to the inhibitory protein (IκB). During this process, LPS is bound to TLR-4 and stimulates the phosphorylation and initiation of IκB kinase (IKK), and IκB is phosphorylated. IκB goes through proteasomal modification, permitting NF-κB to the nucleus by dimerization and translocation where NF-κB initiates the expression of pro-inflammatory cytokines, eicosanoids, and free radicals (Lomnitski 2000b).

The aromatic phenolics including para-coumaric acid in spinach extracts provide notable anti-inflammatory properties (Fan et al. 2011). Fischer rats were supplemented with 0.02% (w/w) dry spinach for 6 weeks and it was observed that the expression of NF-κB mark genes, TNF-α and TNF-β, reduced in the cerebellum region. The anti-inflammatory potential of lutein, which is a carotenoid having increased bioavailability, has been involved with investigation on animal simulations and human trials (Ciccone et al. 2013). BALB/c mice administered with lutein (10 mg/kg via i.p.) tested with LPS exhibited that the plasma concentrations of prostaglandin E2 (PGE2), TNF-α, and IL-1β, along with reduced loads of iNOS and COX-2 enzymes in liver (Anchi et al. 2017). LPS-induced peritoneal macrophages administered with 20 μM lutein showed modified levels of PGE2, TNF-α, and IL-1β. The above results determined that, by eradicating free radicals, lutein reduced the accessibility of H2O2 to trigger IKK-dependent activation of NF-κB, thus reducing NF-κB target gene expression (Vats 2017). Spinach also comprises of significant levels of β-carotene and mimics the anti-inflammatory potential of lutein via similar mechanisms. Further investigation in clinical trials is necessary to validate these findings involving the consumption of spinach and spinach extracts or foods rich in lutein, reducing inflammation in animals via inflection of the NF-κB pathway (Aman et al. 2005).

In gastrointestinal epithelial cells (AGS cells), H2O2-induction and NF-κB activation was inhibited by β-carotene and was observed with reduced levels ofinterleukin-8 (IL-8), a proinflammatory chemokine. Crucially, the bioavailability of β-carotene is much lesser than lutein, since both the carotenoids link up adversely with each other all through the intestinal absorption process. Since the level of lutein in spinach is considerably greater than β-carotene, the anti-inflammatory process is majorly facilitated by lutein. A randomized, double-blind test was conducted, and 25 healthy men and women were provided dried spinach (10.4 g/day) for 8 weeks with placebo-controlled trial for finding the effects of spinach on inflammation. Participants exhibited a substantial rise in serum lutein with the consumption of dried spinach. Therefore, the findings from laboratory animals and cell culture studies suggested that lutein from spinach eradicates the inflammatory reaction by reducing the initiation of NF-κB, and recommended that addition of lutein-enriched foods, like spinach, may possibly suppress inflammation (Madaan et al. 2017).

8.4.2 Antiproliferative Properties

Globally, cancer is a growing lifestyle-based disorder and affects people irrespective of age and gender. Existing statistics indicate that the prevalence percentage of this disease will hike by 70% in the upcoming decades (Yang 2006). It is a primary priority to address this issue with steadfast and effective approaches to restrict and heal cancer. Among the various causes combined with carcinogenesis, the impact of food on hyperplasia is fairly determined. Eating patterns with the incorporation of dark green vegetables are well correlated with a diminished probability for several cancers (Neuhouser 2004). Epidemiological investigations have exhibited a defensive function of spinach intake various types of cancers (Scalbert et al. 2005).

In a case-control study on women who had raw spinach (>52 portions/year) showed 45% reduced chance of breast cancer. Lutein, one of the carotenoids, was particularly connected with a decreased danger of breast cancer (Gaudet et al. 2004). Colon cancer probability was decreased 11% with one serving of spinach (73 g/week) on a regular dietary regimen. In a cohort study of 4,90,802 participants, it was observed that the consumption of spinach in raw or cooked form was linked with a notable decline in esophageal adenocarcinoma (Freedman et al. 2007). The chemoprotective activities of spinach have been examined mutually in animal models and cancer cell lines. Spinach-derived extracts 200 mg/kg/day administered orally increased the proliferation in dorsal and lateral sections of pancreas. Spinach acts as an antiproliferative agent and with consumption of spinach for 2 weeks significantly repressed proliferation of colon epithelial cells that is heme-induced in Wistar rats. These defensive effects were caused due to the soaring content of chlorophyll in spinach (Mutanen et al. 2011). The consistent incorporation of spinach in the diet is chemopreventive in cancer affirmative subjects.

