Phytochemicals in Soybeans

Functional Foods and Nutraceuticals

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Phytochemicals in Soybeans

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Contents

4 Antioxidant Activity and Health Benefits of Anthocyanin of Black Soybeans

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CONTENTS

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

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protein, as a stabilizer in restructured products such as nuggets and sausages, and as a meat-mimicking food in the vegetarian diet.

4.2 UTILIZATION OF BLACK SOYBEAN

Black soybean is one of the soybean varieties and has a dark black seed coat color. Like the yellow soybean variety, black soybean originated from East Asia and began to be domesticated in the period of the Shang Dynasty (1700–1100 BC) in the northern part of China. Based on ancient inscriptions, soybean is one of the five sacred commodities with rice, wheat, millet, and adzuki beans. In ancient times, black soybean was not consumed as food; however, it was widely used as remedies by traditional healers to treat various diseases such as weakness, dizziness, headaches, and digestion problems. Therefore, besides its essential function for consumption, soybean is included as a sacred grain due to its additional value as a remedy to cure various diseases. In ancient times, black soybean could only be consumed by noble families as part of traditional ceremonies. A picture of black soybean is presented in Figure 4.1.

Similar to in China, the utilization of black soybean after spreading to South East Asia, especially Indonesia, is a part of worshipping gods, known as *sesajen*. *Sesajen* is a compulsory traditional gift that is believed to be given by humans to the one who possesses almighty power and rules all living creatures in the world. *Sesajen* consists of various grains, vegetables, fruits, and also animal-based foods. *Sesajen* is available in various ceremonies such as births, birthdays, weddings, funerals, and other socio-cultural ceremonies. The aims of preparing *sesajen* are to seek safety and protection from gods for all the members of society to live in harmony and prosperity. Through being domesticated, the popularity of black soybean has been increasing and is followed by a number of various black soybean–based products, both daily consumption food products and healthy diet food products. In general, black soybean food products are divided into two major categories, which are fermented and non-fermented products. Examples of fermented black soybean products are tempeh, natto, black soybean paste, and soy sauce. Meanwhile, some non-fermented black soybean products are tofu, soy milk, and soy protein isolate. Recent progress in research on the health benefits of black soybean has led to the development of various modern and innovative products such as black soybean tea, black soybean noodle, spaghetti, cookies, and also black soybean drink. Examples of black soybean-based products can be seen in Table 4.1. Meanwhile, pictures of

FIGURE 4.1 Black soybean (*Glycine max* [L.] Merrill).

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.

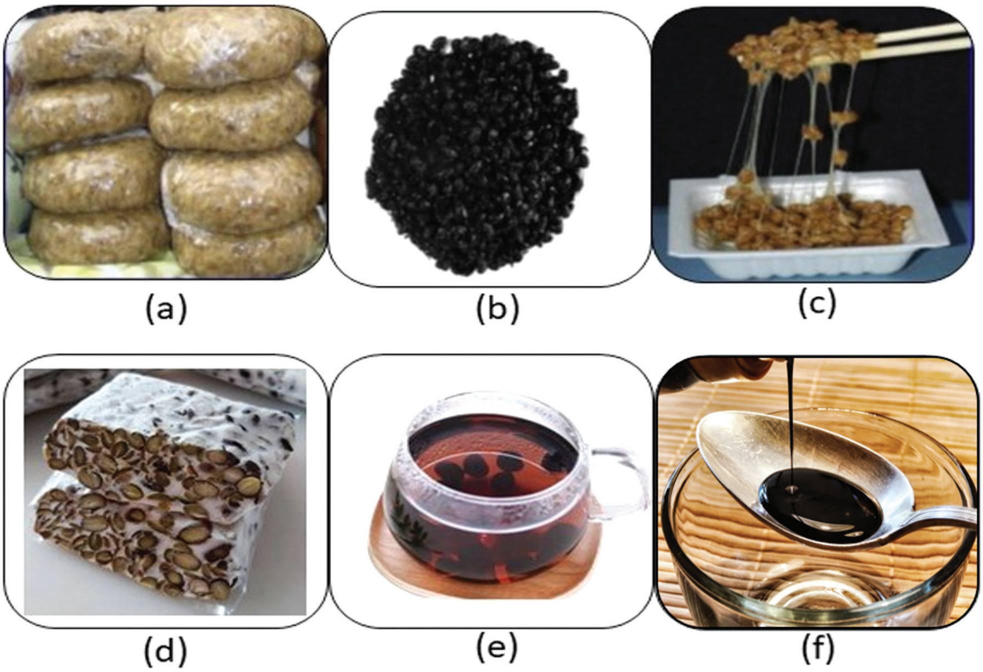

FIGURE 4.2 Examples of some commercial and non-commercial black soybean–based products: (a) *Cheonggukjang*, (b) *douchi*, (c) sweet soy sauce, (d) tempeh, (e) black soybean tea, (f) black soybean snack.

Anthocyanin is a water-soluble pigment containing the substances responsible for the formation of red, blue, and black colors in flowers and any other parts of the plant. Anthocyanin is a secondary plant metabolite included in the polyphenol group because it contains the phenolic ring in its chemical structure. The structures of anthocyanin and cyanidin 3 glucoside (C3G) as the most abundant anthocyanin found in plants are presented in Figure 4.3.

