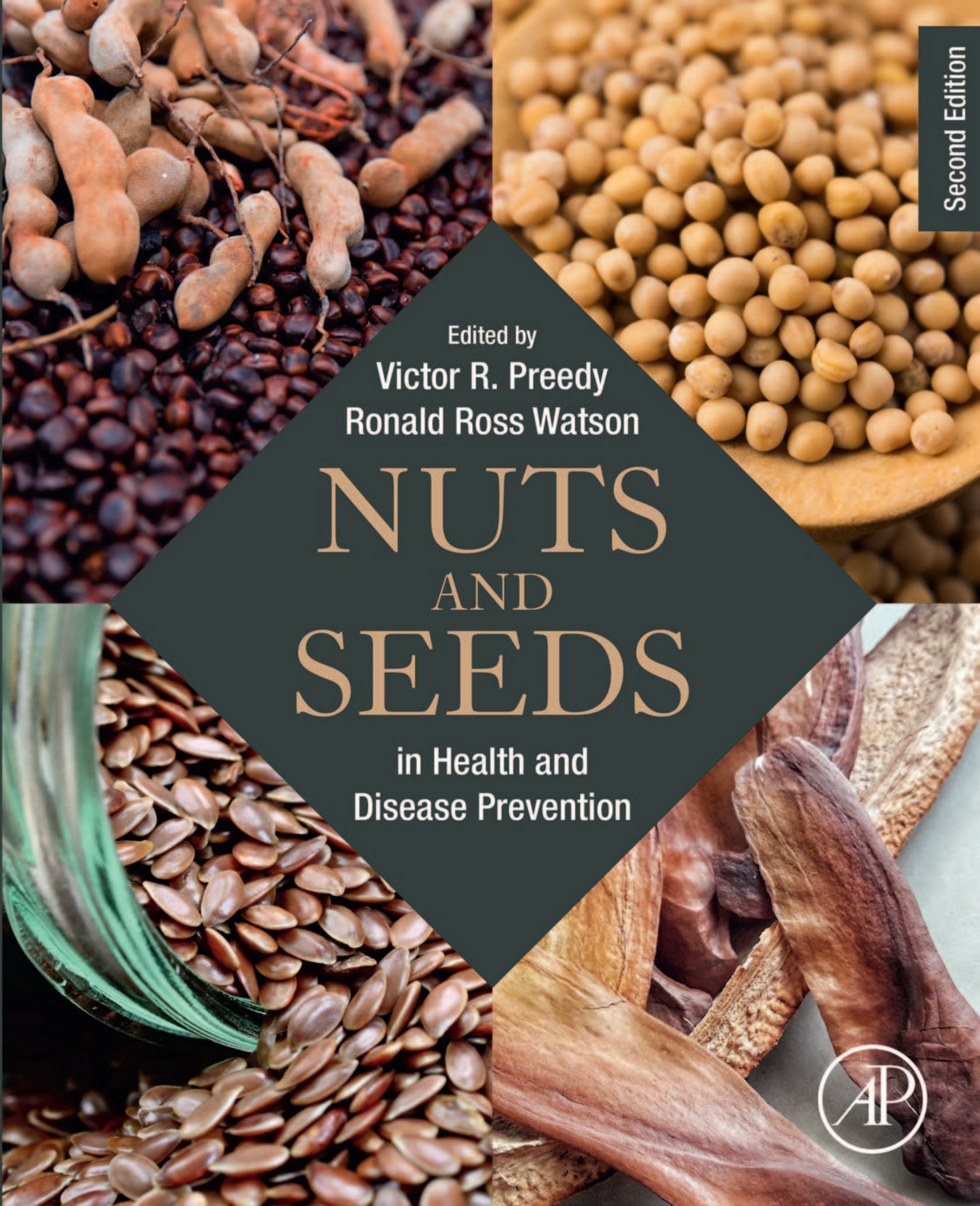

Second Edition

Edited by
Victor R. Preedy
Ronald Ross Watson

NUTS AND SEEDS

in Health and Disease Prevention

NUTS AND SEEDS IN HEALTH AND DISEASE PREVENTION

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NUTS AND SEEDS IN HEALTH AND DISEASE PREVENTION

SECOND EDITION

Edited by

Victor R. Preedy
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Preface

The objective of this book is to bring together scientific material relating to the health benefits and, where appropriate, adverse effects of nuts and seeds. In general, nuts and seeds are important not only from a nutritional point of view but also in terms of their putative medicinal or pharmacological properties. This book aims to describe these properties in a comprehensive way. However, at the same time, it is recognized that harmful effects also arise. Some "nuts" and "seeds," for example, are poisonous when ingested in large quantities, but extracts have putative effects on tissues that may offer some therapeutic potential. Many of the nuts and seeds described in this book are components of traditional remedies without any present-day evidence to support their claims; their properties await rigorous elucidation and scientific investigation. Thus, the book embraces nuts and seeds in an unbiased way. The Editors also recognize that there is a wide interpretation of the terms nuts and seeds, and indeed some authorities have claimed that there are at least 12 seed types. The Editors have largely excluded cereals (grains) and other staple food crops, unless there was cause to include them, such as with buckwheat seeds. They have also selected some specific legumes, where there is some therapeutic potential in their extracts or interesting properties.

The book *Nuts and Seeds in Health and Disease Prevention* is divided into two parts. Part I, General Aspects and Overviews, contains holistic information, with sections on Overviews, Composition, Effects on Health, and Adverse Aspects. In Part II, Effects of Specific Nuts and Seeds, coverage is more specific. Each chapter in Part II contains sections entitled Botanical description, Historical cultivation and usage, Present-day cultivation