Chapter

Anthocyanins and Proanthocyanidins in Natural Pigmented Rice and Their Bioactivities

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Abstract

Natural pigmented rice is mainly black, red, and dark purple and contains a variety of flavones, tannins, phenolic, sterols, oryzanols, and essential oils. Anthocyanins and proanthocyanidins belonging to plant flavonoids are thought of as the major functional components found in black, red, and purple rice and contribute to the intense color of many fruits, vegetables, and pigmented cereals such as blueberries, grapes, red cabbages, and purple sweet potatoes. Recent data have indicated the potential for isolating and characterizing the nutrition and non-nutritive components in colored fruits, vegetables, and cereals for their potential chemopreventive and pharmaceutical agents. This chapter provides up-to-date coverage of pigmented rice in terms of the bioactive constituents, isolation, extraction and analytical methods, and related bioactivities. Special focus has been placed on the anti-inflammation, anticancer, and antiaging processes of the major components found in pigmented rice, especially with regard to germ and bran extracts.

Keywords: anthocyanins, proanthocyanidins, pigmented rice, nonpigmented rice, bioactivities of black rice, bioactivities of red rice

1. Introduction

Although white rice is consumed as a major staple food worldwide, quite a few countries in Southeast Asia (SEA) also consume pigmented cultivars such as red, black, purple, and brown rice. Rice cultivars that originated in Southeast Asia (SEA) have been classified in the species of *Oryza sativa* L., which differs from the *Oryza glaberrima* Steud. species that is cultivated in West Africa. In Thailand, the total area of cultivation has been recorded at 56.3 million Rai (22.3 million acres) with the majority being comprised of white rice cultivars (90%), while pigmented rice is only 0.1% or makes up approximately 62,000 Rai (24,506 acres) [1]. The largest cultivated area is located in the northeast of Thailand (63.10%) followed by the northern region of Thailand (21.93%), the central area (14.5%), and the south (0.47%). India and Indonesia have more cultivated area of pigmented rice than any other SEA countries, although they report a smaller proportion than that of the white rice cultivar. The total cultivated area in India has been recorded at

43.77 million acers (29.4% of the global rice area) with a production of 90 million tons [2]. The world production of rice is estimated at around 680 million tons, which is equivalent to that of wheat [3]. The color intensities of pigmented rice are obtained from the value of lightness, redness, and yellowness and seem to be correlated to the indicators of its bioactive compounds [4–6].

Recently, pigmented rice varieties have received increased amounts of attention from consumers for their high bioactive compounds that present potential nutraceutical benefits to health. It is also well known that these compounds are primarily located in the outer layer of the rice grain, which is regarded as a rice by-product. The by-products of rice processing are rice germ and bran, along with the rice hulls which protect the rice seeds during growth. These account for 20% of the rice crop. These by-products are frequently used as animal feed in developing countries. However, recently, significant amounts of data have revealed the beneficial nutritional impacts of these by-products on human health. The major bioactive compounds that are found in red, black, purple, and brown rice include gallic, protocatechuic, hydroxybenzoic, and vanillic acid, cyanidin 3-O-glucoside, peonidin-3-O-glucoside, proanthocyanidin, flavanol, catechin and epicatechin, carotenoids, and γ-oryzanol content. Several research findings have reported on the biological modulating effects of pigmented rice seeds and bran phytochemicals, including anti-inflammatory activities [7, 8], anticancer activities that have suppressed tumor growth in mice and several human cancer cell lines [9–13], the anti-metastasis properties of cancer cell invasion [14–16], antiaging effects with the reduction of oxidative stress in both in vitro and in vivo models [17, 18], the modulation of serum lipid profiles and the enhancement of mRNA expression levels of fatty acid metabolism-related genes [19], a reduction of platelet hyperactivity and hypertriglyceridemia in dyslipidemic rats [20], and skin antiaging treatments [21–24].

In this current review, we have focused on the health benefits of pigmented rice and the relevant bioactive compounds. We have tried to present the information in this chapter in a way that is easy to understand, even for readers who are not experts in this field of research. The bioactive compounds found in pigmented rice display significant immersion potential with regard to a range of beneficial health effects and also provide significant informative data that could lead to the expansion in the growing of pigmented cultivated areas in Thailand and other Southeast Asian countries. There is also the prospect of additional practical implications, not only for agriculture expansion but in the food industry as well. Several pigmented rice varieties have been used to create new nutraceuticals, and these seem to hold a promise in terms of potential cosmeceutical utilization in the new global business era.

2. Pigmented rice and bioactive compounds

The rice processing industry is well-developed and produces a number of products from rice kernels or grains (70%) along with a large quantity of rice by-products. These by-products include rice bran (8–9%), rice germ (1–2%), and rice husks (20%). Figures on rice paddy composition are presented in **Figure 1**. These by-products are frequently used as animal feed in developing countries [25], but the demand for these by-products in terms of their human nutritional impacts has increased due to their potential health benefits. Rice kernels are primarily a good source for the energy intake of carbohydrates and proteins in humans. Rice bran makes up the outer layer of the rice kernel and is mainly comprised of a pericarp,

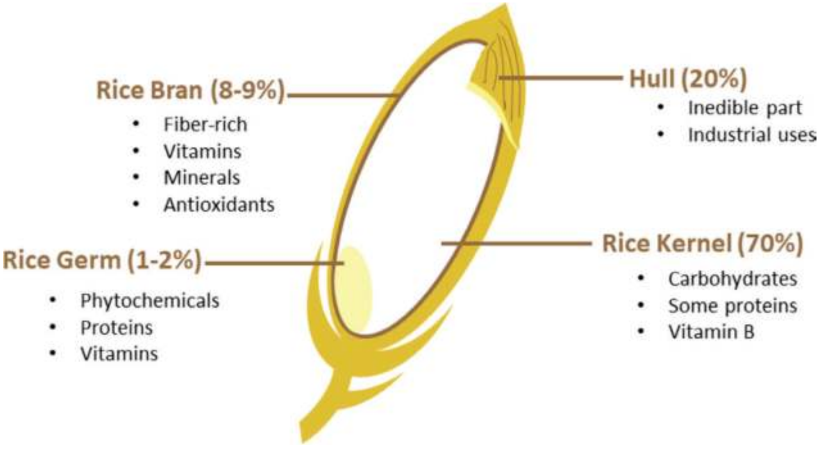

Figure 1.
Rice paddy composition.

aleurone, sub-aleurone layer, and germ. Rice bran and germ contain appreciable quantities of fiber, vitamins, minerals, unsaturated fatty acids, tocopherols, γ-oryzanol, and tocotrienols, which offer potent antioxidant content along with a range of other potential health benefits [26, 27].