The production of anthocyanin by plants has several biological functions, such as attracting pollinators and frugivores. Anthocyanin plays a critical task in attracting pollinators and frugivores using their appealing color. Thus, pollination can be conducted. On the other hand, the anthocyanin colors also act as a repellent for herbivores and parasites. They provide a signal for herbivores and parasites that the plants contain toxic substances or signal a negative impression of unpalatable food. Anthocyanin could also contribute to plants as camouflage, a defensive mechanism to protect itself from insects and any other destructive organism.

Among several functions of anthocyanins in plants, the most investigated is the ability to act as an antioxidant with sunscreen properties due to the function of anthocyanins in protecting leaves in plants facing unfavorable conditions, such as various stressors. In a stress condition, the metabolism of the plant will be in an unbalanced state, thus resulting in an excessive oxidation rate. The anthocyanin plays an essential role as an antioxidant, which could help stabilize the reactive oxygen species due to its keen ability to act as an antioxidant in the plant system. Numerous investigators postulate that anthocyanin could also become a contributor to human health in the form of fruits, vegetables, and legumes, including black soybean rich in anthocyanins, which are consumed in the diet.

A study on anthocyanin formation in black soybean plants was first reported by Nagai (1921). Black soybeans contain a high content of anthocyanins in their seed coats. Various reports have been published in the determination of the anthocyanin content of black soybean seed coats. In agreement, the previously published research revealed that the anthocyanin content of black soybean seed coat is equal to other accessible sources of anthocyanin such as blueberry, blackberry, and

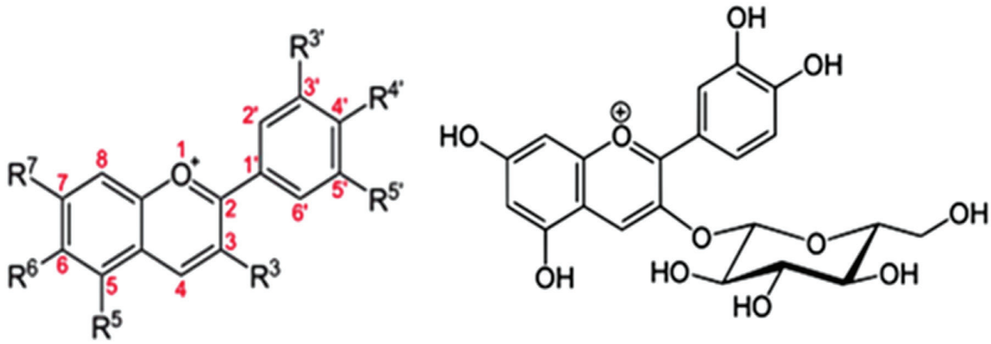

FIGURE 4.3 Chemical structure of anthocyanin (left) and cyanidin 3 glucoside (right).

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 4.3
Individual Anthocyanins of Black Soybean

Black Soybean Varieties	Source	Individual Anthocyanin	References
Cheongja 3	Korea	Cyanidin-3-O-glucoside, petunidin-3-O-glucoside, delphinidin-3-O-glucoside	Jang et al., 2010
A3	Sichuan, China	Cyanidin 3 glucoside, petunidin 3 glucoside, delphinidin 3 glucoside, peonidin 3 glucoside	Wu et al., 2017
Black Tokyo	Serbia	Cyanidin 3 glucoside, pelargonidin 3 glucoside, delphinidin 3 glucoside	Kalusevic et al., 2017
Cheongja 4 ho	Miryang, Korea	Cyanidin-3-O-glucoside, petunidin-3-O-glucoside, delphinidin-3-O-glucoside	Ryu & Koh, 2018
852	Heilongjiang, China	Cyanidin 3 glucoside	Xie et al., 2018

Source: Jati (2020).

beneficial health properties. Matsukawa et al. (2015) investigated the antidiabetes effect of C3G from black soybean on mice. It shows that exposure of adipocytes to C3G induces the differentiation of 3T3-L1 preadipocytes into smaller, insulin-sensitive adipocytes, which induced skeletal muscle metabolism. Another study on rats with breast cancer indicated that the isolate of C3G could inhibit cancer cells' development through the increase of apoptosis process activation (Cho et al., 2017). Meanwhile, dietary C3G significantly reduced body weight gain by enhancing energy expenditure, maintained glucose homeostasis, and increased insulin sensitivity in the obese mice by upregulating brown adipose tissue (BAT) mitochondrial function (You et al., 2017).

From previously published research, the beneficial health properties of C3G are postulated to be due to its radical scavenging capacity, epigenetic action, competitive protein-binding, and enzyme inhibition; thus it could act as an antioxidant and have several anti–degenerative disease capacities. The capability of C3G to act as an antioxidant is believed to be due to the two hydroxyl groups on the B ring that can donate their hydrogen atoms to stabilize free radicals (Khoo et al., 2017). Meanwhile, the activity of C3G to inhibit cancer formation and progression is due to its epigenetic action. C3G can perform epigenetic modification to regulate gene expression in various cancer cells (El-Ella & Bishayee, 2019). Moreover, the protein binding properties and enzyme inhibition capacity of C3G have been previously reported. C3G has a strong capability to bind with macromolecules, such as protein (Wiese et al., 2009). Therefore, in the metabolism system, C3G could act as an enzyme inhibitor by binding to the enzyme's active site (Balasuriya & Rupasinghe, 2011; Bräunlich et al., 2013; Sui et al., 2016). The capability to inhibit the work of enzymes is the reason behind the antidiabetic, hypolipidemic, antihypertension, and other metabolism-related disease inhibition capacities of C3G. For example, it uses glucosidase and amylase enzyme inhibition in converting carbohydrates to glucose and inhibiting hypertension-related enzymes such as an angiotensin-converting enzyme.