Some significant understandings into the cancer protecting processes of spinach have established from cell line studies. Various human cancer cell lines were observed to have dose-dependent and time-dependent antiproliferative activity with spinach extracts (Dutta 2015). Spinach leaves constitute major amounts of glycolipids compared to other glycolipid-rich foods. Monogalactosyl diacylglycerol (MGDG) is widely present followed by digalactosyl diacylglycerol (DGDG) and sulfoquinovosyl diacylglycerol (SQDG). Glycolipids from spinach showed antiproliferation action in varied cancer cells acquired from diverse cancer cell lines including gastric cancer (NUGC-3), leukemia (HL-60), lung cancer (A549), cervix cancer (HeLa), B-cell acute lymphoblastoid leukemia (BALL-1), T-cell acute lymphoblastic leukemia (Molt-4), and colon cancer (Colon-26). Glycolipids reduced the activity of markers involved in cell proliferation and terminated the cell progression, triggering apoptosis. SQDG fractions were against cell proliferation by preventing the replicative process of purified DNA polymerase-α (Javadi 2018). In BALB/c mice, spinach glycolipids reduced colon adenocarcinoma tumor growth and sarcoma tumor volume. Glycolipids (20 mg/kg/day) for 2 weeks pretreated animals with Colon-26 cells implantation revealed a 48.9% decline in tumor growth. Additional investigations analyzing the assimilation of spinach glycolipids are required to be established if whole glycolipids, their absorption products, or a blend of both are accountable for the anticancer activities in the gastrointestinal tissues (Roberts and Moreau 2016). It has been proved that spinach-derived glycolipids have antiproliferative potential by preventing DNA replication during tumor growth. Nearly all investigations that analyzed the defensive impacts of spinach on cell proliferation were performed in laboratory rodent and cell lines. The cumulative results recommend that spinach reduces the probability of esophageal, colon, and breast tumors in humans. Nevertheless, there is a crucial requirement for human intervention studies intended at exploring the anticancer potential of spinach and its derivatives.

8.4.3 Antiobesity Properties

Improper lifestyle and unhealthy dietary patterns lead to altered BMI and result in obesity. Obesity statistics are on the elevated side with approximately 300 million adults across the world suffering from obesity with a BMI $\geq$ 30 (Swinburn et al. 2011). Various measures related to public health are provided to control the epidemic. Bringing about a change in lifestyle with change in dietary pattern blended with exercise and behavioral therapy are the requisite measures adapted by the medical world to combat serious health issues such as obesity. There is rising concern in the incorporation of nutraceutical ingredients in our daily diet to curb orexigenic signals, which minimizes the intake of calories.

In humans, a patented spinach extract comprising of thylakoids was reported to influence satiety as well as decrease consumption of food in animals: 100 g of spinach-derived thylakoid includes protein, fat, carbohydrate, salt, chlorophyll, lutein, zeaxanthin, β-carotene, vitamin A, vitamin K, vitamin E, folic acid to a level of 23.5 g, 11.9 g, 41.7 g, 3.5 g, 3000 mg, 27.9 mg, 730 μg, 4760 μg, 21 μg, 1313 μg, 6.07 mg, and 166 μg, respectively (Li et al. 2019). Female Sprague-Dawley rats supplemented with fat rich diet supplemented with thylakoids reduced their food intake (Ca. –30 kcal/day) in a 13-day study helped in lowering body weights in comparison with control ones. In another study of 100 days, incorporation with thylakoids helped in inhibiting food consumption, body fat as well as body weight in female apoE-deficient mice (Köhnke et al. 2009a, b). Thylakoids have also been reported to slow down the digestion and fat absorption and promote satiety by stimulating satiety signals (Emek et al. 2011). In healthy humans, it was demonstrated that consumption of diet comprising 25 g, or 50 g spinach thylakoids led to a rise in hormone cholecystokinin after 6 h of food intake.

In another significant study, levels of glucagon like pepetide-1 were reportedly increased (44%) postprandially when women were overnight treated with thylakoid-treated (5 g/day). In addition to cholecystokinin and glucagon-like pepetide-1, the satiety hormone leptin derived from adipose was substantially enhanced after a time period of 6 h subsequent the ingestion of a food including 25 g or 50 g thylakoid. Post intake of a thylakoid supplemented meal, there was a sharp decline in serum levels of ghrelin, a hunger hormone derived from stomach in humans after a period of 2 h.

