Antioxidant Activity and Health Benefits of Anthocyanin of Black Soybeans

by Ignasius Radix Ap Jati

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4.1 INTRODUCTION

Soybean (*Glycine max* [L.] Merrill) is one of the world's most important agricultural commodities. The production of soybean globally reaches 362.76 million metric tons (USDA, 2020). The United States, Brazil, and Argentina are the highest producers of soybean. Approximately 68% of global soybean production is used to meet demand in the food sector.

Soybean belongs to the family *Fabaceae*, genus *Glycine*, and subgenus *Soja* (Moench). *Glycine soja* is the name of wild soybean founded in China and neighboring countries. The domesticated soybean is known as *Glycine max* (L.) Merrill. The seed coats have different colors, such as the most commonly grown yellow, green, and black seed coats.

According to Kumudini et al. (2008), the structures of the soybean plant leaf are characterized as the seed (cotyledon) leaves, the primary (unifoliolate) leaves, the trifoliolate leaves, and the prophylls. Meanwhile, the shape of the mature soybean seed is oval and consists of a seed coat surrounding a large embryo. Even though the planting properties are similar to those of the yellow soybean, in that it can be planted in various well-drained soils, favoring a slightly acidic soil (pH 6.0–6.5); needs a salinity threshold of approximately 5 ds/m; and needs the temperature between 10° C and 40° C during the growing season, thus being considered a short-day plant, the black soybean is reported to be more resistant to disease and environmental stress (Lee et al., 2020).

In recent years, the popularity of black soybean is increasing rapidly due to its health properties. Due to its similar characteristics with common yellow soybean, various products can be made from black soybean, for example, vegetable oil and its derivatives, such as margarine, salad dressing, and mayonnaise. In addition, black soybean can also be used as an alternative to meat or animal-based

DOI: 10.1201/9781003030294-4

DPPH and ABTS methods. During fermentation, the isoflavone aglycone and malonylglycoside were increased, which is believed to contribute to the higher antioxidant activity of *cheonggukjang*. Meanwhile, a published study of *doenjang* was done by Kim et al. (2009). *Doenjang* was made from black soybean fermented using *Bacillus subtilis*. The result revealed that black soybean fermented paste *doenjang* exhibits higher antioxidant activity and phenolic compounds compared to the unfermented black soybean. The fermentation process is responsible for the increase in the beneficial properties of *doenjang*. The maximum level of phenolic and antioxidant activity is observed for 110 days of fermentation.

Due to the popularity of the food fermentation process, fermented soybean products are widely developed. In China, *douchi* is one of the traditional black soybean fermented products which are also commercially available. A comprehensive report conducted by Xu et al. (2015) investigates 28 commercially available soybean-based fermented products. Among all the samples, black soybean *douchi* products show the highest antioxidant activity examined by the DPPH method. This could be due to the conversion of isoflavone glycoside to their aglycone form. Moreover, it was presented that there was an increase of essential amino acids, which could be used as an indicator of the availability of bioactive peptides that can also act as antioxidants. Research from Japan, conducted by Jiang et al. (2019), investigated the antioxidant activity of black soybean supplemented in rice miso. The result shows that the products have high antioxidant activity and peptides content. The fermentation process could degrade the amino acids to their smaller peptides, which provides bioactive properties in the inhibition of the oxidation process.

The fact that black soybean and its products are provided free radical scavenging capacity is widely acknowledged. Besides, the promising abilities of black soybean seed and its products were also clearly observed. However, the *in vitro* free radical scavenging examinations using reagents are not sufficient to reach an agreement on the health benefit effects of the black soybean. An in-depth investigation is needed using various tests *in vitro* as well as *in vivo* using animal and human studies on health effects such as anti-inflammatory, anticancer, anti-atherosclerosis and coronary heart disease, antidiabetic, and anti-obesity activity.