2.1 Extraction methods for bioactive compounds in pigmented rice

Several common extraction techniques that are used in the process of rice extraction include the method of solvent extraction, which is a conventional technique used to extract bioactive compounds from pigmented rice, supercritical fluid extraction, and subcritical water extraction. With regard to the conventional technique, a number of organic solvents are commonly used such as acetone, methanol, ethanol, butanol, and water in certain proportions as the extraction solvent [28–30]. In our study, 50% ethanol was used as an extraction solvent at a proportion of 1:5 grain or bran liquid, and extraction was carried out at room temperature for 3–12 h. The extracts were then concentrated with a rotary evaporator until all ethanol residues were removed and then further partitioned against saturated butanol to obtain the medium polar bioactive compounds of the black rice extract [31] or red rice extract [7, 15, 16]. The bioactive compounds present in these fractions shall be described in a later section. In another study, 60% ethanol containing 0.1% HCL was used as an extraction solvent with a 1:10 feed to liquid proportion, and extraction was carried out for 3–12 h. The extracts were then concentrated and further partitioned against petroleum ether [8]. In another study, the rice bran was extracted with 70% ethanol for 30 min repeated three times and was then further partitioned with ethyl acetate at pH 2–3 [32]. The same method was used to extract soluble phenolic compounds in white rice, brown rice, and germinated brown rice [33].

Supercritical fluid extraction has been widely used for the extraction of functional active compounds from medicinal plants including rice and cereals. This was in common with the use of supercritical carbon dioxide as an extraction solvent in other successful experiments. Kim et al. [34] used the method of supercritical fluid extraction of rice bran oil from pigmented rice, which provided higher yields of polyunsaturated fatty acids than the conventional use of organic solvent extraction. In yet another study, supercritical carbon dioxide extraction was used, and yields of 17.5% oil were achieved from powdered rice bran, and a yield of 37% of γ-oryzanols was also obtained, which was characterized as 85% of the extraction efficiency [35].

Another extraction technique is the subcritical water extraction method that has been developed for the extraction of bioactive compounds from pigmented rice through the use of hot water at temperatures between 100 and 374°C under high pressure to maintain a liquid status. This technique is considered to be very friendly to the environment because no organic solvents are used, and this can potentially alleviate some of the problems associated with the conventional methods [36, 37].

There were differences in the extraction procedure and the varieties of the rice cultivars that were used to detect the amounts of bioactive compounds in different portions of rice such as in the whole grains, kernels, endosperm, husks, rice, and bran. More than 1000 published studies have been reviewed to make up the cited data based on this information. Some data on rice composition have been selectively recorded elsewhere [27].

2.2 Various bioactive compounds present in black rice

Phytochemical profiles of black rice are characterized by the presence of anthocyanins, which are a group of reddish to purple flavonoids that exist in black rice and other pigmented cereal grains. The main anthocyanins in black rice were found to be present in quantities more than 95% and were cyanidin 3-O-glucoside (2.8 mg/g) and peonidin-3-O-glucoside (0.5 mg/g) followed by flavones and flavonols (0.5 mg/g) and flavan-3-ols (0.3 mg/g) [38]. The concentrations of total anthocyanins in black rice cultivars significantly varied from one report to another, while much higher concentrations of anthocyanins were detected in Chinese black-purple rice that contained cyanidin 3-O-glucoside (6.3 mg/g) and peonidin 3-O-glucoside (3.6 mg/g) [39]. The variations of the anthocyanin content in the reports on black rice might be due to the use of different cultivars and the variety of differing growing conditions. The anthocyanidins or aglycons, the basic structure of anthocyanins, consist of an aromatic ring (A) that is bonded to a heterocyclic ring (C) that contains oxygen, which is bonded by a carbon–carbon bond to a third aromatic ring (B). When the anthocyanidins are bonded to a sugar moiety in the glycosidic linkage, they are known as anthocyanins. More than 500 different anthocyanins and 23 anthocyanidins have been reported. Anthocyanins exist as mono-, di-, or tri-O-linked glycosides and acyl glycosides of anthocyanidins in plants. The sugar moiety may be substituted by aliphatic, hydroxybenzoic, or hydroxycinnamic acids. The structural characteristics of anthocyanins make them highly reactive toward the reactive oxygen species (ROS) [27]. The basic structure of this is shown in **Figure 2**. Major flavone and flavonol glycosides present in black rice are taxifolin, quercetin, apigenin, and luteolin, which are comprised of monomeric and oligomeric constituents. The concentrations of the flavone and flavonol contents were

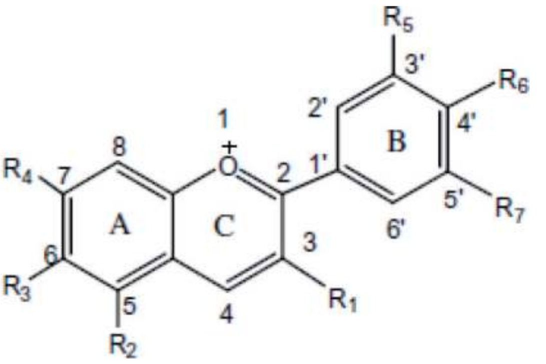

Figure 2.
General structure of anthocyanins.

significantly higher in black rice than in red, brown, or white rice. This was especially true with regard to taxifolin O-hexoside, quercetin 3-O-glucoside, and quercetin 3-O-rutinoside, which were detected only in black rice [38]. Abdel-Aal et al. [40] also reported that the mean anthocyanin content in black rice (3.276 mg/g) was about 35-fold higher than that of red rice (0.094 mg/g). Additionally, the contents of anthocyanin present in Northern Thai black rice cultivar obtained from Doi Saket, Chiang Mai, were 8.1 mg/g extract, which was considered very high when compared to the anthocyanin content found to be present in the Northern Thai red rice cultivar obtained from Dok Khamtai [31].