and usage, Applications to health promotion and disease prevention (the main article), and, finally, Adverse effects and reactions. The Editors were faced with a difficult choice in organizing the chapters in Part II, and this was done using the simplest method available. Thus, in Part II, the nuts and seeds are listed alphabetically in terms of their common names, although each chapter contains full botanical terminology. We realize this is not perfect, for example, there are numerous types of cabbage seeds, and some nuts and seeds may have as many as 20 common names depending on the country where they are grown, but navigation and the retrieval of specific information is aided by a comprehensive index system.

This book is designed for health scientists, including nutritionists and dietitians, pharmacologists, public health scientists, those in agricultural departments and colleges, epidemiologists, health workers and practitioners, agriculturists, botanists, health care professionals of various disciplines, policymakers, and marketing and economic strategists. It is designed for teachers and lecturers, undergraduates, and graduates.

The Editors

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CHAPTER

12

Black Soybean Seed: Black Soybean Seed Antioxidant Capacity

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List of Abbreviations

CAM Cellular adhesion molecule
CHD Coronary heart disease
DNA Deoxyribonucleic acid
DPPH Diphenyl-β-picrylhydrazyl
FRAP Ferric reducing antioxidant power
IRF-1 Interferon regulatory transcription factor-1
LDL Low-density lipoprotein
LPS Lipopolysaccharide
mRNA Messenger RNA
PPARγ Peroxisome proliferator-activated receptor γ
ROS Reactive oxygen species
TBARS Thiobarbituric acid reactive substance
TNF Tumor necrosis factor
UVB Ultraviolet B
VCAM Vascular cell adhesion molecule

Introduction

Soybeans have been consumed in Asian countries since ancient times, especially in China, Japan, Korea, and Indonesia. Foods based on soybeans, such as tofu, natto, and tempeh, are an integral part of the Asian diet, contributing a high amount of protein intake along with meat-based foods. For centuries, black soybeans have been known and used as traditional