Various studies have acknowledged the potential of the satiety hormones, leading to a sharp decline in appetite. In a pivotal work, thylakoid incorporation (3.7 g or 7.4 g) in a meal led to inhibition in postprandial hunger and eating tendency in healthy overweight women. Thylakoid incorporation was also pronounced on anthropometric parameters of body composition in overweight women in single-blinded intervention study, which was carried for 12 weeks. It was concluded from this study that there was a significant reduction in body weight of subjects, which were fed on 5 g thylakoids on daily basis as compared to the placebo group. There was no major change in parameters such as fat-free mass, body fat, and circumference of waist with spinach administration (Montelius et al. 2013). Hence, it can be concluded that the supplementation of diet with spinach-derived thylakoids was reported to inhibit interim hunger, intake of calories, and gain in body weight in healthy population via the alteration of cholecystokinin and glucagon-like pepetide-1 and ghrelin secretions.

8.4.4 Hypoglycemic Activity

Hyperglycemia is highly predominant worldwide and is recognized as one of the clinical indications of metabolic disorder as well as a prime factor to develop type-2 diabetes. These are considered as the alarming indications, which necessitated the need for adopting innovative nutritional and therapeutic methodologies to monitor glycemia and reduce insulin resistance.

Insulin sensitivity has been also reported to be improved by the consumption of spinach and derived compounds out of it. Various cell culture experiments have proved the insulin-sensitizing mechanism of extracts isolated from spinach (Amirinejad et al. 2019). Effects of raw spinach either in juice form or ethanolic extracts on the variation of 3 T3-L1 preadipocytes were studied in a significant study. It was analyzed that spinach juice and ethanolic extract were able to induce 3 T3-L1 cell differentiation in the presence as well as absence of insulin. In in vitro assays it was also found that spinach as juice as well as ethanolic extract inhibited the assimilation of disaccharides (−19.6%) by intestinal α-glucosidase.

Several animal studies have been reported so far regarding the hypoglycemic potential of spinach-derived composites. In one of the study, Wistar rats administered with alloxan monohydrate in order to abolish β-cells from pancreas and roots insulin deficiency followed by administering daily dosage of 70% ethanolic extract of spinach (100 mg/kg body weight) for 12 successive days led to a noticeable reduction of plasma glucose levels (152 mg/dl). In crossbred pigs supplied with high-fat diet, spinach-derived thylakoid (0.5 g/kg body weight) had the potential to curb blood sugar level to a considerable extent. In an extended investigation, when Sprague-Dawley rats were supplemented with spinach-derived thylakoid for 10 days, plasma levels of insulin dropped in a 2-hour OGTT in comparison to control food lacking thylakoid.

In the medical trial, thylakoids were reported to modulate the postprandial insulin response in healthy subjects. People who were fed with a food containing 25 g or

50 g of thylakoids observed a significant reduction in insulin levels despite the fact that the test meal had higher protein and carbohydrate than control. Decrease in the insulin response in the form of reduction in glucose uptake was reported, since thylakoids reduced the transport of methyl-glucose throughout the intestinal brush border line of rats in vitro by affecting intestinal permeability by attaching to it. In case of obese people, ingestion of a meal including 5 g of thylakoids resulted in significant hike in postprandial plasma glucose concentrations in a time period of 2 h as compared to the placebo-containing meal (Singh et al. 2018a, b). Intake of 5 g of thylakoids before the consumption of a test meal led to lowering of postprandial plasma glucose and insulin levels at 15 minutes followed by slowly lowering of insulin concentrations after a time period of 4 h (Stenblom et al. 2015). To conclude, thylakoids can be utilized as a great tool in regulating postprandial glycemia and insulinemia by inhibiting glucose absorption and decreasing insulin production.

8.4.5 Lipid-Lowering Properties

Hypertriglyceridemia in clinical terms can be defined as the condition when blood triglyceride levels go beyond 150 mg/dL, resulting in ailments such as pancreatitis, liver disease, and cardiovascular diseases as well. It is predominant in developed countries, including the United Kingdom, France, South Korea, and the United States to a level of 27.5%, 27%, 25%, and 24%, respectively, but it is also considered as a public health concern in India (47%) (Selçuk 2020). Hypertriglyceridemia can be corrected by weight management accompanied with increased physical activity, nutritional supplementation with fish oil or niacin, and medication intervention as well. Spinach especially spinach thylakoids and its extracts as well can be a great source of alternate choices to these measures. Thylakoid membranes derived from leaves of spinach are a great source of lowering down blood lipids.

