# U.K.S. Kushwaha

# Black Rice

Research, History and Development



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This Springer imprint is published by SpringerNature The registered company is Springer International Publishing AG Switzerland I am grateful to the almighty, the unseen inspirational power that compels me to write. This book is dedicated to those who love the diversity of rice especially black rice.

## Preface

Black rice is popularly known as forbidden rice. Research shows that black rice is considered as the twenty-first century super food. It prolongs life, thus it is also known as long-life rice. Really, this peculiar rice has wide health benefits. The historical evidence along with modern molecular research also tells the truth about black rice, which was hidden by emperors for a long time. Only consumption of black rice to a certain amount saves us from many serious diseases and illnesses. This is because black rice contains extra amount of antioxidants, which are more than blueberries.

The main motto of writing this book on black rice is to introduce black rice to a wide circle of people. Although a lot of research has been conducted on different aspects of black rice, yet no books are available among us. This book covers all the aspects of black rice from research, history, to its development. The manuscript is prepared with a wide range of people in mind. The main feature of this book is that it has different chapters and every chapter is different from the others. Readers can choose their particular chapter of interest to study this book. This book will be of benefit to rice researchers, rice scientists, food nutritionists, medical researchers, medical doctors, and common people who are willing to gain more information and knowledge about black rice.

Black rice consumption is increasing day-by-day around the world. Thus, it is clear that people want to know more about black rice, but no books are available yet. Therefore in the coming years, the demand for this book will increase. People will be curious to know more about black rice. Many people and researchers will benefit by studying it and this book will work as a reference for further research.

The primary audiences of this book include rice breeders, rice researchers, food nutritionists, and medical researchers. The secondary audiences of this book are medical doctors, students of food nutrition, agricultural students, biochemistry researchers who work on rice, medical students, and the general people. This book would be suitable for use in master's level agricultural university courses on rice. The book can also be used in rice-related workshops and seminars. Bachelor's level food nutrition courses can also have this book chapter as supplementary material.

This book describes all the aspects of black rice in brief. Chapter 1 describes rice and different rice types. Chapter 2 gives an introduction to black rice, its origin, and genetics, Chap. 3 gives the black rice history, Chap. 4 describes the nutrition profiles of black rice, Chap. 5 gives the difference between black, brown, and red rice varieties, Chap. 6 gives its economic importance, Chap. 7 gives the black rice application, Chap. 8 describes the black rice cultivation methodology, Chap. 9 describes the health benefits of black rice, and Chap. 10 gives black rice.

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# Contents

Rice.		1
1.1	Rice Background	1
1.2	Taxonomy and Genetics	2
1.3	Rice Domestication	4
1.4	Conditions of Growth	5
1.5	Rice Cropping Seasons	5
1.6	Rice Grain Structure	6
1.7	Types of Rice	6
	1.7.1 Long Grain Rice	8
	1.7.2 Medium Grain Rice	8
	1.7.3 Short Grain Rice	8
1.8	Other Rice Types	9
	1.8.1 Sweet Brown Rice	9
	1.8.2 Wehani Rice	10
	1.8.3 Brown Basmati Rice.	10
	1.8.4 Himalayan Red Rice	11
	1.8.5 Colusari Red Rice	12
	1.8.6 Purple Thai Rice	12
	1.8.7 Chinese Black Rice	13
	1.8.8 Sweet or Waxy Rice	13
	1.8.9 Aromatic Rice	13
	1.8.10 US Arborio Rice	14
1.9	Rice Forms	15
	1.9.1 Rough Rice	15
	1.9.2 Brown Rice	16
	1.9.3 White Rice	17
	1.9.4 Paraboiled Rice	17
	1.9.5 Precooking Rice	18
Refer	ences	19