4.4.2 Anti-Inflammatory and Anticancer Activity

Among several health property investigation methods, anti-inflammatory and anticancer activities from the natural compound are the most commonly examined. Inflammation has been widely investigated because it is associated with various types of diseases, for example, cancer, atherosclerosis, arthritis, and allergy. Early work on the anti-inflammatory effect of black soybean, especially its anthocyanin, was performed by Nizamutdinova et al. (2009), which suggested that anthocyanin plays an important role in the inhibition of pro-inflammatory cytokines and also stimulates wound healing in fibroblasts and keratinocytes. As postulated by Wang et al. (2013), inflammation is a natural biological process conducted by the human body in response to the abnormal condition of infection, irritation, or other injuries. The mechanism of anti-inflammatory activities of natural products is widely investigated since natural products or extracts have been commonly used to treat patients with inflammatory symptoms since ancient times. Inflammation is a process when the immune system responds to abnormal conditions by releasing pro-inflammatory cytokines such as interleukin (IL)-1b, IL-6, and tumor necrosis factor-alpha (TNF-a) sequentially. These pro-inflammatory cytokines' production should be inhibited to prevent or reduce the risk of inflammatory disease incidence. The inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) are also inflammatory mediators involved in various inflammatory processes. The evidence can be seen in the presence of those inflammatory mediators in the inflammatory processes–related cells. Therefore, research has been conducted to suppress the activity or down-regulate the expression of inflammatory mediators using various plants containing the bioactive compound extract. Black soybean, rich in polyphenol, anthocyanin, isoflavone, and bioactive peptides, has also been investigated for potency as an anti-inflammatory agent. Research by Jeong et al. (2013) revealed

On the other hand, the total phenolic content was decreased. Thus, it can be proposed that flavonoids, including anthocyanin, are responsible for black soybean soy milk antioxidant activity. Meanwhile, Ma and Huang (2014) investigated the bioactive compound and antioxidant activity of different soybean-based milk, including black soybean milk. The result revealed that black soybean milk had higher antioxidant activity and total phenolic content than yellow soybean milk. The black seed coat of black soybean contributes to the high content of phenolic as well as anthocyanin. Therefore, the antioxidant capacity was higher compared to the yellow soybean milk. A study by Tan et al. (2016) produced black soybean soy milk with different grinding methods. The result shows that even though black soybeans had gone through several steps of processing to become soy milk, the soy milk product maintained its antioxidant activity to scavenge free radicals. The heating process, which is widely accepted as a factor contributing to the significant decrease of various bioactive compounds and antioxidant activities of food products, only slightly reduced the ability to scavenge free radicals of cooked soy milk.

Another popular soybean-based food product is tofu, a yellow soybean–based product manufactured by the curdling process of soy proteins. Due to the increased focus on the health properties of black soybean, it is common to produce tofu using black soybean. An investigation by Shih et al. (2002) revealed that black soybean tofu exhibits higher antioxidant potential in inhibiting the formation of peroxide compared to the common yellow soybean. There was no significant difference observed in the antioxidant activity of the black soybean and black soybean tofu. This result is possibly due to the combined action by anthocyanin, phenolic, isoflavone, and other bioactive compounds, including peptides in tofu.

The development of black soybean products has been expanding from traditional products to new and popular products such as black soybean tea, spaghetti, and crackers. Research on the bioactive compounds and antioxidant activity of black soybean-based crackers shows that the bioactive compound content and antioxidant activity of black soybean crackers is higher than that of the yellow soybean crackers (Slavin et al., 2013). The high content of anthocyanin in black soybean played a crucial role in maintaining the antioxidant activity of crackers even though a decrease was observed on phenolic and anthocyanin. Moreover, the contribution to the antioxidant activity of crackers could be due to the isoflavone content, which was not significantly affected by the heating process. This research suggested that moderate temperature in crackers manufacture is needed to retain its phenolic and anthocyanin contents.

The effect of roasting in producing black soybean snacks and beverages was also investigated. Shen et al. (2019) performed research on the effect of roasting on the antioxidant activity of small black soybean. Small black soybean is known as a remedy or herb for the traditional treatment of diseases. Unlike the common fact that the heating process will reduce the bioactive compound and antioxidant activity, the roasting process increases the phenolic content and antioxidant activity measured by DPPH and ABTS. The release of phenolic compounds from its matrices by roasting is believed to be responsible for the result. Thus, the reaction with DPPH and ABTS resulted in higher antioxidant activity than the unroasted black soybean. On the other hand, a study by Zhou et al. (2017) revealed that the pre-treatment process of soaking before roasting on black soybean could decrease the antioxidant activity as measured by DPPH, ABTS, and FRAP methods. The soaking process is responsible for the leaching of anthocyanin and phenolic compounds, thus decreasing its antioxidant activity. Based on the fact that roasted black soybean is popular to be consumed as a snack and health food supplement, the preparation and cooking process should be done cautiously.