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remedies to treat common colds and fevers along with their symptoms such as headaches. Soybeans are also used for people who have irritable bowel syndrome and experience an uncomfortable sensation in the chest.[1] Investigations into the biological activities of black soybeans are rapidly increasing because of reports revealing that black soybeans have a high content of anthocyanin in their seed coat.[2] Anthocyanin is a secondary metabolite of plants that is responsible for the formation of their black, purple, and red color. Studies on anthocyanin in black soybean seed coats also show that black soybean seed coat extract has antioxidant activities postulated to contribute to the prevention of degenerative diseases such as cancer, coronary heart disease, and diabetes because of their ability to inhibit the rate of oxidation in human metabolism.[3] Studies on elucidating the mechanism of anthocyanin in preventing and treating such diseases have become the focus of scientists. This chapter provides information on black soybeans as a plant and a source of food products and describes the ability of the extract of anthocyanin of soybeans and its food products to prevent diseases and promote human health.

History, cultivation, and use

According to ancient scripture, black soybeans are believed to have been planted and cultivated in Asia. The period of the Shang Dynasty (1700−1100 BC) was postulated to be the earliest time of black soybean cultivation, especially in northern China.[4] Together with rice, wheat, millet, and adzuki beans, black soybeans were named as one of the five sacred grains mainly owing to their importance in the daily life of the Chinese, although black soybeans were rarely consumed as a staple food but were commonly used as a medicinal food and remedies. It is believed that the inclusion of black soybeans as a sacred grain was for mythological and supernatural reasons as a grains from God that has the ability to cure numerous diseases. The increased amount of international trading in the 16th century led to the spread of black soybean cultivation in other Asia regions such as Japan, Korea, and Indonesia. Beside its use as a food, black soybeans have become an essential part of many traditional ceremonies. In Indonesia, black soybeans are used in traditional ceremonies such as weddings and funerals.[5]

The soybean (*Glycine max* L. Merril) is a species of plant belonging to the Leguminoseae group also known as soja max and glycine soja. There are numerous varieties of soybean, based on the color of their seed coat. The most common are yellow, green, and black seed coat soybeans. In addition to the black seed coat, black soybeans have a yellow seed interior (cotyledons) and a near-spherical shape. To obtain anthocyanin, the seed coat should be removed from the cotyledons. This can be done by soaking, boiling, and then peeling or drying followed by coarse milling of the black soybean. Compared with other beans, the hilum of soybeans is longer and thinner. Soybean species are considered a short season crop that usually needs 3−5 months of growth from germination until harvest. As a rainfed bush crop, an average of 350−450 mm rainfall is needed for the black soybean plant for optimal growth and yield.[6] Despite its demand for a sufficient amount of water, excess water can have a detrimental effects on the plant, such as impaired germination, leading to anaerobic respiration, and an increase in the incidence of pathogenic activity. Although

TABLE 12.1 Black Soybean-Based Food Products.

Product	Description	Country of Production
In si, tau si	Dried by-product of mashed black soybean sauce fermented with *Aspergillus oryzae*	China, the Philippines
Tempeh	Traditional food from black or yellow soybean fermented with *Rhizopus oligosporus*	Indonesia
Tofu	Protein gel-like product from soybeans	Asian countries
Soy sauce	Sauces fermented with *A. oryzae* and *Aspergillus sojae*, used as condiment	Asian countries
Natto	Traditional Japanese soybean product fermented with *Bacillus subtilis*	Japan
Chungkookjang	Steamed black soybeans fermented with *Bacillus* species	Korea

black soybeans are commonly found in tropical areas, based on their nature, they can be cultivated within a wide range of temperatures. The development of fermentation methods has led to an in black soybean-based food products.[7–10] An example of black soybean-based food products is presented in Table 12.1.

Although black soybeans are popular as a food with medicinal properties, their cultivation and use are limited compared with yellow soybean. There are few large-scale farming areas as well as food industries for black soybeans. A report from Indonesia revealed that there is cooperation between large-scale industry and farmers to plant black soybeans for use in soy sauce, which is a popular condiment among Indonesians.[11] This cooperation has become part of community empowerment to increase the population's livelihood and reduce the poverty of traditional farmers who have only a small farm, by providing the seeds and purchasing the yield at a fair price. There is still much future potency that can be optimized from black soybean seeds especially related to their health-promoting properties. For example, industries could extract anthocyanin from the black soybean seed coat. From the seeds, bioactive peptides could be isolated, and both could potentially be used as an alternative medicine.