4.4 ANTIOXIDANT ACTIVITY AND HEALTH BENEFITS

The number of reports concerning antioxidant activity and its health benefits has been increasing rapidly in the last decades. This condition is related to the progressive rate of incidence of various diseases such as Alzheimer's, cancer, cardiovascular diseases, and diabetes. The changes of traditional healthy to modern unbalanced lifestyles in terms of workplace stress, quality of food intake, dietary habits, and environmental pollution are believed to play a crucial role in the occurrence of 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

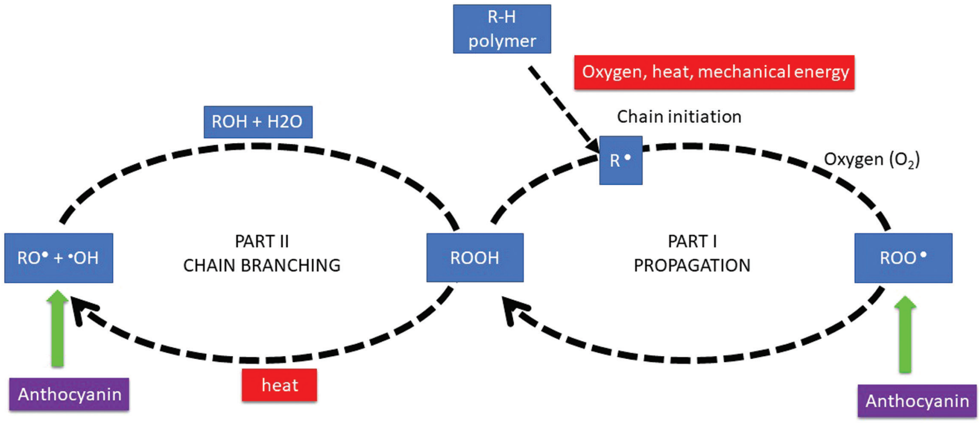

FIGURE 4.4 Antioxidative mechanism of anthocyanin.

mL (expressed as half-maximal effective concentration/EC_{50}), from 17.5 to 105.8 units/g, and from 42.5 to 1,834.6 µmol Trolox equivalent/g, respectively. Thus, this finding is scientifically supported by the traditional belief of the Chinese in using black soybean as an herb for the treatment of various diseases. The high content of the antioxidant compound could be the main reason that black soybean has beneficial properties for human health. Although black soybean is mostly popular in Asian countries, research on black soybean antioxidant activity is also reported from the black soybean grown in Central Europe. Two varieties of black soybean from Serbia were examined by Malencic et al. (2012) and compared with common yellow soybean and other colored soybeans. It is shown that both varieties of black soybean had higher total polyphenols, anthocyanin, and flavonoid contents than other varieties. In accordance, the free radical scavenging activity determination using the DPPH method exhibits a positive correlation between the total polyphenol content and the ability to scavenge DPPH radicals. Meanwhile, a recent study by Lee et al. (2020) on 172 samples of black soybean landrace in Korea shows that all of the samples exhibit free radical scavenging activity using DPPH, ABTS, and FRAP methods. The majority of published research with a wide variety of black soybean cultivars is from Korea. Compared to other countries, black soybean is a common food ingredient in Korea. Traditional food such as black soybean paste is a top-rated food product among Koreans. Meanwhile, in other countries, soybean-based food products mostly use yellow-colored soybean.

The exploration of the health benefits of soybean, especially the black-colored soybean, played an essential role in the increase of black soybean utilization as a top-rated product because soybean in Western countries was mainly explored for the oil content and also used for non-food applications. The continuous research on black soybean seeds' beneficial properties leads to the awareness of essential methods of processing used as one of the crucial steps before consumption. Black soybean processing procedures have been in the spotlight as a critical factor in maintaining the bioactive compound content and antioxidant capacity of black soybean products. A number of studies investigated the effect of different processing methods on the bioactive compounds and antioxidant activity of black soybeans, such as the production of soy milk, black soybean beverage, fermented products such as *cheonggukjang*, tempeh, natto, miso, yogurt, *petit suisse*, tofu, roasted black soybean, and germinated black soybean. Investigation of black soybean soy milk was performed by Xu and Chang (2009), which compared traditional processing with the modern ultrahigh temperature (UHT) method. The result suggested that the antioxidant activity examined by DPPH and FRAP methods was higher in the UHT and traditional processing methods than in the raw soy milk. This result could be due to the increase of the flavonoid content of black soybean soy milk resulting from the heating process.

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 germination. A similar trend was observed by Lee et al. (2018) using DPPH and ABTS methods. The seventh day of germination yielded the highest ability to act as an antioxidant. Nevertheless, it was suggested that the degraded polysaccharides with an additional hydroxyl group, especially uronic acid, were responsible for increasing antioxidant activity due to the capability to scavenge free radicals during germination.