Another popular method to utilize black soybean, especially as a medicinal food, is to germinate the black soybean to become a black soybean sprout. Many studies show that germination increases the beneficial properties of legumes due to the increased rate of enzyme activities during germination, which leads to the leaching of various nutritional and functional compounds from their matrices. Research by Kumari et al. (2015) revealed that germination could increase the antioxidant activity of black soybean sprouts by approximately 403% due to the release of anthocyanin, phenolic, and isoflavone, as well as the vitamin C content of black soybean due to enzyme activity during

In addition to the *in vitro* study using cell culture, *in vivo* research was also widely conducted to examine the anti-inflammatory properties of the black soybean. Research by Kanamoto et al. (2011) shows that the administration of black soybean extract in high-fat diet-fed mice resulted in reduced gene expression of major inflammatory cytokines such as tumor necrosis factor-R and monocyte chemoattractant protein-1. Although the cellular mechanism is unclear, the results provide a promising potency of black soybean to be developed as a functional food, reducing the inflammatory process.

Meanwhile, the anthocyanin study in downregulating pro-inflammatory cytokine expression was conducted by Park et al. (2015). By consuming anthocyanin-rich extract from black soybean, the COX-2 expression in the normal-diet-fed mice was significantly reduced by the addition of anthocyanin extract. The prominent contributor of anthocyanin to inhibit the inflammatory process is cyanidin 3 glucoside. It is postulated that such a role was not merely attributed to the extract's antioxidant activity but also the ability of individual anthocyanin to interfere with a signaling pathway by a direct blockage. Besides, the lower serum concentration of PGE2 in mice was also observed by anthocyanin extract supplementation. PGE2 is known as a metabolite of COX-2. Thus, supplementation of anthocyanin from black soybean could have beneficial effects in reducing the inflammatory incidence, and therefore, black soybean could potentially be developed as a functional food.

Another *in vivo* study was also performed using rats induced with a high-fat diet to investigate the capability of consuming black soybean seed coat extract in the inhibition of obesity-related inflammatory processes (Kim et al., 2015). The result shows that administering black soybean seed coat extract could remarkably suppress the gene expression of TNF-α and IL-6, which are pro-inflammatory adipocytokines that play a role in the adipogenesis pathway. The gene suppression capability could be due to the cyanidin 3 glucoside in black soybean, which could activate the AMPK pathway by decreasing TNF-α expression and contribute to the significant decrease of body fat accumulation (Kwon et al., 2007). This result suggested that black soybean can be optimized as food for the diet in obesity prevention.

The inflammatory process is also closely related to the onset of various diseases, including cancer. As reported by the World Health Organization, cancer is the second leading cause of death globally (WHO, 2008). Therefore, significant research has been conducted in various fields, including medicine, pharmacology, and pharmacognosy, to elucidate the complex mechanism of cancer and investigate drugs and plant bioactive compounds that could potentially be used as drugs or remedies for cancer treatment. Black soybean, which has long been known as a traditional herb for various diseases, has also been investigated for its anticancer properties.

An early study was performed by Shon et al. (2007) on the anticancer activity of fermented black soybean extract on the HeLa, HepG-2, HT-29, and MCF-7 cancer cells. The result showed that fermented black soybean extract has strong potential as an anticancer agent contributed by the anthocyanin and phenolic content known to have high antioxidant activity. The anticancer result was positively correlated with the antioxidant activity measured by DPPH, ABTS, reducing power, and the inhibition of NO production. In agreement, research by Zou and Chang (2011) revealed that black soybean extract was capable of suppressing the proliferation of human AGS gastric cancer cells due to the polyphenol content in black soybeans such as phenolic acid, anthocyanin, isoflavone, and flavonols. Those bioactive compounds could induce the apoptosis process in cancer cells by altering the ratio of Bax to Bcl-2 and activation of caspase-3, followed by cleavage of PARP. Meanwhile, a study on the anticancer activity of black soybean paste *doenjang* was performed by Park et al. (2015). HT-29 human colon cancer cells were used to examine the anticancer activity of the extract. It was reported that black soybean extract exhibited an anticancer effect on HT-29 cells by MTT assay. It was suggested that this activity could be closely related to the ability of black soybean *doenjang* to reduce the inflammation process by downregulating the pro-inflammatory cytokines such as TNF-α, IL-6, and COX-2. Although the molecular mechanism of cancer cell growth inhibition remains unclear, it is strongly believed that bioactive compounds in black soybean *doenjang*, such as phenolic acid and anthocyanin, are playing a vital role in such accomplishment.

could be used as functional food related to their anti-inflammatory and anticancer activities and polyphenols content, especially anthocyanin and the low molecular bioactive peptides available.