Anthocyanin

Black soybeans are not as popular as yellow soybeans, but researchers have investigated the ability of black soybeans to provide health-promoting properties as conceived of in several Asian countries for centuries. One factor with a key role in the ability of black soybean to be used as a remedy is the anthocyanin content in the seed coat. Anthocyanin is part of phenolic substances, which are the secondary metabolites of plants. Numerous studies reported that polyphenol substances exhibit significant antioxidative activities; they are suggested to be responsible for preventing several degenerative diseases.[12] Anthocyanin is a class of polyphenols that is a water-soluble pigment responsible for the red, blue, and black colors of flowers and plants. Anthocyanin is widely known to have bioactive properties and substantial pharmaceutical activity. Fig. 12.1 shows the chemical structure of anthocyanin.

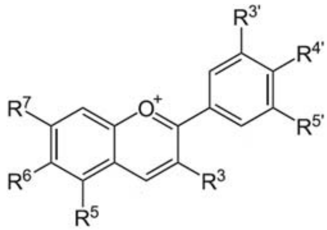

FIGURE 12.1 Chemical structure of anthocyanin.

A study of the anthocyanin content of black soybeans was first reported in 1921 by Nagai regarding its formation in plants.[13] Research on anthocyanin in plants including black soybeans has been growing rapidly. These findings includes the elucidation of individual anthocyanins and their biological activities.[14] Information about individual anthocyanins in black soybeans is presented in Table 12.2. As shown in Table 12.2, the major anthocyanin found in black soybean seed coat is cyanidin-3-glucoside. Other individual anthocyanins such as malvidin, delphinidin, and petunidin-3-glycoside, which are considered new anthocyanin, are also presented. There are variations in anthocyanin content in the black soybean seed coat. This could be because of the species, climatic conditions, and the geographical location.[15] However, compared with other plant foods such as rice, sorghum, berries, and grapes, the anthocyanin content of black soybean seed coats is relatively high. The anthocyanin contents of different varieties of black soybean are illustrated in Table 12.3.

Antioxidant activity of black soybeans and black soybean–based food products

Studies on the antioxidant activity of plant foods are rapidly increasing. In the human system, antioxidants are is believed to have the important function of stabilizing free radicals, whereas free radicals are substances commonly found in humans as a result of metabolism.[16]

TABLE 12.2 Individual Anthocyanin of Black Soybeans.

Black Soybean varieties/Sources	Individual Anthocyanins	References
Cheongja 3/Korea	Cyanidin-3-O-glucoside, petunidin-3-O-glucoside, delphinidin-3-O-glucoside	Jang et al. [61]
A3/Sichuan, China	Cyanidin 3 glucoside, petunidin 3 glucoside, delphinidin 3 glucoside, peonidin 3 glucoside	Wu et al. [62]
Black Tokyo/Serbia	Cyanidin 3 glucoside, pelargonidin 3 glucoside, delphinidin 3 glucoside	Kalušević et al. [63]
Cheongja 4 ho/Miryang, Korea	Cyanidin-3-O-glucoside, petunidin-3-O-glucoside, delphinidin-3-O-glucoside	Ryu and Koh [64]
852/Heilongjiang, China	Cyanidin 3 glucoside	Xie et al. [65]

TABLE 12.3 Anthocyanin Content of Different Varieties of Black Soybeans.