The total procyanidin content in black rice has been found to be present in high variations depending on the grain cultivar; however, it is noteworthy to mention that procyanidins are typically observed in red rice but not in black rice varieties [41–45]. Interestingly, some black cultivars have shown the presence of oligomeric procyanidins with a 2–10 degree of polymerization [38]. Furthermore, black and red rice were found to contain only one flavan-3-ol monomer, catechin. Additionally, a Canadian black rice variety also contained catechins at levels four times higher than epicatechin. Furthermore, the concentration of catechin was much higher in red rice (92 μg/g) than in black rice (20 μg/g) [46]. Other phytochemicals have been detected in black rice including all four derivatives of γ-oryzanol, such as 24-methylenecycloartenol, campesterol, cycloartenol, and β-sitosterol ferulates, along with lower levels of carotenoids. The main carotenoids detected in black rice were xanthophylls, lutein, and zeaxanthin, while lycopene and β-carotene could be detected but were found to be present as a minor component [38]. The value of the carotenoid content in black rice kernels is lower than the carotenoids found to be present in black rice bran. It was reported that values in a range of 33–41 μg/g of carotenoids were found in the bran extracts of four varieties of Thai black rice [47]. A range of phenolic compounds including vanillic acid, protocatechuic acid, chlorogenic acid, ferulic acid, and coumaric acid has been detected in black rice with the dominant phenolic acids being present in red and black rice bran [7, 31]. The contents of phenolic compounds, flavonoids, catechins, anthocyanins, and proanthocyanidins, are summarized in **Table 1** as examples of the phytochemicals that were detected in Doi Saket Thai black rice cultivar. The germ and bran extracts of the black and red rice varieties were found to have the greatest phytochemical content with decreasing amounts occurring in the rice hull and even less in the seeds or kernels. Additionally, the expected low levels of these phytochemicals were found in white rice as a consequence of the milling process.

2.3 Various bioactive compounds present in red rice

Red rice was characterized by a high quantity of oligomeric procyanidins (0.2 mg/g) with more than 60% of total phytochemicals found in the rice seeds. Proanthocyanidins are high molecular weight polymers or complex flavan-3-ol polymers that consist mainly of catechin, epicatechin, gallocatechin, and epigallocatechin units that can also be found in rice germ and bran, particularly in pigmented rice. The degree of polymerization varied, and the reddish colored test was associated with the presence of a class of polymeric compounds of the proanthocyanidins. These could be in the sum class of the oligomer and polymer contents of the total proanthocyanidins present in the red rice bran extract fraction. The degree of polymerization and galloylation can affect their bioactivity and proanthocyanidin profiles differently depending on the food sources [27, 48]. Proanthocyanidins can be classified into several classes depending on the degree of hydroxylation of the constitutive units and the linkages between them. Our research group has reported on the type of proanthocyanidins found in the red rice that was collected from Dok Khamtai

Compound	(mg/g extract)
Total phenolic content	117.6 ± 14.6
Vanillic acid	4.2 ± 0.4
Protocatechuic acid	2.3 ± 0.1
Gallic acid	ND
Coumaric acid	0.5 ± 0.2
Ferulic acid	1.4 ± 0.0
Chlorogenic acid	1.7 ± 0.3
Total flavonoid content	42.9 ± 2.1
Anthocyanin	8.1 ± 1.9
Catechin	ND
Proanthocyanidin	ND

Values are mean ± S.D., ND = not detectable.

Table 1.
Phytochemical content of black rice extract (polar fraction).

cultivar, Northern Thailand, as a type B proanthocyanidin. The monomeric units of proanthocyanidin in the acid hydrolysis of the red rice extract fraction were found to be catechins, epicatechins, gallocatechins, and epigallocatechins [16]. The results revealed that the proanthocyanidin types were procyanidin (catechin and/or epicatechin) and prodelphinidin (gallocatechin and/or epigallocatechin), while the degree of polymerization was recorded at approximately 4. Interestingly, the majority of proanthocyanidins in our red rice extract were of the oligomer with the same degree of polymerization that was found in grape seed extracts [49]. As has been mentioned previously, red rice has a high content of catechins and proanthocyanidins, but some of the black rice cultivars found in France and Canada have revealed the presence of catechins in their black rice cultivars (four times less than the red rice cultivars). It is worth mentioning that many other records have shown that procyanidins have been typically observed in red but not black rice varieties, including in the Northern Thai black rice cultivar obtained from Doi Saket, Chiang Mai [31]. The general structure of proanthocyanidins is shown in (**Figure 3**).

The other active compounds were γ-oryzanol and carotenoids at 27%, whereas flavones, flavonols, and anthocyanins were present in a much less quantity at less than 9% [38]. The main carotenoid detected in red rice bran was lutein, while xanthophylls and zeaxanthin were the carotenoids that were found to be present in lesser quantities. A range of phenolic acids including gallic, protocatechuic, hydroxybenzoic, vanillic, and ferulic acids in red, black, and brown rice have been detected as the dominant phenolic acids present in red and black rice bran [50, 51]. The contents of the phenolic compounds, flavonoids, catechins, anthocyanins, and proanthocyanidins, are summarized in **Table 2** as an example of the phytochemicals that were detected in Dok Khamtai Thai red jasmine rice cultivar. The contents of these bioactive compounds can be used to determine the antioxidant activities that may then provide health benefits.

2.4 Various bioactive compounds present in brown and white rice

The rice bran of whole grain brown rice (unpolished) has been acknowledged as a potential source of edible oil. Although rice bran oil is not very popular worldwide, its demand is increasing due to numerous reports on its health benefits.