Consumption of a meal comprising thylakoids (50 g) reduced serum free fatty acids postprandially than the subjects without consumption of spinach thylakoid. This is well in line with the findings that thylakoid-rich diets decreased serum triglycerides and free fatty acids significantly in both Sprague-Dawley rats and apoE-deficient mice (Köhnke et al. 2009a, b). In a rodent study, there was a reduction in lipid levels with the consumption of daily dose of 70% ethanolic spinach extract (100 mg/kg). Rats treated with spinach extract were reported a reduction in serum triglycerides. It was able to stabilize plasma triglycerides to a level of 83 mg/dL in nondiabetic rats than the diabetic ones. Some of the animal studies have shown the efficiency of spinach-derived thylakoids and phytochemicals in diminishing blood triglycerides (Roberts and Moreau 2016). However, less study has been devoted to investigate the potential of cooked and raw spinach on post-prandial blood triglyceride level. Due to less literature available, the impacts of spinach and its components on blood lipids needs more exploration.

8.5 Conclusion

Spinach (*Spinacia oleracea* L.) is a leafy vegetable hailing from goosefoot family. Spinach is known to represent several antioxidative, antiproliferative, anti-inflammatory, and hepatoprotective effects. It has also been acknowledged to exhibit flavonoids, carotenoids, and phenolic compounds as well. Various common chronic illnesses find their causative agents in the form of free radical and inflammatory markers, and spinach has been known to play a role in the eradication of these ailments. Consumption of spinach strengthens the antioxidant defense mechanism. Spinach could be a great source of therapeutically effective extracts. A high level of research is conducted in order to emphasize on the therapeutic uses of spinach leaves. Prospective intervention investigations should address various functional properties to inform the best knowledge derived from clinical experience of spinach as well as its bioactives.

References

Adamson MW (ed) (2004) Food in medieval times. Greenwood Publishing Group, Westport

Aman R, Schieber A, Carle R (2005) Effects of heating and illumination on trans− cis isomerization and degradation of β-carotene and lutein in isolated spinach chloroplasts. J Agric Food Chem 53(24):9512–9518

Amirinejad A, Hekmatdoost A, Ebrahimi A, Ranjbaran F, Shidfar F (2019) The effects of hydroalcoholic extract of spinach on prevention and treatment of some metabolic and histologic features in a rat model of nonalcoholic fatty liver disease. J Sci Food Agric 100(4):1787–1796

Anchi P, Khurana A, Bale S, Godugu C (2017) The role of plant-derived products in pancreatitis: experimental and clinical evidence. Phytother Res 31(4):591–623

Bergman M, Perelman A, Dubinsky Z, Grossman S (2003) Scavenging of reactive oxygen species by a novel glucurinated flavonoid antioxidant isolated and purified from spinach. Phytochemistry 62(5):753–762

Ciccone M M, Cortese F, Gesualdo M, Carbonara S, Zito A, Ricci G, Pascalis FD, Scicchitano P, Riccioni G (2013) Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care. Mediators Inflamm 1–12.

Dutta S (2015) Natural sources as potential anti-cancer agents: a review. Int J Pharm Drug Anal 3 (5):156–164

Elvira-Torales LI, Periago MJ, González-Barrio R, Hidalgo N, Navarro-González I, Gómez-Gallego C, García-Alonso FJ (2019) Spinach consumption ameliorates the gut microbiota and dislipaemia in rats with diet-induced non-alcoholic fatty liver disease (NAFLD). Food Funct 10 (4):2148–2160

Emek SC, Akerlund HE, Clausén M, Ohlsson L, Weström B, Erlanson-Albertsson C, Albertsson PA (2011) Pigments protect the light harvesting proteins of chloroplast thylakoid membranes against digestion by gastrointestinal proteases. Food Hydrocoll 25(6):1618–1626

Fan D, Hodges DM, Zhang J, Kirby CW, Ji X, Locke SJ, Prithiviraj B (2011) Commercial extract of the brown seaweed Ascophyllum nodosum enhances phenolic antioxidant content of spinach (*Spinacia oleracea* L.) which protects Caenorhabditis elegans against oxidative and thermal stress. Food Chem 124(1):195–202