Black Soybean varieties/Sources	Total Anthocyanin content (mg/G)	References
Mallika	13.63	Astadi et al. [46]
Cikuray	14.68	Astadi et al. [46]
Cheongja 3/Korea	12.11	Jang et al. [61]
A3/Sichuan, China	3.95	Wu et al. [62]
QWT31/Yunnan, China	4.96	Wu et al. [62]
QWT5/Guizhou, China	3.01	Wu et al. [62]
JJ16/Chongqing. China	3.62	Wu et al. [62]
Black Tokyo/Serbia	1.92	Kalušević et al. [63]
Cheongja4ho/Miryang, Korea	1.68	Ryu and Koh [64]
852/Heilongjiang, China	6.96	Xie et al. [65]

The few free radicals in a normal condition usually can be neutralized by antioxidants synthesized by the human body, such as superoxide dismutase or glutathione peroxidase. However, pollution, an unbalanced diet, excessive exposure to sunlight, and smoke could multiply the number of free radicals, which need additional antioxidants from the diet to prevent excessive oxidation.[17] Free radicals attack lipids, protein, and DNA, leading to the development of various diseases such as atherosclerosis, cancer, and coronary heart disease.[18]

Rapid progress in research on antioxidant activity is supported by the development of antioxidant activity assays. In vitro procedures such as diphenyl-β-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity, thiobarbituric acid reactive substance (TBARS), linoleic bleaching system, superoxide anion, hydroxyl radical antioxidant capacity, total reactive antioxidant potential, potassium ferricyanide reducing power, and cupric reducing antioxidant capacity were created to mimic biological processes in the human body.[19–21] These procedures could assist researchers in the early stages of exploring the potency of samples of antioxidants. Among a number of procedures, DPPH is the most commonly used method for examining the antioxidant activity of black soybean seed coat extract. Results showed that black soybean seed coat extract possesses high antioxidant activity owing to anthocyanin and other phenolic content that could act as hydrogen donors that donating hydrogen to stabilize free radicals.[22–24] The 2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) method is also widely used to determine black soybean antioxidant activity. The results are in line with the DPPH method, which shows that black soybean extract is able to donate a hydrogen atom.[25,26] Another commonly method used is FRAP. Reports suggest that black soybean seed coat extract significantly reduces iron, which means that it has high antioxidant content.[27,28] The antioxidant activity of black soybeans can also be examined in a lipid system using linoleic acid or other lipids. It was proven that black soybean extract was able to reduce lipid oxidation, as measured by the inhibition of TBARS formation.[29,30] This simple in vitro technique assists in the research of the biological activity of black soybeans using cell cultures, animal experiments, and human subjects to explore the mechanisms of health-promoting and disease-preventing properties of black soybean seeds.

Beside the investigation into the antioxidant activity of raw black soybean seed extract, there are also reports on the ability of black soybean seed food products to act as antioxidants. A study on the antioxidant activity of tofu as the most popular soybean-based product was conducted using black bean tofu. Tofu from black bean showed relatively high antioxidant activity when it was examined using the thiocyanate method. Moreover, from the lipid peroxidation assay, black bean tofu inhibited the rate of lipid peroxidation, which affected the shelf life of tofu to be longer than that of yellow soybean tofu.[31]

Meanwhile, research on *chungkookjang*, a traditional Korean paste made of fermented black soybeans, revealed that fermented black soybeans could scavenge DPPH radicals better than unfermented ones. It was also postulated from an in vivo study that a diet of *chungkookjang* could increase superoxide dismutase and catalase activity as an antioxidant within the body, and thus could stabilize free radicals. Moreover, hepatic TBARS was also reduced. The higher antioxidant activity of fermented black soybeans results from the increase in polyphenol content caused by partial cleavage of the glycosides by fermentation.[32] Research on food products shows that the antioxidant activity does not significantly decrease with processing.[33–35] This finding can be seen in Fig. 12.2, which shows that different processing times and temperatures did not significantly decrease the antioxidant activity of black and yellow soybean crackers. This provides a promising future for the development of functional foods from black soybean seeds. Thus, consuming black soybean seed products should be promoted to increase the intake of healthy food.

Health-promoting and disease-preventing effects of black soybean seed

Black soybean seed has been used as a medicinal food and remedy for centuries. However, there is only limited scientific research to support such claims. Much research has been done to elucidate the mechanism of black soybean seed for promoting health and preventing disease. Factors related to the development of many diseases have been found, and the researchers elaborated on the results to investigate the health-enhancing properties of black soybean seed against inflammatory disease, atherosclerosis, diabetes, obesity, coronary heart disease, cancer, and so on.