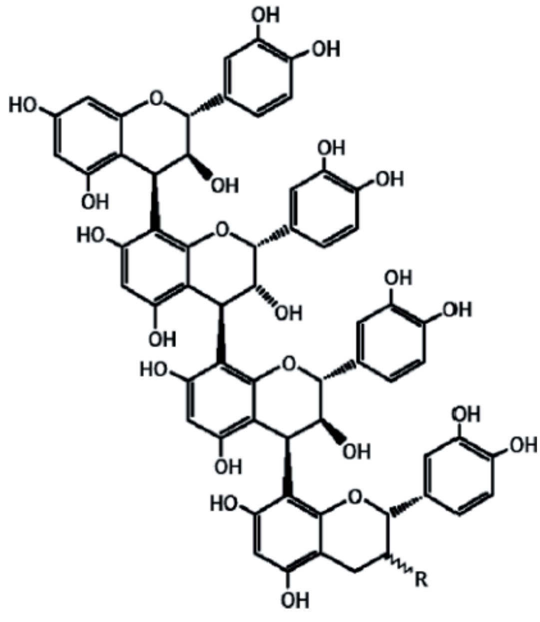

Figure 3.
General structure of proanthocyanidins [16].

Previously, rice bran obtained from brown rice has received a significant amount of attention from the nutraceutical industry as brown rice bran is recognized as the primary source of oil extraction. On this issue, agro-industrial by-products are gaining special attention from the food processing industry because rice bran oil presents a positive fatty acid profile along with the presence of other phytochemicals like ϒ-oryzanol, tocopherols, and tocotrienols. Basically, rice bran is rich in carbohydrates (34–62%), lipids (15–20%), proteins (11–15%), and dietary crude fiber (7–11%) [52]. The health benefits of rice bran include the strong antioxidant potential of rice bran oil. This is not only a consequence of the presence of significant quantities of linolenic acid (34%), oleic acid (38.4%), and other unsaturated

Compounds	**(mg/g extract)**
Total phenolic content	237.78 ± 17.26
Vanillic acid	1.53 ± 0.19
Protocatechuic acid	0.35 ± 0.03
Gallic acid	ND
Coumaric acid	0.2 ± 0.01
Ferulic acid	0.56 ± 0.04
Chlorogenic acid	ND
ϒ-Tocotrienol	ND
ϒ-Oryzanol	1.75 ± 0.23
Anthocyanin	ND
Catechin	6.65 ± 0.57
Proanthocyanidin	53.45 ± 3.23

Values are mean ± S.D., ND = not detectable.

Table 2.
Phytochemical content of red rice extract (polar fraction).

fatty acids but also occurs as a result of the high contents of γ-oryzanol, tocopherols, and tocotrienols that reveal strong oxidative stability along with a range of other health benefits [53, 54].

The protein content present in rice bran of brown rice is characterized as a good source of protein that is nutritionally superior and hypoallergenic in nature. Rice bran is a rich source of essential amino acids such as lysine, which seems to be present in minute quantities in other cereal grains [55, 56]. The proteins in rice bran are highly digestible and can be utilized as an effective food ingredient. Rice bran is rich in dietary fiber, and, consequently, the rice bran by-products of rice processing are now often present in food commodities and functional foods that have been marketed for the ability to add dietary fiber to the diets of consumers and to offer health benefits in terms of daily nutrition. Additionally, brown rice possesses high contents of a variety of nutrients, such as fiber, vitamins, and minerals that are lost during the process of refining and milling in the production of white rice within the rice agro-industry. Notably, brown rice possesses four times as much dietary fiber as white rice [57].

White rice is a major source of energy nourishment for the world's population, especially in Asian countries. However, the carbohydrate content in white rice accounts for 80% of its makeup, which is considered a higher amount than wheat. Wheat is a popular grain among European countries and contains a lessor proportion of carbohydrates (approximately 50–70%) [58]. For this reason, there are concerns that white rice possesses a high glycemic content and that it may not be a suitable source of carbohydrates for people who have weight problems. It is interesting to note that white rice does not contain anthocyanins and proanthocyanidins, which are the important phytochemicals that are found in black rice and red rice, respectively, particularly in portions of rice germ and bran extracts. While total flavonols and phenolic compounds are observed to be significantly high in pigmented rice, nonpigmented rice such as white rice possesses a minute quantity of flavone/flavanol content [50].

3. Pigmented rice and bioactivities that benefit health

Phytochemicals found in pigmented rice (brown, black, purple, and red rice) are not present in white rice because many valuable phytochemicals, fiber, vitamins, and nutrients are lost during the processes of refining and milling [57]. Since brown rice contains higher dietary fiber and nutrients, previous studies have revealed that when compared to a white rice diet, a brown rice diet was found to significantly reduce weight, body mass index (BMI), and the circumference of the waist and hips, as well as to lower diastole blood pressure and inflammatory biomarkers such as C-reactive proteins (CRP). Arabinoxylan and β-glucan, prebiotics that are found in brown rice, are beneficial for human gut microbiota such as *Bifidobacterium* and *Lactobacillus*. They are considered as contributing factors in producing an anti-obesity effect [57, 59]. Moreover, in terms of their antidiabetic effects, brown rice was used as an intervention for preventing type 2 diabetes. This is likely because one of their components, ϒ-oryzanol, plays an important role in controlling high-fat diet-induced ER stress in the hypothalamus, which helps in reducing the preference for fatty foods [60]. ϒ-Oryzanol in brown rice has also been found to prevent the apoptosis of pancreatic β cells and to reduce levels of blood cholesterol [61]. Dietary rice brans that give brown rice its brown color also reveal potent anticancer activities through their antioxidant activity, as well as offering antiproliferation, immune modulation, and mucosal protection [62, 63].

Natural pigmented rice, such as black and red rice, may even offer more health benefits than brown rice. Not only is natural pigmented rice higher in the beneficial antioxidant activities of black and red rice, but it also displays strong anti-inflammatory activities as well as anticancer and anti-metastasis activities. The antiaging properties of the major components found in pigmented rice may be anthocyanins and proanthocyanidins, which have been found to be especially rich in content in the germ and bran extracts of black and red rice, respectively. The details of which will be described in greater detail in the following section.