4.4.3 Anti-Atherosclerosis and Coronary Heart Disease

Coronary heart disease and related cardiovascular diseases have become the highest cause of mortality globally. The blockage of blood circulation causes this disease by the accumulation of plaque in the blood vessel, thus blocking delivery to the heart. The blockage of blood vessels is usually caused by a fat deposit. The process of depositing fat in the artery walls is known as atherosclerosis. Atherosclerosis is formed by three consecutive processes: Fatty streak formation, atheroma formation, and atherosclerotic plaque formation (Rafieian-Kopaei et al., 2014). One of the contributors to the formation of plaque is low-density lipoprotein (LDL) cholesterol. The oxidation of LDL increases the formation of foam cells, which then accumulate in the arteries. Therefore, the antioxidant substances play an important role in inhibiting the initial step of atherosclerosis by preventing the oxidation of LDL, as shown in Figure 4.6 (Moss et al., 2018). Anthocyanin is an antioxidant that could inhibit LDL oxidation and also reduce the incidence of inflammation, which, as a result, could decrease atherosclerotic formation in the blood vessel. Various studies of plant herbs and medicine capability on the inhibition of LDL oxidation have been published, including black soybeans. A study by Takahashi et al. (2005) investigated the antioxidant activity of black soybean and yellow soybean seed coat on the capability to inhibit LDL oxidation. The result shows that the extract of black soybean seed coat could prolong the lag time of LDL oxidation compared to the yellow soybean seed coat. This condition describes the ability of black soybean extract to delay the propagation phase after the initial phase. This result is probably due to the higher anthocyanin content in black soybean compared to yellow soybean. Moreover, hydrolyzing soybean with β-glucosidase has successfully increased the inhibition rate of LDL oxidation due to the fact that hydrolyzed soybeans are rich in aglycone which has higher antioxidant activity. Aglycone is also prominently found in fermented soybean. Therefore, the consumption of fermented soybean products such as tempeh, natto, miso, and soybean paste is recommended to decrease the risk of atherosclerotic formation. In agreement, Astadi et al. (2009) examined two local Indonesian varieties of black soybean, which were Mallika and Cikuray. Both black soybeans could decrease the LDL oxidation. The ability of black soybeans Mallika and Cikuray was higher than that of BHT, a synthetic antioxidant used as a positive control. The anthocyanin content of black soybean is believed to be responsible for the antioxidative action. The most dominant anthocyanin in black soybean, cyanidin 3 glucoside, is

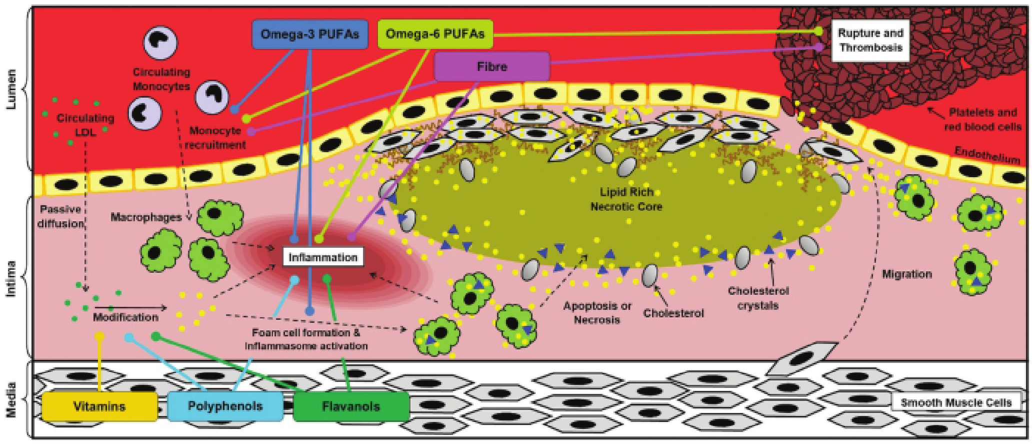

FIGURE 4.6 Role of anthocyanin in inhibiting atherosclerotic formation. Source: Moss et al. (2018).

binding to the active side of α-glucosidase enzyme and preventing the enzyme from hydrolyzing the complex carbohydrate into glucose.