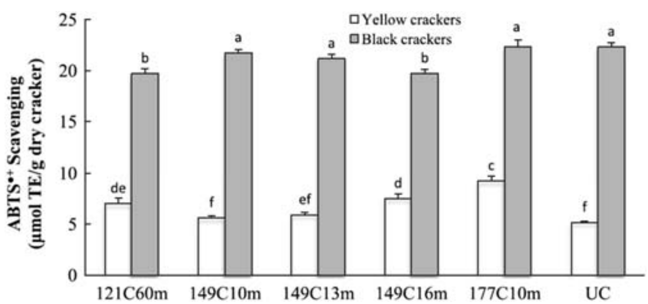

FIGURE 12.2 Effects of different types of processing on antioxidant activity of soybean crackers. *ABTS*, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; *TE*, Trolox Equivalent; *UC*, Unbaked Control.

Atherosclerosis and coronary heart disease

Atherosclerosis is a condition in which the blood vessels are partially or fully blocked by the accumulation of plaque; thus, blood cannot circulate throughout the body. The development of atherosclerosis is believed to be caused by the oxidation of low-density lipoprotein (LD)L. As a transport mechanism for cholesterol, LDL is potentially oxidized and may accumulate in the lining of blood vessels. This condition leads to the development of diseases such as coronary heart disease (CHD). The beneficial properties of anthocyanin in reducing CHD risk have been reported.[36–38] The ability of black soybeans to prevent the oxidation of LDL is proposed to be related to the delay of plaque formation. An early report also suggested that the daily intake of soybean protein is associated with a decrease in cardiovascular disease risk.[39] Other studies showed that the polyphenol content of soybean seed coats could prolong the lag time of LDL oxidation and that the ability of black soybean for this activity compared with yellow soybeans.[40] Research on the Malika and Cikuray varieties of black soybean in Indonesia revealed that the seed coat extract had the ability to prevent isolated human LDL oxidation. This beneficial properties could be due to the ability of anthocyanin in the extract to scavenge free radicals and thus inhibit the reaction between LDL and free radicals.[41] By using an in vitro monocyte-endothelial cell adhesion assay, researchers proved that black soybean extract had the ability to prevent atherosclerosis. This method is usually used to examine the potency of a sample to inhibit the development of atherosclerosis by mimicking the first phase of atherosclerosis.[42] In the early stage of atherosclerosis, cellular adhesion molecules (CAMs) on the vascular endothelial cells are activated by different factors, especially inflammatory conditions. After that, CAMs are bound by leukocytes such as monocytes.[43] Both the seed coat extract and the embryo extract of Yak Kong black soybean from Korea were able to attenuate the adhesion of THP-1 monocytes to LPS-induced human umbilical vein endothelial cells by up to 40% compared with the lipopolysaccharide (LPS)-stimulated control group. This research was done within a nontoxic dose of extract (5–20 mg/mL).[44]

Obesity and diabetes

Obesity has become an enormous problem in both developed and developing countries. Moreover, the rate of obesity in children is increasing rapidly. Obesity is believed to have an important role as a risk factor for diabetes.[45] A study on the effect of consuming black soybean anthocyanin in rats revealed that anthocyanin is suggested to have antiobesity properties as well as provide hypolipidemic effects. Supplementation of black soybean anthocyanin in high-fat diet rats could moderate weight gain in the liver and decrease epididymal and perirenal fat pads. Moreover, black soybean anthocyanin supplementation improved the lipid profile of rats by decreasing cholesterol and triglyceride serum levels and increasing the high-density lipoprotein cholesterol concentration.[46]