Meanwhile, fermentation has long been known as one of the oldest methods of food processing. In the beginning, fermentation was conducted to preserve foods. Several published studies suggested a strong correlation between consuming fermented food products and the inhibition of various diseases (Pala et al., 2011; Kriss et al., 2018; Gille et al., 2018; Song & Giovannucci, 2018). The most recent development of soybean-based food products is fermented soy milk, and unlike the other traditional soy food products, fermented soybean milk is believed to be originated from Western countries (Shurtleff & Aoyagi, 2004). Lee et al. (2015) investigated the effect of fermentation of black soybean soy milk using *Lactobacillus acidophilus* ATCC 4356, *Lactobacillus plantarum* P8, and *Streptococcus thermophilus* S10 as starter cultures on the bioactive compound and antioxidant activity of soy milk. The result shows that the phenolic compound of fermented black soybean milk was higher compared to the non-fermented soy milk. Fermentation using *Streptococcus thermophilus* resulted in the highest phenolic compound compared to other starter cultures. In support of the increase of phenolic content by fermentation, the antioxidant activity of fermented soy milk was higher than that of non-fermented soy milk, as examined by the DPPH method. The higher content of phenolic compounds in fermented soy milk, which contributes to the higher antioxidant activity, could be because fermentation will decompose the substrate of the fermented product, which also breaks the matrix of foods, thus resulting in the release of phenolics content. Moreover, the antioxidant activity of fermented soy milk was also contributed by the isoflavone aglycone, which increased due to fermentation. This result is in line with previous work conducted by Cheng et al. (2013), which examined the antioxidant activity of black soybean milk extract using the DPPH method. It is reported that fermentation of black soybean milk by *Rhizopus oligosporous* exhibits higher capability in scavenging DPPH radicals due to the breakdown of the matrix in the soy milk. Therefore, the bioactive compound was released thus could donate its hydrogen atoms to stabilize the DPPH radicals.

Other black soybean food-based products reported their antioxidant activity in black soybean yogurt fermented by *L. delbrueckii* subsp. *bulgaricus* 1.1480 (Lb) (þ) and *S. thermophilus* ys14(St) (þ). The black soybean yogurt had higher antioxidant activity measured by DPPH and FRAP methods than cow milk (Ye et al., 2013). Moreover, Moraes-Filho et al. (2014) conducted research on the antioxidant activity of black soybean cheese manufactured using a mixture of *Lactobacillus acidophilus*, *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and exopolysaccharide (EPS) producer *Streptococcus thermophilus*. It shows that black soybean milk and its cheese product had high phenolic and isoflavone content, which resulted in the high antioxidant activity examined by DPPH and ABTS methods. The fermentation process is believed to play an essential role in the hydrolysis of isoflavone and the release of the phenolic compound from the food matrix, thus affecting the antioxidant activity of the product.

Other black soybean fermented products that are scientifically reported for their antioxidant activity are black soybean fermented paste, which is commonly used in the daily dishes of Korea. Examples of black soybean fermented paste are *chunjang*, *doenjang*, *daemaekjang*, and *cheonggukjang*. A report by Kwak et al. (2007) indicated that black soybean paste *cheonggukjang* shows a stronger antioxidant activity in scavenge DPPH radicals and inhibits lipid peroxidation compared to unfermented steamed soybean. The antioxidant activity of black soybean paste was positively correlated with the increase of phenolic and isoflavone aglycone and malonylglycoside contents in the fermented paste. This finding was then confirmed by Hwang et al. (2013), which investigated two varieties of Korean black soybean as the main ingredients to produce *cheonggukjang*, using potential probiotic *Bacillus subtilis* CSY191 for their antioxidant activities. The result shows that fermentation could increase the free radical scavenging activities of *cheonggukjang* examined by

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 that anthocyanins from black soybean were able to downregulate lipopolysaccharide-induced inflammatory responses in BV2 microglial cells. The anthocyanin mechanism in downregulating inflammatory response is by suppressing the NF-κB and Akt/MAPKs signaling pathways. Thus, anthocyanin from black soybean can be suggested to be used as therapeutic remedies for the condition of neurodegenerative disease. In this research, nitric oxide (NO) and prostaglandins E2, as well as TNF-α and interleukin (IL)-1β as LPS-induced pro-inflammatory mediators, were inhibited by anthocyanin. Downregulating the capability of anthocyanin was also shown in the expression of inducible NO synthase, cyclooxygenase-2, TNF-α, and IL-1β in LPS-stimulated BV2 cells. The ability of anthocyanin of black soybean as an anti-inflammatory agent is in agreement with other work conducted by Kim et al. (2013), which examined the activity of anthocyanin from black soybean to inhibit *Helicobacter pylori*–induced inflammation in human gastric epithelial AGS cells. *Helicobacter pylori* is well known for commonly infecting the gastric epithelial cells and leads to an inflammation process and various pathological incidences. Moreover, the infection of gastric epithelial cells by *Helicobacter pylori* will increase ROS, iNOS, COX-2, and IL-8 as inflammatory-associated gene expression. ROS plays a significant role in the oxidative damage of DNA, protein, and lipid. Anthocyanin of black soybean in this research can decrease the production of ROS. Besides, anthocyanin could inhibit the expression of iNOS and COX-2 as well as reduce the IL-8 production by 45.8%. Therefore, it can be suggested that anthocyanin could have a strong protective effect against gastric damage triggered by *Helicobacter pylori* infection. Both of these studies support a previous study conducted by Kim et al. (2008), which explored the capability of anthocyanin of black soybean to reduce the rate of inflammation *in vitro* using colorectal cancer cells and also *in vivo* with an animal model. The colorectal cancer cell was preferred because colorectal cancer is one of the most commonly observed cancer incidences in humans. Besides, previous research suggested that there was a negative correlation between the rate of consuming legumes and the incidence of colorectal cancer. The results show that iNOS and COX-2 expression were suppressed by anthocyanin, possibly by reducing the cellular oxidative stress. This ability is due to the hydroxyl group's presence at the 3 position of the B ring in the anthocyanin structure. Meanwhile, for the *in vivo* study, anti-inflammatory effects were also observed. Nevertheless, the anti-inflammatory properties could not solely be contributed by anthocyanin content because a similar result was also obtained for yellow soybean. A possible explanation was that the role of isoflavone and bioactive peptides in both black and yellow soybean could also act as an anti-inflammatory agent.