3.1 Antioxidant activities

The antioxidant activities of black and red rice and their crude extracts have been studied, and the results demonstrated that the addition of the pigmented rice could increase antioxidant capacity, both in vivo and in vitro [64–66]. In a study involving the supplementation of diets with black rice pigment fractions, the diets that attenuated atherosclerotic plaque formation in apolipoprotein E-deficient mice [66] and the anthocyanin-rich extract of the black rice might play an important role in the enhancement of atherosclerotic plaque stabilization [8]. In another study, a mixture of brown and black rice improved the lipid profiles and antioxidant status in rats [67]. Another animal study also demonstrated that black rice bran pigment effectively escalated hepatic antioxidant enzyme activities including superoxide dismutase and glutathione peroxidase in high-cholesterol-fed rats [68]. In addition to the in vivo studies, in a cell culture experiment, superoxide anions and reactive oxygen species were significantly suppressed after black rice extract exposure in HepG2 hepatocellular carcinoma [17]. When the antioxidant activities of pigmented rice were compared with those of nonpigmented rice in several studies [30, 41], the results demonstrated that the extracts from pigmented rice displayed higher antioxidant activity than did the nonpigmented rice. In another study, the radical scavenging activities of the extracts from white, black, and red rice were tested. The highest activity was observed in red rice (2.77 μmol of Trolox or vitamin E equivalents/ml), followed by black (0.92 μmol) and white (0.26 μmol) [41, 42]. Polymeric proanthocyanidins play an important role as radical-scavenging components in red rice. The relationships between the antioxidant activities and the components of pigmented rice were explored [41, 69, 70]. The antioxidant activities correlated well with the content of polyphenols and phytochemicals that contribute to the intense color of the pigmented rice. Interestingly, some studies have shown that the antioxidant activity of black rice may be reduced by up to 53% during cooking [71–73].

3.2 Anti-inflammatory properties

Inflammation is an important mechanism of immune pathogenesis, which is our body's response to tissue injury, infection, and stress. Importantly, the prolonged production of inflammatory mediators by macrophage can cause damage to the host and can contribute to the pathology of many diseases including inflamm-aging, arthritis, asthma, cancer, diabetes, and atherosclerosis. Macrophage plays a key role in response to an immediate defensive mechanism of our body against attacking foreign agents, especially with a microbial lipopolysaccharide (LPS) [74]. Macrophage is activated and produces many kinds of inflammatory mediators including nitric oxide (NO), prostaglandins, and many cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6), and tumor necrosis factor (TNF)-α [75]. Many researchers have studied in vitro and in vivo models to elucidate that natural products are able to ameliorate the inflammatory response in LPS-stimulated macrophage.

During the last decade, it has been shown that anthocyanins reduce the risks of cardiovascular diseases and cancers with inflammatory, antioxidant, and chemo-protective properties [15, 76, 77]. Some reports have demonstrated that lipophilic phytochemicals contained in pigmented rice germ and bran, such as γ-oryzanol and vitamin E derivatives, exert anti-inflammatory activities [78, 79]. On the other hand, pigmented rice contains high amounts of medium polar or hydrophilic compounds such as phenolics, bioflavonoids, anthocyanin, and proanthocyanidins that have been reported for their anti-inflammatory properties, in both in vitro and in vivo models [80–82].

Pigmented rice contains a variety of bioactive compounds with anti-inflammatory properties; however, there have been quite a few reports employing experimental designs that provide direct evidence to support using the extracts of pigmented rice. For the first time, our research group has demonstrated the molecular mechanisms underlying the anti-inflammatory effects. The anthocyanin-rich fraction of black rice extract significantly inhibited LPS that induced many pro-inflammatory mediators in RAW 264.7 macrophage white blood cells [31]. The pro-inflammatory mediators in this study were NO, TNF-α, and IL-6, and they effectively reduced the expression of two important inflammatory enzymes, the inducible NO synthase (iNOS) and the inducible cyclooxygenase-2 (COX-2). These results were regulated by an inhibition of the mitogen-activated protein kinase signaling pathway (MAPK pathway), leading to a decreased nuclear translocation of NF-κB and AP-1, two major transcription factors involved in the inflammation process. In testing the anti-inflammatory properties of anthocyanin and hydroxybenzoic acid, the major components were detected in the black rice extracts based on our extraction protocol, and similar results were obtained. A schematic diagram of the proposed mechanism of the anti-inflammatory properties of black rice anthocyanin is presented in **Figure 4**. In a study on cyanidin-3-glucoside and protocatechuic acid, no beneficial effects were found against inflammation induced by LPS [73]. Therefore, the anti-inflammatory properties of black rice might require the synergistic action of many phytochemicals, which are rich in anthocyanin and other phenolic compounds that play a role in this process. Interestingly, the same study has demonstrated that the cooking process did not alter the anti-inflammatory potential of black rice. In another study, other researchers reported that cyanidin-3-glucoside displays anti-inflammatory effects [8]. Our group also conducted a study on the anti-inflammatory effects of proanthocyanidin-rich red rice extract via the suppression of the MAPK, AP-1, and NF-κB pathways in RAW 264.7 macrophages that induced inflammation by LPS [7]. It was found that the red rice medium polar fraction that was enriched with polyphenols and proanthocyanidins exerted potent anti-inflammatory activities by inhibiting the production of TNF-α, IL-6, and NO in LPS-activated macrophage, whereas the red rice nonpolar fractions displayed no anti-inflammatory properties. All of the above results indicate that black rice that is rich in anthocyanins and red rice that is rich in proanthocyanidins exhibit therapeutic potential for the treatment of inflammatory diseases.