Research on the antiobesity properties of *Monascus pilosus* fermented black soybean was reported. Using adipocytes and high-fat diet-induced obese mice, the research revealed that lipid accumulation in 3T3-L1 adipocytes was inhibited by fermented black soybeans. Consuming fermented black soybeans could decrease body weight gain in the mice. Meanwhile, consuming fermented black soybeans significantly lowered the messenger RNA (mRNA) levels of adipogenesis-related genes such as peroxisome proliferator activated receptor, fatty acid synthase, and fatty acid binding protein.[47] Another report on the relation between consuming black soybeans and obesity prevention was reported in Korea. Consuming black soybeans decreased the intake of food, fat accumulation, and lipogenesis gene expression such as acetyl CoA carboxylase and CCAAT-enhancer-binding protein α. Fig. 12.3 shows the fat weight and adipocyte cell size in control and high-fat diet mice treated with black soybeans, and high-fat diet mice treated with orlistat, respectively. The results revealed that black soybean treatment decreased the fat weight and adipocyte cell size of high-fat diet rats. Black soybean intake was also closely related to the increase level of lipoprotein lipase, hormone-sensitive lipase, and adenosine monophosphate–activated protein kinase as a lipolysis protein. Although strong evidence of the effect of black soybean on lipogenesis gene expression and a decrease in fat accumulation was clearly observed, the mechanism of action of black soybean seed coat extract remains unclear.[48]

Adipocyte differentiation is closely related to the incidence of diabetes. A study on the antidiabetes effect of black soybean and its anthocyanin cyanidin 3 glucoside was done on the adipocyte differentiation of mice. Intake of black soybean and its anthocyanin decreased

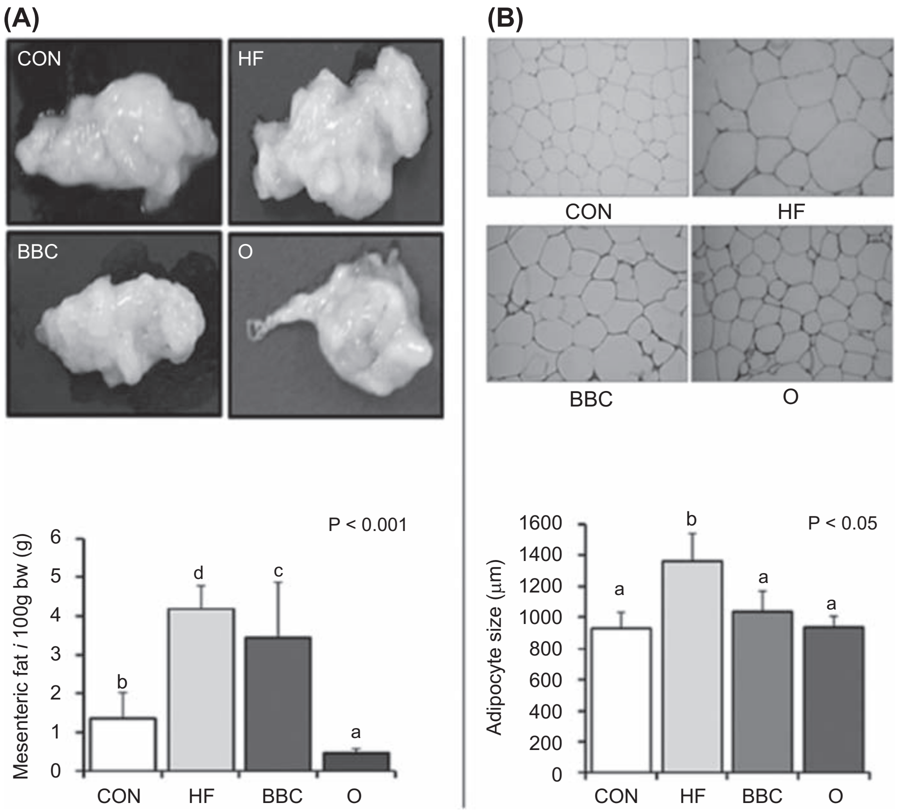

FIGURE 12.3 Mesenteric fat weight and adipocytes cell size of mice. *BBC*, high-fat diet with black soybean treatment; *CON*, control; *HF*, high-fat diet; *O*, high-fat diet with orlistat treatment.