In support of this finding, Kim et al. (2017) investigated the downregulation of LPS-induced inflammatory markers of nitric oxide (25.01%), TNF-α (76.78%), IL-1β (58.99%), and IL-6 (84.48%) by the extract from germinated black soybean. The significant decreases of inflammatory markers were possibly due to the low molecular weight of peptides and free amino acids in the extract, which can suppress the inflammation process. However, the mechanism of low molecular peptides and free amino acids to inhibit the inflammatory process remains unclear. A more recent study conducted by Kim et al. (2018) on bioactive peptides which could act as anti-inflammatory agents revealed that black soybean could inhibit the gene expression of NO, TNF-α, IL- 1β, and IL-6 and showed that the germinated black soybean will release the smaller bioactive peptides which are readily available for antioxidative reaction. Thus, it could significantly reduce the expression of the pro-inflammatory cytokines.

Kim et al. (2017) revealed that black soybean anthocyanins significantly decreased LPS-stimulated production of ROS, inflammatory mediators such as nitric oxide (NO) and prostaglandin E2, and pro-inflammatory cytokines, including tumor necrosis factor α and interleukin-6. The capability of black soybean extract to decrease the production of ROS, PGE-2, and nitric oxide is due to the free radical scavenging activity of anthocyanin, especially cyanidin 3 glucoside, which is the most abundant anthocyanin found in the black soybean seed coat. Meanwhile, anthocyanin's mechanism in reducing the expression of NO and PGE-2 is possibly contributed by the ability of anthocyanin in inhibiting the expression of protein enzymes responsible for NO and PGE-2 production.

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.

In addition to the famous polyphenol content, black soybean, similar to the conventional yellow soybean, is a rich source of protein. Six low molecular weights of the protein were found in black soybean and harmful amino acids (Chen et al., 2019), which is responsible for the antioxidant and anticancer activity of black soybean. The different protein content, the prevalence of acidic amino acids, and the limited content of hydrophobic amino acids are parameters responsible for inhibiting ovarian cancer cell growth. Meanwhile, a recent study by Chen et al. (2018) shows that bioactive peptides isolated from black soybean by-products could inhibit the growth of cancer cells using human liver (HepG2), lung (MCF-7), and cervical (Hela) cancer cell lines. From the extensive studies on the anti-inflammatory effect of black soybean, different polyphenols, including anthocyanin, flavonoids, and other phenolic compounds, were suggested to be responsible for such a significant effect. The mechanism of action of polyphenols to inhibit the inflammatory process is described by Zhang et al. (2019). There could be positive interaction among polyphenols available (Figure 4.5). Based on the various published research, black soybean in raw, fermented, and by-product forms