3.3 Anticancer properties

Cancer is one of the leading causes of morbidity and mortality worldwide. Notably, only 10% at the most of all cancers are due to genetic factors, while 90% are directly or indirectly correlated with an individual's lifestyle and dietary habits [83]. Many scientific reports have shown that a healthy lifestyle, including a diet rich in natural products, such as herbs, cereals, fruits, and vegetables, can help reduce the risk of cancer [84, 85]. Some of the phytochemicals found in these natural products

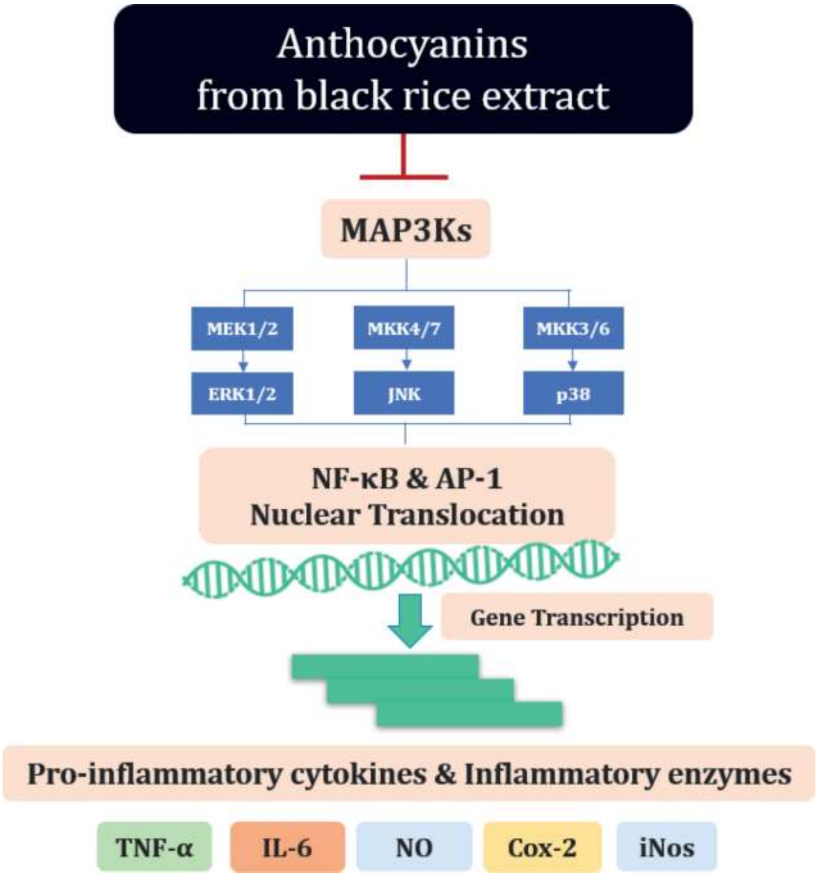

Figure 4.
Schematic diagram of anti-inflammatory properties of black rice anthocyanin.

are secondary metabolites, including phenolic compounds, bioflavonoids, terpenoids, and alkaloids. In this chapter we shall focus more on the presence of phenolic compounds and flavonoids, including anthocyanins and proanthocyanidins, as the major compounds found in pigmented rice, especially in rice germ and bran.

Active components of pigmented rice bran have demonstrated anticancer properties in in vitro cancer cell models, including those involving leukemia, colon, breast, liver, and stomach cancer cells. In a study on the anticancer potential of rice bran against the proliferation of leukemic cell lines, the antioxidant activities of the active compounds found in rice bran were noted for this beneficial effect [10]. Another investigation on the tumor suppression activities of rice bran from different pigmented and nonpigmented rice varieties reported that 70% ethanolic extract of the pigmented rice bran inhibited phorbol ester-induced tumor promotion in a better manner when compared to the nonpigmented rice bran variety [11]. In yet another study, the growth inhibitory effect of rice bran polyphenols, mainly γ-oryzanol and its derivatives, has been reported in human colorectal adenocarcinoma [86]. The anticancer activity of rice bran could be varied considerably in different rice cultivars or varieties in accordance with the different chemical profiles of the active compounds. In addition, the second study had analyzed seven varieties of rice bran for their growth inhibition potential against human colorectal cancer cells and reported on variations in the degree of growth inhibition depending upon the rice bran variety [9]. Some evidences have indicated that cyanidin-3-glucoside and peonidin-3-glucoside obtained from black rice anthocyanin can be combined with doxorubicin to inhibit cancer cell growth, while both anthocyanin compounds could inhibit cancer invasion into other tissues through the downregulation of the degradative enzymes MMP-2 and MMP-9 [14]. Interestingly, Chen et al. [87] compared the relationship of the bioactive compounds with the growth inhibitory effects of pigmented rice bran extracts. The results revealed that the light brown bran had no effect, the purple bran exhibited a minor effect on leukemia and cervical cancer cells, and the red bran exhibited strong inhibitory effects on leukemic, cervical, and stomach cancer cells. High concentrations of protocatechuic acid and anthocyanins in purple bran and proanthocyanidins in red rice bran have been singled out for their growth inhibitory effects against human cancer cells.

Many studies on anticancer properties have been reported in Thai rice cultivars. In an important study, Kum Phayao black rice cultivar was found to be highly cytotoxic to human HepG2 cells when compared with other Northern Thai purple rice cultivars [12]. In yet another study, the alcoholic extracts of black-purple rice grain cultivar Kum Doi Saket demonstrated an antimutagenic activity against aflatoxin B1 in Ames tests [88]. The therapeutic potential of black rice anthocyanin for treating inflammatory diseases that are associated with cancer has been proposed for its mechanism via the inhibition of the MAPK signaling pathway [31]. A very recent study conducted by our research group revealed that the proanthocyanidin-rich fraction isolated from the red rice germ and bran of the Kum Doi Saket cultivar grown in the northern part of Thailand significantly reduced the cell viability of HepG2 cells (IC_{50} value at 20 μg/ml) [13]. The proanthocyanidin-rich fraction could inhibit cell proliferation and induce cell apoptosis by increasing the apoptotic proteins, such as cleaved PARP-1, cleaved caspase 8, and cleaved caspase-3, and decreasing the anti-apoptotic protein survivin without p53 protein changes. A schematic diagram of this mechanism is presented in **Figure 5**. In addition, our previous studies have demonstrated that red rice grain extracts with high proanthocyanidin content displayed an anti-metastasis effect on invasive human breast carcinoma cells MDA-MB231 [16] and human fibrosarcoma HT1080 cell lines [15]. In addition, proanthocyanidins in other colored plants, such as grapes and blackberries, have demonstrated anticancer, anti-inflammatory, and antioxidant activities to a similar extent as the proanthocyanidins that are found in red rice germ and bran [7–9, 13].