Freedman ND, Park Y, Subar AF, Hollenbeck AR, Leitzmann MF, SchatzkinA ACC (2007) Fruit and vegetable intake and esophageal cancer in a large prospective cohort study. Int J Cancer 121 (12):2753–2760

Frewer L, Scholderer J, Lambert N (2003) Consumer acceptance of functional foods: issues for the future. Brit Food J 105(10):714–731
Gaudet MM, Britton JA, Kabat GC, Steck-Scott S, Eng SM, Teitelbaum SL, Gammon MD (2004) Fruits, vegetables, and micronutrients in relation to breast cancer modified by menopause and hormone receptor status. Cancer Epidemiol Biomark Prev 13(9):1485–1494
Guha D, Das S (2008) CNS depressive role of aqueous extract of *Spinacia oleracea* L. leaves in adult male mice albino rats. Indian. J Exp Biol 46(3):185–190
Hedges LJ, Lister CE (2007) Nutritional attributes of spinach, silver beet and eggplant. Crop Food Res Confidential Rep 1928
Heim KE, Tagliaferro AR, Bobilya DJ (2002) Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutri Biochem 13(10):572–584
Hu J, Mou B, Vick B (2007) Genetic diversity of 38 spinach (*Spinacia oleracea* L.) germplasm accessions and 10 commercial hybrids assessed by TRAP markers. Genet Resour Crop Ev 54:1667–1674
Javadi B (2018) Diet therapy for Cancer prevention and treatment based on traditional Persian medicine. Nutr Cancer 70(3):376–403
Joseph JA, Shukitt-Hale B, Denisova NA, Prior RL, Cao G, Martin A, Bickford PC (1998) Long-term dietary strawberry, spinach, or vitamin E supplementation retards the onset of age-related neuronal signal-transduction and cognitive behavioral deficits. J Neurosci 18(19):8047–8055
Kaur D, Kamboj A, Shri R (2016) Comparative evaluation of anxiolytic effects of various extracts of oats (Avena sativa), rice bran (Oryza sativa) and spinach (*Spinacia oleracea)* in experimental animals. Int J Pharm Sci Drug Res 7(10):4110
Khare CP (2007) Indian medicinal plants, 1st edn. Berlin/Heidelburg, Springer, pp 622–623
Kirtikar KR, Basu BD (2005) Indian medicinal plants. In: Bio-geen books, vol 8, 2nd edn. International Book Distributors, Dehradun, pp 2078–2079
Ko SH, Park JH, Kim SY, Lee SW, Chun SS, Park E (2014) Antioxidant effects of spinach (*Spinacia oleracea* L.) supplementation in hyperlipidemic rats. Prev Nutr Food Sci 19(1):19
Köhnke R, Lindbo A, Larsson T, Lindqvist A, Rayner M, Emek SC et al (2009a) Thylakoids promote release of the satiety hormone cholecystokinin while reducing insulin in healthy humans. Scand J Gastroenterol 44(6):712–719
Köhnke R, Lindqvist A, Göransson N, Emek SC, Albertsson PÅ, Rehfeld JF et al (2009b) Thylakoids suppress appetite by increasing cholecystokinin resulting in lower food intake and body weight in high-fat fed mice. Phytother Res 23(12):1778–1783
Li Y, Cui Y, Lu F, Wang X, Liao X, Hu X, Zhang Y (2019) Beneficial effects of a chlorophyll-rich spinach extract supplementation on prevention of obesity and modulation of gut microbiota in high-fat diet-fed mice. J Funct Foods 60:1034–1036
Lomnitski L, Carbonatto M, Ben-Shaul V, Peano S, Conz A, Corradin L, Nyska A (2000a) The prophylactic effects of natural water-soluble antioxidant from spinach and apocynin in a rabbit model of lipopolysaccharide-induced endotoxemia. Toxicol Pathol 28(4):588–600
Lomnitski L, Foley JF, Grossman S, Shaul VB, Maronpot RR, Moomaw CR, Nyska A (2000b) Effects of apocynin and natural antioxidant from spinach on inducible nitric oxide synthase and cyclooxygenase-2 induction in lipopolysaccharide-induced hepatic injury in rat. Toxicol Pathol 87(1):18–25
Madaan T, Choudhary AN, Gyenwalee S, Thomas S, Mishra H, Tariq M et al (2017) Lutein, a versatile phyto-nutraceutical: an insight on pharmacology, therapeutic indications, challenges and recent advances in drug delivery. Pharma Nutri 5(2):64–75
Manabe I (2011) Chronic inflammation links cardiovascular, metabolic and renal diseases. Circ J 75 (2011):2739–2748
Metha D, Belemkar S (2014) Pharmacological activity of *spinacia oleracea* linn.-a complete overview. Asian J Pharam Res Develop 2(1):83–93
Montelius C, Osman N, Weström B, Ahrné S, Molin G, Albertsson PÅ, Erlanson-Albertsson C (2013) Feeding spinach thylakoids to rats modulates the gut microbiota, decreases food intake and affects the insulin response. J Nutri Sci 2(20):1–9