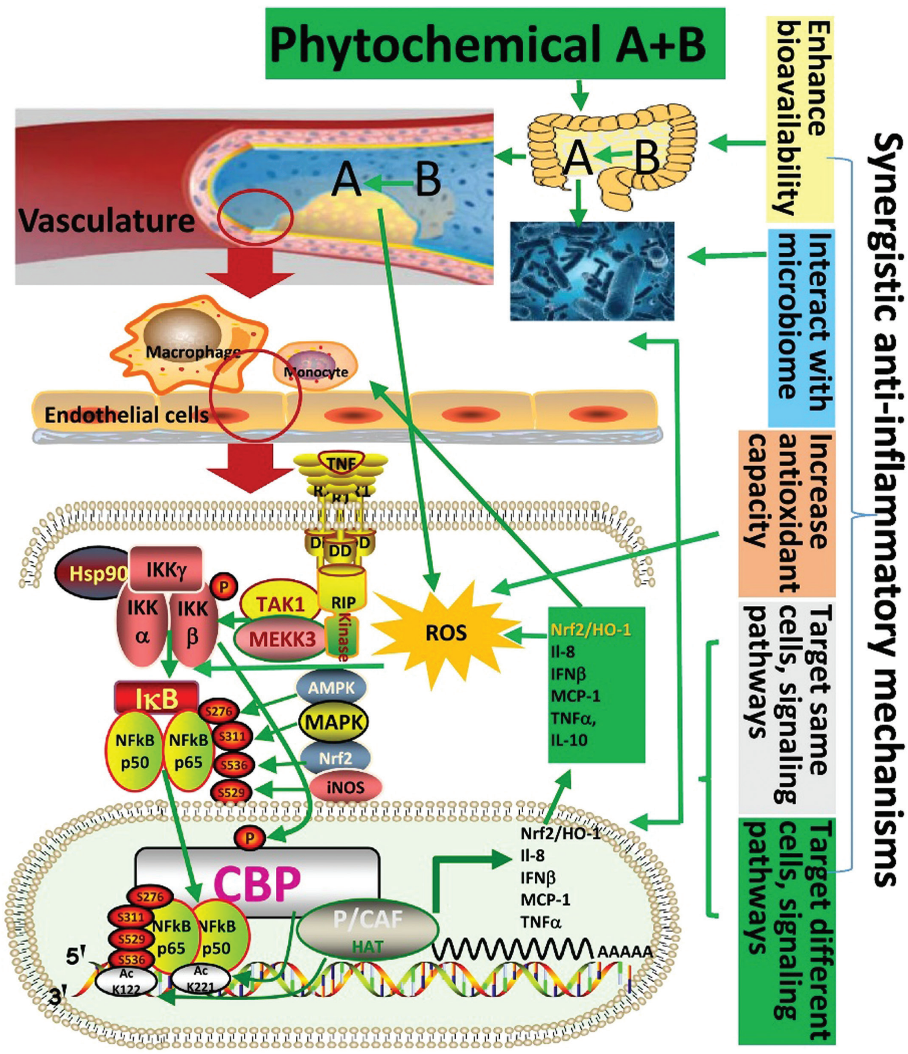

FIGURE 4.5 Mechanisms of the synergistic anti-inflammatory effect of combined phytochemicals. Source: Zhang et al. (2019).

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

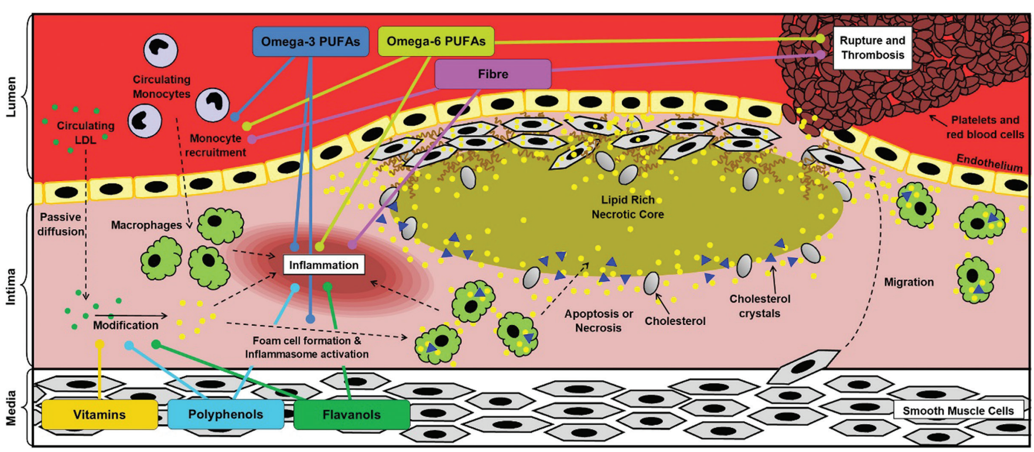

FIGURE 4.6 Role of anthocyanin in inhibiting atherosclerotic formation. Source: Moss et al. (2018).

reported to have potent antioxidant activity, preventing the oxidation of LDL. Published work by Chen et al. (2011) revealed that the fermentation process could increase the LDL oxidation inhibition by black soybean. Black soybean was fermented with *Aspergillus awamori*, and this process is usually used to make miso. The result shows that total phenolic content and amino nitrogen were significantly increased by fermentation due to the hydrolysis of the black soybean matrix and the release of bioactive compounds. This process is believed to be responsible for fermented black soybean's ability to inhibit LDL oxidation. It can be suggested that besides bioactive compounds such as anthocyanin, phenolic acid, isoflavone, and, amino nitrogen and other small peptides contribute to the beneficial health effects of black soybean and other legumes. Meanwhile, Kim et al. (2013) published their work on the antiplatelet aggregation of the black soybean. Platelet aggregation is one of the processes in atherosclerosis formation. Therefore, antiplatelet aggregation is an important property to decrease the risk of cardiovascular diseases. The inhibition of platelet activation is contributed by the anthocyanin, peptides, isoflavone, and adenosine. An *in vivo* study by Chao et al. (2013) was published on the effect of black soybean as prevention of the atherogenic process in the hypercholesterolemic rabbit. Black soybean could prolong the lag time of LDL oxidation, which can inhibit LDL oxidation. Moreover, the atheroma region in the aortic arch of the rabbit was significantly decreased by consuming a black soybean diet. The beneficial effects of the black soybean diet are several powerful bioactive compounds such as anthocyanin, phenolic, isoflavone aglycone, and small peptides available in black soybeans. Meanwhile, research on the prevention of atherosclerosis by black soybean was conducted by Lee et al. (2017). In this research, the monocyte-endothelial cell adhesion method, associated with atherosclerosis progression, was used. The result explained that black soybean could lower the monocyte-endothelial cell adhesion because of the isoflavone and proanthocyanidin content. Although the report is only *in vitro* study, several other works has been published on the correlation between consuming isoflavone and proanthocyanidin-rich foods with the decreased rate of coronary disease. Further study by Lee et al. (2018) revealed that black soybean could also suppress the TNF-α stimulated the expression of vascular cell adhesion molecule-1 and monocyte chemotactic protein-1 and phosphorylation of IκB kinase and IκBα involved in the initiation of atherosclerosis in HUVECs. This ability is due to the contribution of a daidzein metabolite, 7,8,4'-trihydroxyisoflavone (7,8,4'-THI), bioavailable in the blood of rats administered with soybean embryo extract and isoflavone.