A similar conclusion was provided by Jang et al. (2010) who worked on both *in vitro* and *in vivo* investigations of black soybean peptides' capability to improve insulin resistance. The *in vitro* study focused on the effect of black soybean peptides on endoplasmic reticulum (ER) stress. ER stress contributed by obesity, which then affects insulin resistance, leads to DM type 2. The result shows that black soybean peptides could decrease the ER stress and, therefore, could ameliorate insulin resistance. Furthermore, the *in vivo* study using mice suggested that the intake of black soybean peptides could reduce blood glucose and improve the animal model's glucose tolerance. Bioactive peptides in black soybean are possibly improving the signaling pathway of insulin and inhibiting the glucosidase enzyme. Therefore the homeostasis of blood glucose could be maintained. Besides, a human clinical trial was performed by Kwak et al. (2010) on the ability of the peptide to improve glucose control in prediabetes, and newly diagnoses subjects with DM type 2. The result revealed that subjects with 12-week supplementation of black soybean peptides tended to have lower fasting glucose levels and a significant reduction in two hours post-load glucose compared to the placebo group. Although the mechanism of the decrease of blood glucose level by black soybean peptides is still unclear, it is suggested that peptides can be bound to various sites of α-glucosidase, which then inhibit their capacity to hydrolyze carbohydrates.

Meanwhile, work by Kurimoto et al. (2013) focused on the ability of black soybean seed coat extract, which is rich in polyphenol content, to improve the hyperglycemia condition and insulin sensitivity in diabetic mice. The result suggested that the intake of black soybean seed coat extract could ameliorate the hyperglycemia shown by the decrease in blood glucose levels. The insulin sensitivity was improved through the activation of AMP-activated protein kinase (AMPK) in the skeletal muscle and liver of the animal model. Besides, the upregulation of glucose transporter 4 in the skeletal muscle and the downregulation of gluconeogenesis in the liver were observed. The beneficial effects of black soybean seed coat extract were caused by cyanidin 3 glucoside and proanthocyanidin, which are abundantly available. In agreement, cyanidin 3 glucoside was reported to have antidiabetic activity via the initiation of differentiation of preadipocytes into a smaller size and improved insulin sensitivity (Matsukawa et al., 2015). The administration of black soybean seed coat extract reduces the body and the white adipose tissue weight and decreases the size of adipocytes in white adipose tissue. The mechanism was revealed using 3T3-Ll cells treated using black soybean seed coat extract and individual cyanidin 3 glucosides. The result shows that smaller adipocytes were observed as a result of 3T3-L1 differentiation. Furthermore, PPARγ and C/EBPα gene expressions and adiponectin secretion were increased. On the other hand, the tumor necrosis factor-α secretion was decreased. Meanwhile, the insulin signaling was activated and improved, and the glucose uptake was increased.

Besides peptides and anthocyanin, phenolic compounds could also contribute to the improvement of DM type 2 by inhibiting the work of α-amylase and α-glucosidase enzymes (Tan et al., 2017). All of the crude extracts, semi fractionated and fractionated, show better inhibition capacity than the commercial inhibitor. An interesting finding is that the fractionated extracts provide a different result for both enzymes. For example, myricetin could significantly inhibit α-amylase but shows no significant differences observed for α-glucosidase. Thus, it can be suggested that the synergistic effect among phenolic compounds is crucial in the inhibition of both enzymes.

4.4.5 Anti-Obesity

Obesity in recent years has become an international concern due to its progressive development rate. Obesity is not only prevalent in developed countries but also spread widely in developing countries. Diabetes, atherosclerosis, coronary heart diseases, and cancer are diseases that closely relate to obesity. It is believed that obesity is playing a significant role in the occurrence of such morbid diseases. Balancing the diet could contribute to the reduced risk of obesity along with physical activities. The development of biochemistry and genetic-related research leads to the elucidation of the mechanism