2	Black	<b>Rice</b>
	2.1	Introduction
		2.1.1 Chinese Black Rice 24
		2.1.2 The Forbidden Rice 25
		2.1.3 The Emperor's Rice
	2.2	Origin and Domestication of Black Rice 26
		2.2.1 Domestication Process
		2.2.2 Black Conversion Process
		2.2.3 Spread Process 29
	2.3	Genetics of Black Rice 29
	2.4	Health Benefits 31
	2.5	Pecularity of Black Rice
		2.5.1 Rich in Iron
		2.5.2 Rich in Protein
		2.5.3 A Naturally Gluten Free Grain 37
		2.5.4 Black Rice Versus Blueberries
		2.5.5 Power Up 38
		2.5.6 Delicious
		2.5.7 Affordable 38
		2.5.8 Easy to Prepare
		2.5.9 Taste and Smell
	2.6	Popularity of Black Rice
	2.7	Black Rice Cons, Side Effects and Negatives 40
	2.8	Ways to Call Black Rice in Different Languages 40
	Refere	ences
3	Histor	ry
	3.1	History of Black Rice 49
	3.2	Discovery of Forbidden Rice
	Refere	ences
4		
4	Nutri	Notificated Different Direct Transformer 52
	4.1	Nutrition of Different Rice Types
	4.2	Nutritional Facts of Black Kice    51      Eastern Affecting Disc Nutrition    57
	4.5	Chamicala Dresent in Diack Diac
	4.4	A 4.1 Antheoryanin Drafile of Plack Rice
		4.4.1 Anunocyanin Prome of Black Rice
	15	4.4.2 Cyalifulii-5-Olucoside (CSO)
	4.5	on Nutrition
		45.1 Washing or Pinsing Pice Pafera Cooking
		4.5.2 Cooking Cite Delote Cooking
		4.5.3 Wet Milling 60
		454 Parboiling 70
		455 Fermentation 71

	4.6	Rice C	ooking Methods and Anthocyanin Content	71
	4.7	Nutrier	nt Content and Degree of Milling	74
	4.8	Aroma	tic Chemistry of Black Rice	75
	Refer	ences		79
5	Black	, Browi	n, and Red Rices	85
	5.1	Major	Differences Among Different Rice.	85
		5.1.1	White, Long-Grain Rice	86
		5.1.2	Colored Rice	86
	5.2	Brown	Rice	91
	5.3	Red Ri	ce	93
	5.4	Black 1	Rice	94
	5.5	Differe	nce in Black and Brown Rice.	96
		5.5.1	Difference in Calories	96
		5.5.2	Differences in the Carbohydrates, Protein and Fat	96
		5.5.3	Difference at the Minerals	97
		5.5.4	Difference in Antioxidant Power	97
	Refer	ences .		98
6	Econ	omic Im	portance	101
	Refer	ences	-	103
7	Black	Rice A	pplications	105
	7.1	Natural	Food Coloring Dye	106
	7.2	Future	Uses of Black Rice.	107
	Refer	ences		113
8	Black	Rice C	Cultivation	115
	8.1	Black 1	Rice Varieties	116
		8.1.1	Typical Characteristics of Black Rice	118
	8.2	Method	d of Cultivation.	119
		8.2.1	Broadcasting Method	119
		8.2.2	Drilling Method	122
		8.2.3	Transplantation Method.	122
		8.2.4	Japanese Method	122
		8.2.5	Upland Cultivation	123
		8.2.6	Lowland Cultivation	123
		8.2.7	Transplanting Methods in Detail	123
	8.3	Disease	es	127
		8.3.1	Blast	127
		8.3.2	Bacterial Leaf Blight	128
		8.3.3	Sheath Blight	130
		8.3.4	Brown Spot	130
		8.3.4 8.3.5	Brown Spot	130 131
		8.3.4 8.3.5 8.3.6	Brown Spot. Stem Rot.   Stem Rot. False Smut	130 131 132