3.4 Anti-inflamm-aging properties

Inflamm-aging, a state of chronic, low-level systemic inflammation, is a widespread feature of human aging and a major risk factor for disabilities and mortality in aging individuals [89, 90]. Inflamm-aging is characterized by an overall increase in plasma levels of pro-inflammatory cytokines, such as IL-6 and TNF-α, and

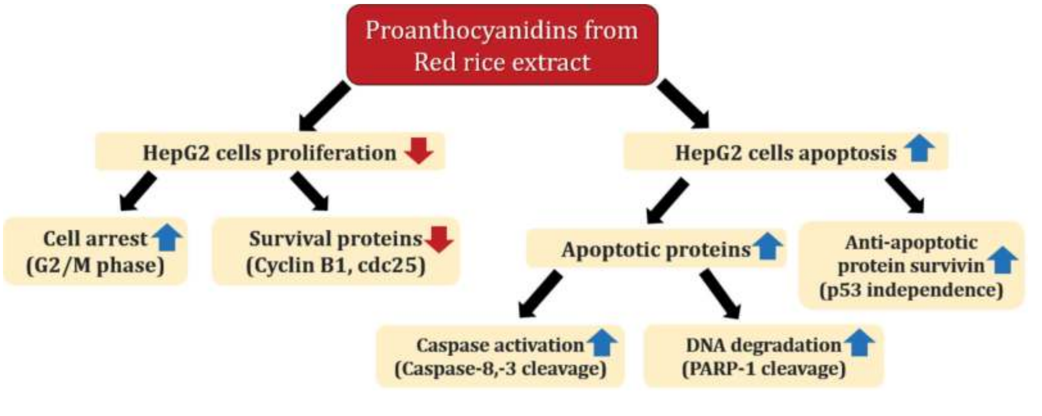

Figure 5.
Schematic diagram of anticancer properties of red rice proanthocyanidins.

subsequently can increase major inflammatory markers such as C-reactive protein (CRP) and serum amyloid A. This generalized pro-inflammatory status potentially triggers the onset of the most important age-related diseases such as cardiovascular diseases, atherosclerosis, metabolic syndrome, type 2 diabetes, obesity, neurodegeneration, sarcopenia, frailty, and cancer [91, 92].

Since the anti-inflammatory effects of phytochemical components in black rice and red rice (anthocyanins and proanthocyanidins) are able to target many inflammatory signaling pathways, such as the MAP kinase and AMP-activated protein kinase (AMPK) and mTOR pathway, the result can also decrease free radical production by their antioxidant activity, inhibiting NF-κB activation and reducing the expression of inflammatory mediators (NO, iNOS, and pro-inflammatory cytokines) [7, 31, 93, 94]. Therefore, this has made natural pigmented rice a promising candidate as an anti-inflamm-aging agent. Some relevant studies have found that a Mediterranean diet (a diet involving high consumption of vegetables, fruits, and whole grains such as pigmented rice, olive oil, and fish, but low in the intake of saturated fats and other animal fats) can modulate the multi-interconnected processes that are involved in inflammatory responses such as free radical production, NF-κB activation, and the expression of inflammatory mediators by balancing between pro- and anti-inflamm-aging activities as well as maintaining healthy gut microbiota homeostasis and epigenetic modulation of oncogenesis through specific microRNAs [95, 96].

Several studies have identified a number of actions of anthocyanins in a phytochemical diet in the context of neuroinflammation and neurodegeneration in aging individuals. It was also recently reported in an experimental model of multiple sclerosis that anthocyanins (100 mg/kg) could effectively suppress the secretion of pro-inflammatory mediators and protect cellular components against oxidative damages that were induced by demyelination [97]. Anthocyanins also protect neuronal cells from prooxidant and pro-inflammatory damage via the modulation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and the inhibition of NF-κB pathways [98]. Moreover, anthocyanins also exhibited a similar degree of anti-inflammatory effects, and these compounds suppressed the expression and secretion of pro-inflammatory mediators in macrophages by inhibiting the nuclear translocation of NF-κB [99].

Red rice extracts that contain high levels of proanthocyanidins were also found to have neuroprotective effects and anti-inflamm-aging effects that are similar to those of anthocyanins. Previous studies have found that in primary hippocampal neuronal cells that had been treated with proanthocyanidins (14 μg/ml) and exposed to LPS, the major neuroprotective effects of proanthocyanidins were involved with a reduction of NF-κB, p38, and JNK [100]. In brief, the consumption of foods rich in polyphenols has been associated with the prevention of chronic diseases. In particular, anthocyanins, proanthocyanidins that act through various mechanisms that modulate the inflammatory signaling pathways, result in a reduction of inflammation that is often seen in aging individuals. A schematic diagram of the proposed mechanism of anti-inflamm-aging properties of black rice and red rice is presented in **Figure 6**. From the aforementioned results, it has been determined that black rice and red rice with their anti-inflamm-aging properties have a therapeutic potential that would likely need to be further investigated in geriatrics and gerontology fields.

3.5 Skin anti-aging properties

Many studies have shown that bioactive compounds found in pigmented rice, such as proanthocyanidin, catechin, vanillic acid, and oryzanol, may be useful in the cosmetic and nutraceutical industries as skin antiaging agents. As mentioned