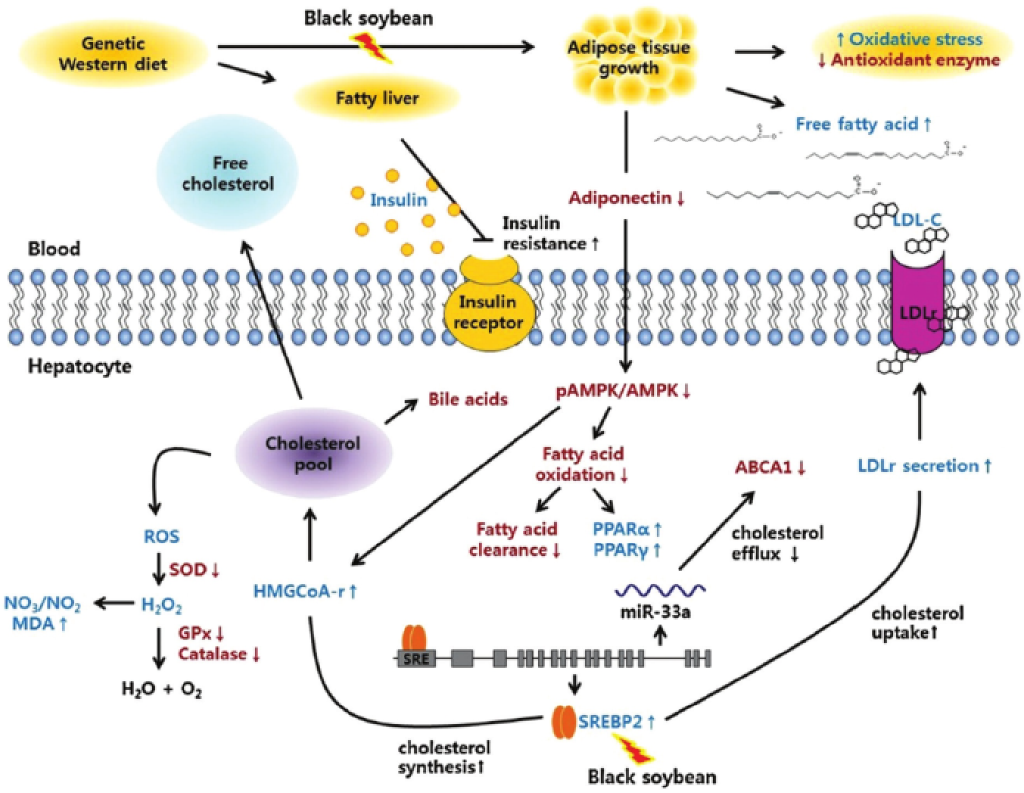

FIGURE 4.7 The mechanism of black soybean against non-alcoholic fatty liver disease. Source: Jung et al. (2013).

high-fat-diet-induced obese mice were supplemented by both the extract and the powder of fermented black soybean. The anti-obesity capacity was also performed in adipocytes. The result shows that fermented black soybean could decrease the body weight gain of mice and also suppress the mRNA expression of adipogenesis-related genes such as peroxisome proliferator-activated receptor γ (PPAR γ), fatty acid-binding protein 4 (FABP4), and fatty acid synthase (FAS). Meanwhile, the lipid accumulation of 3T3-L1 adipocytes was also decreased by the presence of fermented black soybean. Fermentation could increase the level of isoflavone glycoside, which is known to play an essential role in lipid metabolism and is also responsible for part of hydrolysis of protein to smaller peptides that also could give beneficial effects for the obesity condition. Furthermore, anthocyanin and other phenolic compounds available in black soybean also contribute to the ability of black soybean to improve obesity in the animal model.

In line with previously published studies, anthocyanin of black soybean seed coat was proven to contribute to the improvement of lipid profile, level of abdominal fat, and also low-density lipoprotein content of Korean overweight/obese adults in a randomized controlled trial (Lee et al., 2016). The result suggested that anthocyanin from the black soybean seed coat is responsible for reducing body weight and lipid accumulation. Its essential task is to activate the AMPK pathway in the white adipose tissue, skeletal muscle, and liver, and thus promote the catabolic and inhibit the anabolic pathways of lipids.

Meanwhile, work by Jing et al. (2018) explored the effect of black soybean intake on the lipid and also gut microbiome profile of high-fat-diet-induced mice. The result shows that the improvement of the lipid profile was observed, and a significant decrease of triglyceride, total cholesterol, and low-density lipoprotein content was found in mice in the black soybean supplemented diet such diseases. An unhealthy lifestyle contributes to human metabolism by creating an unbalanced status and increasing susceptibility to the onset of various diseases. For example, oxygen metabolism, which is an ordinary process under normal circumstances to generate reactive oxygen species (ROS), could shift to excessive production of ROS as a response from the body to the abnormal oxidation process. The ROS production, which is usually used for cell signaling and homeostasis, had become uncontrollable. Therefore, it is also called free radicals.

Free radicals tend to attack other molecules in order to be stable. DNA, lipids, and proteins are the most vulnerable substances in the presence of free radicals. This process is suggested to be the start of various diseases' development. Free radicals can be stabilized by substances known as antioxidants through different pathways, such as donating their hydrogen to scavenge free radicals, known as a primary antioxidant, thus breaking the chain reaction, and also decomposing hydroperoxide radicals into non-reactive substances, known as the secondary antioxidant. The human metabolism system has its defense mechanism against free radicals through the numbers of enzymes with antioxidant capacity called indigenous antioxidants such as catalase, superoxide dismutase, and glutathione peroxidase.