		8.3.8 Tungro Virus 12	33
		8.3.9 Khaira Disease	34
	8.4	Insect Pests 1	34
		8.4.1 Stem Borer 1	35
		8.4.2 Gall Midge 1	36
		8.4.3 Rice Hispa 1	36
		8.4.4 Leaf Roller 1	37
		8.4.5 Armyworm	37
		8.4.6 Mole Cricket	38
		8.4.7 Green Leaf Hopper	38
		8.4.8 Plant Hopper	39
		8.4.9 Swarming Caterpillar 1	39
		8.4.10 Gundhi Bug	40
		8.4.11 Rats	40
	8.5	Harvesting and Threshing 14	47
	8.6	Post-harvest Loss	48
	8.7	Yield 14	49
	Refer	ences $\ldots \ldots 1^4$	49
0	II.a.l4	h Danafta of Dlash Diag	51
9		Exhauses Health and Insurance I an excite	51
	9.1	Ennances Health and Increases Longevily	55
	9.2	Protects Heart Health	54 57
	9.5	Reduce Atheroscierosis	57
	9.4	Control Hypertension	39 50
	9.5	Reduces Subke Kisk Levels in Wollien	39 50
	9.0	Anti informatory Action	39 60
	9.7		00 61
	9.8	Deterrifice the Deter	01 62
	9.9	Ligid Drafts Language 1	02 64
	9.10		64
	9.11	Reduces Risk of Diabetes    1      0.11.1    Vision and Exec Health	04 65
		9.11.1 Vision and Eye Health	03 65
		9.11.2 Cardiovascular Health	03 65
		9.11.3 Glucose Metabolism	65 65
	0.12	9.11.4 Weight Loss	03 66
	9.12	Improves Eye vision	00 66
	9.13	Help in Satiation and Weight Management	00
	9.14		0/
	9.15	Increases Life Span	/1
	9.16	Protects from Osteoporosis	13
	9.17	Ennances Hair Growth	14
	9.18	Reduces Risk of Asthma	74 74
	9.19	Antioxidative Effects	/4 74
	9.20	Acts as GABA Rice	76
	9.21	Works as Nature's Great Super Foods	76

	9.22	Black Rice is a Panacea 17
	9.23	Other Possible Health Benefits 17'
	Refere	ences
10	Black	<b>Rice Recipes</b>
	10.1	Rice Cooking Methodology 185
		10.1.1 Absorption Method
		10.1.2 Steaming Method
		10.1.3 Boiling Method
	10.2	Basic Directions for Absorption
		10.2.1 Rinsing 180
		10.2.2 Measuring
		10.2.3 Simmering
	10.3	Cooking Black Rice 18
	10.4	Rice Cooking Tips and Its Serving
	10.5	Black Rice Pudding
	Refere	ences
Ind	ex	

# Abbreviations

α-SMA	$\alpha$ -smooth muscle actin
μg	Micrograms
μg/g	Micrograms/gram
a.i.	Active ingredient
%	Percentage
2-AP	2-acetyl-1-pyrroline
8-OHdG	8-hydroxy-2'-deoxyguanosine
ACCase	Acetyl CoA carboxylase
ACS	American Chemical Society
ADFI	Average daily feed intake
ADG	Average daily gain
AEBR	Anthocyanin extract from black rice
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANR	Anthocyanidin reductase
ANS	Anthocyanidin synthase
AST	Aspartate transaminase
BC	Before Crist
BCP	Bran colorant powder/black rice bran colorant powder
BCR	Black-colored rice
BHC	Benzene hexachloride
bHLH	Basic helix-loop-helix
BHT	Butylated hydroxytoluene
BPH	Brown planthopper
BPP	Bioprocessed polysaccharide
BRACs	Black rice anthocyanin content
BRB	Black rice bran
BRE	Black rice extract
BRF	Black rice fraction
BRHA	Black rice husk ash
C3G	Cyanidin-3-glucoside/Cyanidin-3-O-β-D-glucoside