4.4.4 Antidiabetic Activity

Diabetes mellitus (DM), primarily type 2, is a complex metabolic disease that is caused by insulin resistance to the increase of blood glucose level, known as hyperglycemia. This condition will lead to the development of various diseases such as blood vessel–related diseases (heart disease, stroke) and tissue dysfunction (liver, kidney, and pancreas). Nowadays, the number of cases of DM type 2 has increased rapidly and become infamously known as the mother of diseases. The main contributor to DM type 2 is an unbalanced diet and unhealthy lifestyles such as smoking, exposure to pollution, and excessive alcohol consumption. Various drugs have been developed for the prevention and treatment of DM type 2 in regulating blood glucose by direct blood glucose homeostasis intervention or by increasing the insulin response sensitivity. However, this is not an easy task due to the complexity of factors contributing to DM type 2 and the side effects of consuming the drugs that need to be taken into account.

Recently, a number of works were published on the potency of plant food for the prevention and treatment of DM type 2. The bioactive compound of a plant is believed to contribute positively to the signaling response of insulin, for example, anthocyanin. Published work by Chen et al. (2007) reported the ability of fermented black soybean *douchi* to inhibit the activity of the α-glucosidase enzyme. This enzyme is responsible for catalyzing the hydrolysis of carbohydrates to simple sugar, increasing blood glucose levels. Fermented black soybean has anti-α-glucosidase activity through its bioactive compounds, such as the aglycone form of isoflavone, and its anthocyanin compound by 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 behind the onset of obesity. It was reported that bioactive compounds such as anthocyanin and also peptides had anti-obesity effects. Previously published research has also investigated the ability of black soybean, a rich source of anthocyanin and peptides, to prevent the incidence of obesity *in vitro* and *in vivo*.

The anti-obesity and hypolipidemic effect of anthocyanin from black soybean seed coat was reported by Kwon et al. (2007) using high-fat diet-fed rats. The result shows that the intake of black soybean seed coat and black soybean anthocyanin extract lowered the body weight gain, suppressed liver weight gain, and decreased the weight of epididymal and perirenal fat pads. Consuming the black soybean extract could improve the rats' lipid profile, which includes lowering the triglyceride and cholesterol level and increasing the high-density lipoprotein content. The anti-obesity and hypolipidemic effect of black soybean could be contributed by the ability of bioactive compounds to interfere with the gene expression responsible for lipid metabolism. Meanwhile, the fecal excretion rate was also increased. Furthermore, anthocyanin could also take part in starch digestion by inhibiting α-glucosidase enzyme activity, thus reducing glucose metabolism. Anthocyanin can probably affect the triglyceride synthesis in the metabolism and downregulate the mRNA expression of the lipolytic enzyme for lipid hydrolysis.

Besides its anthocyanin content, black soybean is rich in bioactive peptides, suggesting adipogenesis inhibitory activity (Kim et al., 2007). The presence of bioactive peptides identified as tripeptide (isoleucine, glutamine, asparagine) could suppress the differentiation of the 3T3-L1 preadipocyte cells. Thus, it can be postulated that the peptides in black soybean affect the gene expression in adipose tissue, which in result regulates adipogenesis effectively.

Meanwhile, a double-blind, randomized, controlled study in overweight and obese human subjects was performed by Kwak et al. (2012) to investigate the weight reduction effect of black soybean peptides supplementation. After completing the study period, there was a significant reduction in weight, body mass index, and body fat mass in the test group. Moreover, a lower fasting blood glucose level was observed. Furthermore, the supplementation of black soybean peptides lowered the leptin level in the subjects. Leptin is a critical adipose-derived hormone that plays a crucial role in energy intake and energy expenditure and regulates appetite and metabolism, which is usually found at a high level in obese people. Black soybean peptides are suggested to affect the leptin pathway, which then downregulates the energy and lipid metabolism along with the decrease of appetite. Thus, it decreased the body weight, body fat mass, and body mass index of the subjects.

A study by Kim et al. (2012) shows that anthocyanins could reduce adipose tissue mass by acting directly on adipocytes using 3T3-L1 preadipocyte cell line exposed to anthocyanin from black soybean. Moreover, anthocyanin could inhibit the proliferation of pre-confluent preadipocytes and mature post-confluent adipocytes and reduce the number of viable cells. Furthermore, the accumulation of lipids was decreased, and black soybean anthocyanin was able to downregulate the peroxisome proliferator-activated receptor γ, a main transcription factor for the adipogenic gene.

Meanwhile, research by Jung et al. (2013) revealed that black soybean intake could reduce hepatic cholesterol accumulation in high-fat-diet-induced non-alcoholic fatty liver disease rats. Non-alcoholic fatty liver disease is a condition of excess fat in the liver. The published research shows that the intake of black soybean powder could reduce the liver's cholesterol and triglyceride levels. The mechanism of the ability of black soybean to reduce the cholesterol and triglyceride levels, thus potentially reducing the risk of diabetes, liver disease, as well as metabolic disorders, is presented in Figure 4.7 (Jung et al., 2013). The expression of SREBP2 as an indicator of cholesterol metabolism was suppressed by black soybean supplementation, and it can be pointed out that black soybean could decrease the HMG CoA reductase expression. Moreover, black soybean supplementation could increase the work of superoxide dismutase, catalase, and glutathione peroxidase antioxidant enzymes, and thus could balance the oxidation process in the body, resulting in the lower production of ROS and in the long term reducing the risk of atherosclerosis.