The rate of ROS production in the human body due to environmental stress, however, could not be managed by indigenous antioxidants alone. Therefore, an exogenous antioxidant from various sources is needed. Intake of fruits, vegetables, and legumes, which for centuries have been known as healthy food, becomes the researcher's focus to explore the substances responsible for the health effects of such commodities. Among many plants, black soybean is rich in anthocyanin, which could act as a free radical scavenger, having anti-inflammatory, anticancer, and anti-atherosclerosis activity, the ability to prevent coronary heart disease, and antidiabetic and anti-obesity activity.

4.4.1 Free Radical Scavenging Activity

A free radical is defined as an unstable substance due to its unpaired electron configuration. The incidence of various diseases is believed to be caused by free radicals which reactively attack molecules in the human system such as DNA, protein, and lipids. Free radicals can be stabilized by antioxidants through the hydrogen atom donation or free radical scavenging process. Antioxidants can rapidly donate their hydrogen atoms to free radicals, stabilize, and thus terminate the chain reactions. Antioxidant compounds such as anthocyanin have high free radical scavenging activity. The action mechanism of anthocyanin as an antioxidant is available in Figure 4.4. It can be seen that anthocyanin can act as a hydrogen donor that could stabilize free radicals, and is thus called a radical scavenger. Also, anthocyanin could react with hydroperoxide to yield a non-radical product. Different methods have been developed to examine their activities due to the vital function of antioxidants as a free radical scavenger. The examples of the methods are DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (ferric reducing antioxidant power), hydroxy radical scavenging activity, superoxide anion radical scavenging, and ABTS (2,2′-Azino-bis[3-ethylbenzthiazolin-6-sulfonic acid]).

Due to the importance of free radical scavenging assays, combined methods were commonly provided by researchers in their published reports in order to ensure that the substances examined were showing similar trends in free radical scavenging activities using different assay protocols. Numerous studies of black soybean free radical scavenging activities have been published. Such research spreads from the exploration of raw black soybean seeds to black soybean–based food products. Moreover, different processing methods, as well as geographical regions, were also widely investigated. A report by Astadi et al. (2009) examines the antioxidant activity of black soybean seed coat of the Mallika and Cikuray variety using the DPPH method. The result shows that the extract of both varieties could scavenge more than 90% of DPPH radicals. The black soybean Mallika variety is mainly utilized to produce sweet soy sauce products in Indonesia. A study from China by Zhang et al. (2011) on the radical scavenging capacity of 60 different varieties of black soybean revealed that antioxidant properties detected by DPPH, FRAP, and Oxygen Radical Absorbance Capacity (ORAC) methods all showed wide variations ranging from 4.8 to 65.3 μg/100

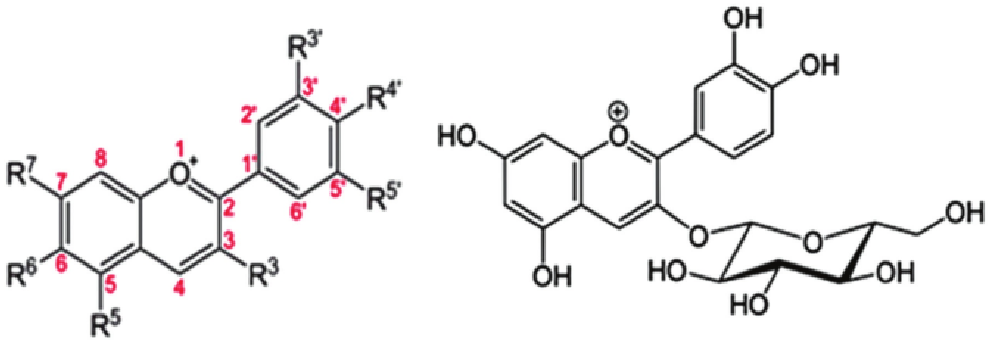

FIGURE 4.3 Chemical structure of anthocyanin (left) and cyanidin 3 glucoside (right).