Ca	Calcium
CA	Crude ash
CAT	Catalase
CF	Crude fat
CF	Crude fiber
CFP	Crude fermentation-polysaccharide
CFP-S	Crude fermentation-polysaccharide-S. cerevisiae
CFU	Colony-forming unit
CHI	Chalcone isomerase
Chr.	Chromosome
CHS	Chalcone synthase
CI	Positive control
CIPDs	Chronic inflammatory proliferative diseases
cm	Centimeter
CNB	China black rice
СР	Crude protein
CRD	Completely randomized design
CTC	Cytosine Thymine Cytosine
Cu	Copper
Cy 3-Glc	Cyanidin 3-O-β-D-glucoside
Cy-3-G	Cyanidin 3-glucoside
DAP	Days to planting
DFR	Dihydroflavonol reductase/Dihydroflavonol-4-reductase
DM	Dry matter
DNA	Deoxyribo nucleic acid
DOM	Degree of milling
DPPH	1,1-diphenyl-2-picrylhydrazyl radical
DW	Dry weight
EC	Endothelial cells
equiv./g	Equivalent/gram
EU	European Union
F3H	Flavanone 3-hydroxylase
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
FBE	Fermented black rice bran extract
FCR	Feed conversion ratio
Fe	Iron
fgr	Fragrance gene
Fig	Figure
FLS	Flavonol synthase
g	Gram
GABA	γ-Amino butyric acid
GGT	Gamma glutamyl transferase

GLH	Green leafhoppers
glyLDL	Glycated low-density lipoprotein
GN	Granules
GPT	Glutamate pyruvate transaminase
GR	Glutathione reductase
GSH-Px	Glutathione peroxidase
GST	Glutathione S-transferase
HDL	High-density lipoprotein
HFCD	High fat/cholesterol diet
HN	Hom nil black rice
HOPE	Heart outcomes prevention evaluation
HPCCC	High-performance countercurrent chromatography
HSCCC	High-speed countercurrent chromatography
HSCs	Hepatic stellate cells
HYV	High-yielding variety
IBS	Irritable bowel syndrome
iNOS	Inducible nitric oxide (no) synthase
IRRI	International rice research institute
IU	International units
Κ	Potassium
kcal	Kilocalorie
kg/ha	Kilogram/hectare
LAR	Leucoanthocyanidin reductase
LDL	Low-density lipoprotein
LDOX	Leucoanthocyanidin dioxygenase
LPS	Lipopolysaccharide
MALDI-MS	Matrix-assisted laser desorption/ionization mass spectrometry
MDA	Malondialdehyde
mETC	mitochondrial Electron transport chain
Mg	Magnesium
mg	Milligrams
mg/g	Milligrams/gram
MMPs	Matrix metalloproteinase
Mn	Manganese
MP	Munpu red rice
mRNA	messenger Ribonucleic acid
Mth	Methuselah
Myb	R2R3-Myb transcriptional factor
Na	Sodium
NC	Negative control
NFBE	Non-fermented black rice bran extract
NO	Nitric oxide
NPK	Nitrogen phosphorus potash
OAVs	Odor activity values

OMT	O-methyltransferase
Р	Phosphorus
PA	Proanthocyanin
PBR	Purple black rice
PC-3	Prostatic cancer cell-3
PD	Niaw Dam Pleuak Dam
РК	Niaw Dam Pleuak Khao
RBE	Red bran extract
ROS	Reactive oxygen species
<b>RP-MPLC</b>	Reversed-phase medium pressure liquid chromatography
RT	Rhamnosyl transferase
SD	Standard deviation
Se	Selenium
SIPS	Stress-induced premature senescence
SOD	Superoxide dismutase
TAC	Total anthocyanin content
TBS	Trap barrier system
TC/TCH	Total cholesterol
TCM	Chinese traditional medicine
TFA	Trifluoroacetic acid solution
TNF-alpha	Tumor necrosis factor alpha
tocol	Tocoferol
ton/ha	ton/hectare
TPC	Total phenolic content
TV	Television
UFGT	Flavonoid-3-O-glucosyltransferase
UGT	UDP-glycosyl transferase
u-PA	Plasminogen activator
US	United States
USA	United States of America
USDA	United States Department of Agriculture
UV	Ultraviolet
VLDL	Very low density lipoprotein
WBPH	Whitebacked Planthopper
WGE	White grain extract
WP	Wettable powder
Zn	Zinc