Morelock TE, Correll JC (2008) Spinach. In: Prohens J, Nuez F (eds) Vegetables I, Handbook of Plant Breeding, vol 1. Springer, New York, pp 189–218

Mutanen M, Niku M, Oikarinen S (2011) Green leafy vegetables in cancer prevention. In: Vegetables, whole grains, and their derivatives in cancer prevention. Springer, Dordrecht, pp 31–45

Neuhouser ML (2004) Dietary flavonoids and cancer risk: evidence from human population studies. Nutr Cancer 50(1):1–7

Nishihara E, Inoue M, Kondo K, Takahashi K, Nakata N (2001) Spinach yield and nutritional quality affected by controlled soil water matric head. Agric Water Manag 51(3):217–229

Pegge S (2006) The Forme of Cury. Bibliobazaar Ltd.

Roberts JL, Moreau R (2016) Functional properties of spinach (*Spinacia oleracea* L.) phytochemicals and bioactives. Food Func 7(8):3337–3353

Scalbert A, Manach C, Morand C, Rémésy C, Jimenez L (2005) Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr 45(4):287–306

Selçuk KT (2020) Epidemiology of inflammation-related diseases. In Role of Nutrition in Providing Pro−/Anti-Inflammatory Balance: Emerging Research and Opportunities: IGI Global, pp 24–44

Simko I, Hayes RJ, Mou B, McCreight JD (2014) Lettuce and spinach. Yield Gains Major US Field Crops 33(4):53–85

Singh N, Rajini PS (2004) Free radical scavenging activity of an aqueous extract of potato peel. Food Chem 85(4):611–616

Singh A, Dubey PK, Chaurasiya R, Mathur N, Kumar G, Bharati S, Abhilash PC (2018a) Indian spinach: an underutilized perennial leafy vegetable for nutritional security in developing world. Energ Ecol Environ 3(3):195–205

Singh J, Jayaprakasha GK, Patil BS (2018b) Extraction, identification, and potential health benefits of spinach flavonoids: a review. In: Advances in plant Phenolics: from chemistry to human health. American Chemical Society, Washington DC, pp 107–136

Stenblom EL, Egecioglu E, Landin-Olsson M, Erlanson-Albertsson C (2015) Consumption of thylakoid-rich spinach extract reduces hunger, increases satiety and reduces cravings for palatable food in overweight women. Appetite 91:209–219

Swinburn BA, Sacks G, Hall KD, McPherson K, Finegood DT, Moodie ML, Gortmaker SL (2011) The global obesity pandemic: shaped by global drivers and local environments. Lancet 378 (9793):804–814

U.S. Department of Agriculture, Agricultural Research Service (2005) USDA National Nutrient Database for standard reference, release 18. Nutrient Data Laboratory Home Page, Accessed at: http://www.nal.usda.gov/fnic/foodcomp

Vats S (2017) Methods for extractions of value-added nutraceuticals from lignocellulosic wastes and their health application. In: Grumezescu AM, Holban A-M (eds) Ingredients extraction by physicochemical methods in food. Academic Press, London, pp 1–64

Wang M (2018) Interactions between dietary flavonoid apigenin and human gut microbiota in vitro (Doctoral dissertation, Rutgers University-School of Graduate Studies)

Yang L (2006) Incidence and mortality of gastric cancer in China. World J Gastroenterol 12(1):17

Zikalala BO (2014) Chemical composition of baby spinach (*Spinacia oleracea* L.) as affected by nitrogen, phosphorous and potassium nutrition. Thesis submitted to University of South Africa, Pretoria. Available at http://hdl.handle.net/10500/18762