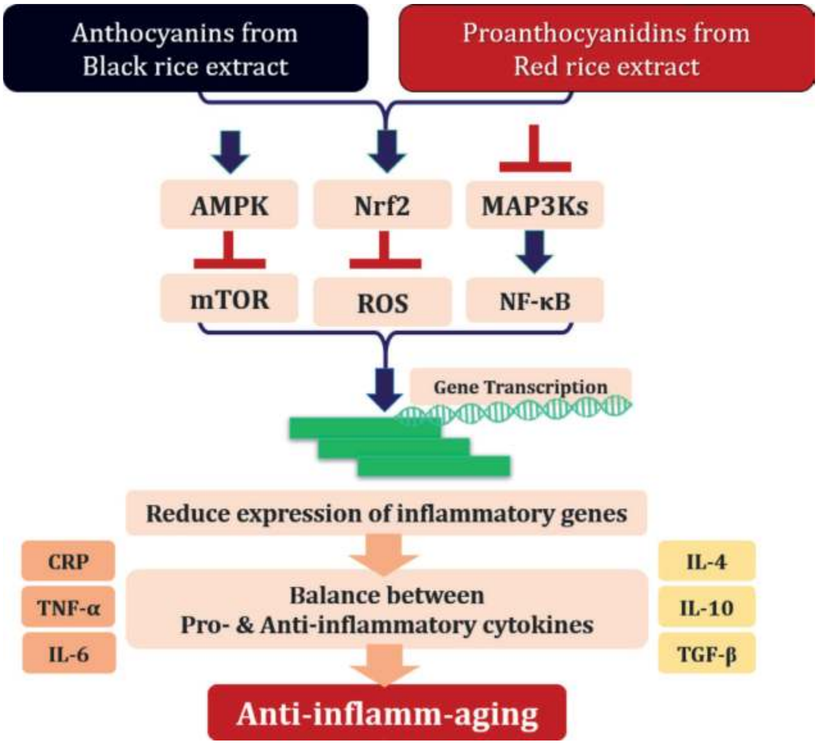

Figure 6.
Schematic diagram of anti-inflamm-aging properties of pigment rice.

in the previous section in this chapter, these bioactive compounds demonstrated antioxidant and anti-inflammatory properties. For the enhancement of the knowledge of skin antiaging properties, the bioactive compounds in the pigmented rice extract have been elucidated in a number of research laboratories. Skin aging is a process characterized by progressive physiological and structural changes in the skin. These changes could be considered as individually intrinsic and extrinsic factors, such as those associated with age, lifestyle, diet, and sunlight. Additionally, certain environmental factors can contribute to skin aging [101]. In the skin aging process, the level of degradative enzymes, such as elastase and collagenase, in skin fibroblasts are elevated, and this can lead to a loss of skin firmness and the appearance of wrinkles [102]. Mature skin in the elderly or those with sun-exposed skin can cause dark spot formations on the skin or result in the over-synthesis of melanin [103]. Hence, natural or herbal products that can exert skin benefits, including scavenging reactive oxygen species (ROS), the suppression of extracellular matrix degradation enzymes, and the inhibition of melanin synthesis, can be applied in skincare products for their beneficial skin anti-aging properties.

As pigmented rice has been reported to possess antioxidant properties, the extracts could be used for skin-anti-aging purposes. In a study by our research group, red rice extract showed anti-photoaging activity by protecting UV-induced collagen and hyaluronic acid degradation in human skin fibroblasts [21]. The red rice extract also inhibited collagenase and MMP-2 activity. In another study our group [22] has elucidated the skin antiaging properties of the main bioactive compounds in red rice extract including proanthocyanidin, catechin, hydroxybenzoic acid, vanillic acid, and oryzanol. The results showed that collagenase and MMP-2 activity were strongly inhibited by proanthocyanidin and catechin, whereas hydroxybenzoic acid, vanillic acid, and oryzanol had no effect. Both proanthocyanidin and catechin significantly induced the synthesis of collagen and hyaluronic acid, which is an important biological target for skin antiaging agents. Proanthocyanidins and γ-oryzanol could reduce the melanin content in B16-F10 melanoma cells. Some studies have proposed the use of red rice callus or stem cells as a source of materials for replenishing the aging body in a series of experiments. The results demonstrated the efficacy of red rice callus in cosmetic products on 28 volunteer subjects aged 30–55 years and proved to promote skin lightening, hydration, and elasticity. On the other hand, a study performed involving five different varieties of Thai pigmented rice demonstrated that all rice crude extracts with 50% ethanol exhibited a weak level of activity on tyrosinase inhibition [23]. This result is similar to our findings which demonstrated that proanthocyanidin and oryzanol could reduce melanin content but had no effect on mushroom tyrosinase activity [22]. However, our results have produced experimental data to support that proanthocyanidin decreased cellular tyrosinase activity leading to a decrease in melanin content. As has been mentioned previously, proanthocyanidin is highly present in red rice germ and bran and is very similar in chemical structure to the oligomers of catechin and epicatechin that are found in grape seeds and red wine. It is noteworthy to cite the findings of a study that found that the oral administration of grape seed extract was effective in lightening UV-induced pigmentation of guinea pig skin by a reduction in the number of 3,4-dihydroxyphenylalanine (DOPA)-positive melanocytes, Ki-67 positive, proliferating cell nuclear antigen (PCNA)-positive melanin-containing cells in the basal epidermal layer of the UV-irradiated skin in grape seed extract-fed guinea pigs. In addition, this study has demonstrated that grape seed extract effectively inhibited mushroom tyrosinase activity and inhibited melanogenesis without inhibiting the growth of culture B16-F10 mouse melanoma cells.

4. Conclusion

In this chapter, the by-products of rice processing, such as germ and bran, contain a wide range of biologically active compounds that can be recovered and used in a variety of approaches in nutraceuticals. This is in correlation with an increasingly deeper understanding of the predominant bioactive compounds found in pigmented rice, particularly anthocyanin and proanthocyanidin found in black and red rice, respectively. The dietary intervention and other high-value applications in functional food and cosmetic products have been attracting ever-growing attention in recent decades. The need for scientific evidence of pigmented rice bioactive compounds in different cultivars is encouraging for future perspectives within the new global business era of nutraceutical and agriculture expansion.

Most of the studies on the biological properties of black or red rice bioactive compounds have been conducted through an in vitro approach; however, only a few reports have been applied in preclinical or in animal studies. Further investigations will be needed to produce evidence on the efficacy of pigmented rice in terms of the anticancer activities and anti-inflammation properties in sub-chronic cases, especially among the aging members of the society in which sub-chronic inflammation commonly leads to noncommunicable diseases in later life. In addition, scientific studies have determined that the skin antiaging properties of pigmented rice should be useful and available in clinical studies for their efficacy and their further development in skincare products.

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Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

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