TABLE 4.2
Anthocyanin Content of Black Soybean and Black Soybean Products

Black Soybean Varieties	Sources	Total Anthocyanin Content (mg/G)	References
Mallika	Indonesia	13.63	Astadi et al., 2009
Cikuray	Indonesia	14.68	Astadi et al., 2009
Cheongja 3	Korea	12.11	Jang et al., 2010
A3	Sichuan, China	3.95	Wu et al., 2017
QWT31	Yunnan, China	4.96	Wu et al., 2017
QWT5	Guizhou, China	3.01	Wu et al., 2017
JJ16	Chongqing, China	3.62	Wu et al., 2017
Black Tokyo	Serbia	1.92	Kalusevic et al., 2017
Cheongja 4 ho	Miryang, Korea	1.68	Ryu & Koh, 2018
852	Heilongjiang, China	6.96	Xie et al., 2018

Source: Jati (2020).

grapes. Some of the newest reports on the anthocyanin content of black soybean seed coats are presented in Table 4.2. It is shown that there were differences observed in anthocyanin content among black soybean varieties due to the variety of species, climatic conditions, and also geographical location. In addition to the anthocyanin content, a number of studies were performed to elucidate the individual anthocyanin of the black soybean seed coat. Such research mainly aims to investigate the prevalent individual anthocyanin found in the black soybean seed coat. Thus, in-depth exploration of the mechanism of anthocyanin's health properties, such as the capability of anthocyanin to inhibit the oxidation process and the role of anthocyanin in combatting degenerative diseases, could be investigated. The number of publications investigating the individual anthocyanin in black soybean seed coat is presented in Table 4.3. As shown in Table 4.3, the most common and abundantly found individual anthocyanin in black soybean seed coat is cyanidin. Meanwhile, other anthocyanins such as delphinidin, peonidin, malvidin, petunidin, and pelargonidin were also present in the black soybean seed coat. However, the concentration of individual anthocyanin depends on the black soybean plant varieties.

4.3.1 Cyanidin 3 Glucoside (C3G)

C3G is the most prominent anthocyanin found in black soybean. Besides its abundant presence, numerous studies have suggested that C3G is the main compound responsible for anthocyanin's

TABLE 4.1
Black Soybean-Based Products

Groups	Example	Countries of Production
Traditional fermented product	• *In si*, *tau si* (Dried by-product of the mashed black soybean sauce fermented with *Aspergillus oryzae*)	China
	• Natto (Traditional Japanese soybean product fermented with *Bacillus subtilis*)	Japan
	• Soy sauce (Sauce fermented with *Aspergillus oryzae* and *Aspergillus soyae*, used as a condiment)	Asian countries
	• Tempeh (Traditional food from black or yellow soybean fermented with *Rhizopus oligosporus*)	Indonesia
	Cheonggukjang, *doenjang* (Steamed black soybeans fermented with *Bacillus* species)	Korea
Traditional non-fermented product	• Tofu (Protein gel-like product from soybean)	Asian countries
	• Soy milk (Soybean-based beverage made by soaking and grinding the soybean, boiling the mixture, and filtering the large-sized particles)	Worldwide
Newly developed commercial product	• Black soybean tea	Japan, Korea
	• Black soybean spaghetti	United States
	• Black soybean snack	Korea

Source: Modified from Harlen and Jati (2018).

black soybean products are presented in Figure 4.2. The rapid progress of the black soybean–based food products market is possibly due to the contribution of the black soybean's health properties. Consumers believe that consuming black soybean will provide a better health condition, which has been done for centuries by their ancestors. Moreover, the traditional belief has been supported by extensive research on the bioactive compounds of food plants, which can inhibit the onset of various degenerative diseases.

4.3 ANTHOCYANIN

Bioactive compounds are substances from food sources commonly consumed by animals and humans that are available in trace amounts and possess biologically active properties, which could affect physiological functions and cellular activities. Consuming bioactive compounds could give health benefits, both as food intake, which provides energy and other essential nutrients, and as remedial agents that contribute to the reduction of inflammation, decrease the rate of oxidative stress, and normalize metabolic disorder (Siriwardhana et al., 2013). The health effects of a high intake of bioactive compounds through the consumption of varieties of plant foods have long been known. For example, the most popular Mediterranean diet, which is based on traditional dietary and lifestyle habits in the Mediterranean region adapted to the new modern lifestyle diet, successfully exhibits potency in reducing the incidence of various degenerative diseases such as cancer, heart disease, stroke, Alzheimer's, diabetes, cataracts, and age-related functional degeneration (Hassimotto, Genovese, & Lajolo, 2009; Siriwardhana et al., 2013). The advancement of research and the awareness of a healthy diet has led to the discoveries and isolation of numerous bioactive compounds from plants such as polyphenolic compounds, including anthocyanin.

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