Research on the effect of fermentation of black soybean on the anti-obesity capacity was published by Lee et al. (2015). Using *Monascus pilosus* as a culture starter of fermented black soybean,

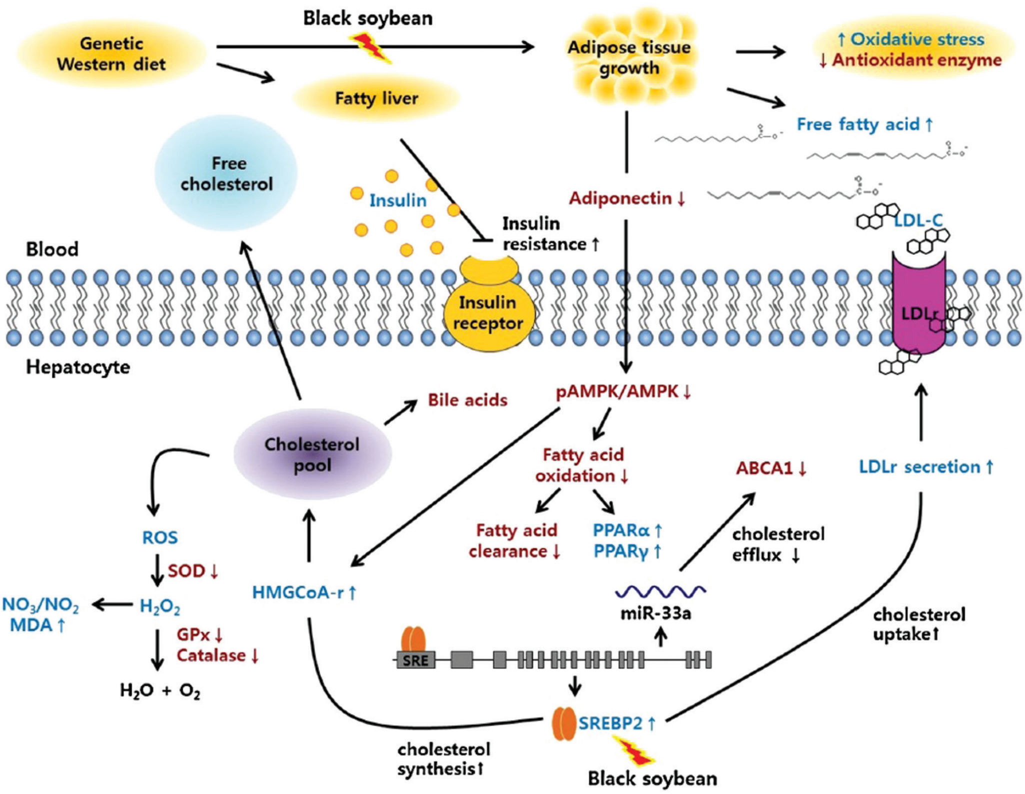

FIGURE 4.7 The mechanism of black soybean against non-alcoholic fatty liver disease. Source: Jung et al. (2013).

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 group. Moreover, the short-chain fatty acid level, especially propionate and butyrate in the feces, was improved by the black soybean diet. Propionate and butyrate are known to be antiobesogenic and able to reduce visceral and liver fat. In agreement with several previously published works, the anti-obesity capacity of black soybean is contributed by their peptides and anthocyanin content. Additionally, this research suggested that the fiber content of black soybean is also giving beneficial effects by altering the gut microbiome profile, which leads to the improvement of lipid metabolism and inhibits lipid accumulation.

4.5 FUTURE POTENCY

Research on the health benefit of black soybean has been widely established. Black soybean is reported to have antioxidant, anti-inflammatory, anticancer, anti-atherosclerosis and coronary heart disease, antidiabetic, and anti-obesity capacity due to certain compounds such as anthocyanin, isoflavone, peptides, fiber, and other polyphenol compounds. Due to various health benefits, the extract of black soybean can be produced and used as remedies for various diseases. Meanwhile, the processing of black soybean to make various products is proven to only slightly reduce some bioactive compounds, and on the other hand, it increases other bioactive compounds and thus could retain the antioxidant properties. Therefore, black soybean is a potential commodity to be developed as a functional food. The already established and well-known black soybean health properties can be essential in producing black soybean food products that can be widely accepted. Thus, it could contribute to the health improvement of society by providing black soybean–based healthy food products.

4.6 SUMMARY

Black soybean is a rich source of anthocyanin, isoflavone, phenolic compounds, bioactive peptides, and fiber. Such health-promoting compounds are responsible for antioxidant activity, as well as anti-inflammatory, anticancer, anti-atherosclerosis and coronary heart diseases, antidiabetes, and anti-obesity capacity. Black soybean, both as a whole seed and the seed coat only, was proven to have health benefit properties as measured by *in vitro* chemical and cell culture assays, *in vivo* animal models, and also clinical trials with humans. The method of processing as an essential factor in consuming black soybean such as soaking, grinding, boiling, and roasting did not significantly affect the antioxidant activities. Moreover, the fermentation of black soybean could increase the bioavailability of the bioactive compounds as well as their beneficial properties. Based on its characteristics, black soybean has the potential to be developed as a functional food.

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