body weight as well as the weight of white adipose tissue and the size of adipocytes in white adipose tissue. Moreover, smaller adipocytes were observed on 3T3-LI cells treated with black soybean extract. This result correlated with the increase in peroxisome proliferator-activated receptor γ and C/enhancer binding protein α gene expression. Moreover, it was suggested that adiponectin secretion increased, tumor necrosis factor (TNF) decreased, insulin signaling was activated, and the uptake of glucose increased.[49]

Inflammation and cancer

Inflammation has long been implicated in the development of cancer. Studies of inflammation related to the onset of various diseases have been widely performed, including the anti-inflammatory effect of black soybean anthocyanin.[50–52] A study showed that anthocyanin from black soybeans could inhibit the antigen-induced TNF-α stimulation of vascular cell adhesion molecule-1 (VCAM-1) by regulating DNA sequence GATAs and interferon regulatory transcription factor-1 (IRF-1). VCAM-1 is believed to be a target for highly metastatic human melanoma cells. These cells have a high-affinity conformation at their cell surface, facilitating adherence and subsequent migration. The IRF-1 and transcription factor genes bind to GATA in the VCAM-1 gene promoter region. These metastatic cells have a pathological role in inflammatory processes that eventually lead to cancer and atherosclerosis. Stimulation of cells with TNF-α increases VCAM-1 expression. Pretreatment of cells with anthocyanins inhibited VCAM-1 expression and reduced the nuclear levels of GATAs and IRF-1.[53]

Antiinflammatory and antifibrotic activities of Cheongja 3 black soybean was reported using an animal model for the treatment of Peyronie disease. The result showed that Peyronie disease plaque formation was reduced. Moreover, strong transforming growth factor-β1 immunoreactivity was observed with the increased expression in the collagenous connective tissue and fibroblast.[54]

Several epidemiological studies consistently suggested that the risk for cancer can be reduced by consuming soybean-based foods containing an antioxidant compound such as anthocyanin.[55,56] A case-control report from Korea revealed that the risk for breast cancer in Korean women was reduced by consuming black soybeans.[57] This is attributed to the anthocyanin content of black soybeans.

Research on the antiinflammatory and antiproliferative effects of black soybean anthocyanin was also done using HT-29 human colon adenocarcinoma cells, which showed that cyanidin and delphinidin significantly inhibited cell growth and suppressed cyclooxygenase-2 and inducible nitric oxide synthase mRNA production.[58]

Anthocyanin was also reported to have the ability to protect skin as an anticancer and anti-aging agent.[59] It was reported that black soybean anthocyanin defended keratinocytes from ultraviolet B (UVB)-induced cytotoxicity and apoptosis. The mechanism behind this ability of anthocyanin is inhibition of the caspase 3 pathway and Bax protein level reduction as a proapoptotic. A study on mouse skin revealed that anthocyanin can prevent apoptotic cell death. This probably results from the ability of anthocyanin to modulate UVB-mediated reactive oxygen species (ROS) production after UVB exposure. ROS has a key role in the apoptosis pathway. Therefore, by modulating ROS production, lipid peroxidation could be reduced, and the oxidative damage of DNA and cellular protein could be obviated.[60]

Summary Points

Black soybeans originated from China and are widely cultivated in Indonesia, Japan, and Korea.

Black soybeans are usually used for traditional medicine, herbs, and remedies.

Black soybeans can be used as ingredients to produce tempeh, natto, miso, sweet soy sauce, tofu, and soy milk.

Black soybeans contain a high amount of anthocyanin in their seed coat, especially cyanidin 3 glucoside, which possesses high antioxidant activity.

Processing does not significantly decrease the antioxidant activity of black soybeans.

Black soybeans have antiobesity, antidiabetes, anticancer, and antiatherosclerotic properties.

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