### Chapter 1 Rice

#### 1.1 Rice Background

Around half of the world's population depends on rice (Oryza sativa L.) as a staple food and, it is considered as a basic source of energy for them (Monks et al. 2013). It is the most important cereal crop taken either directly as human food or indirectly as animal feeds (Saenkod et al. 2013). Rice is grown in more than 100 countries of the world spanning from 45° S to 53° N latitudes (Chang 2003). It is the predominant staple food in at least fifteen countries in Asia and the Pacific, ten countries in Latin America and the Caribbean, one country in North Africa, and seven countries in sub-Saharan Africa (FAOSTAT 2005). Rice is found everywhere except Antarctica. At least half of all the rice produced is consumed within 10 miles of where it was grown. Rice can be grown in almost any environment and is the highest yielding cereal grain. Large portions of the world's population spend up <sup>3</sup>/<sub>4</sub>'s of their incomes on rice alone. Almost 95 % of the rice production is recorded in Asian countries (Bhattacharjee et al. 2002). The word "food" and "rice" are used synonymously in different Asian languages. Recognizing the importance of this crop, the United nations General Assembly declared 2004 as the "International Year of rice" (IYR).

In terms of production, rice is only second to corn. It can produce more than 3000 grains of rice from a single seed. With respect to human nutrition, rice provides more than one-fifth of human calorific intake worldwide. In Asia alone, more than 2 billion people obtain 60–70 % of their daily calories intake from rice (Diouf 2003; Khush 2005). Asian rice (*Orysa sativa L.*) is the major cereal grain for 3 billion people worldwide (FAOSTAT). It represents as much as 70 % of calories intake in Southeast and South Asia and is integral to several cultures in Asia (FAOSTAT). Rice is widely cultivated and consumed in East, Southeast, and South Asia. Through European colonization, it reached Europe and America.

There are about more than 40,000 varieties of rice but only a few varieties are familiar to most of us. All varieties of rice, no matter what their colors are members

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of the grass family *Gramineae*, originally found growing in wild tropical and subtropical regions of the world. It is believed that rice may have been cultivated more than 4,000 B.C. years ago in China, India, and other Asian countries. Recent molecular evidence suggests that rice was first domesticated in China around 8,000–13,500 years ago. The first domestication gave rise to the *Japonica* like varieties from which the *Indica* types subsequently diverged (Molina et al. 2011).

All rices along with black rice are the family of grasses called Gramineae. It is the seed of monocotyledonous plant Oryza sativa L. (Asian rice) or Oryza glaberrima Steud. (African rice). Worldwide, the most consumed members of this family are Oryza sativa. There are more than 25 species of this Oryza variety; most are indica, followed by japonica, and then the javanica varieties. A small amount of Orvza glaberrima, a perennial species is grown in Africa. So-called "wild rice" (Zizania aquatica) is grown in the Great Lakes region of the United States, is more closely related to oats than to rice. Rice is generally considered a semiaquatic annual grass plant. Rice grown in summer/spring season is called boro rice and that rice grown in winter season is known as rabi rice. Once rice is sown, it is harvested at about after 6 months in tropical regions. Conventional rice cultivation needs 2,500 l of water to produce one kg of seeds but now modern rice cultivation methods have drastically reduced water requirements by rice. Flooding at various stages in the plant's life keep pests and weeds away. Depending on the variety, soil, and weather conditions, rice plants can grow anywhere from 3' to 6' tall (1-2 m). Worldwide, most consumed rice is long grain and polished white rice. Many people think of rice as being white or brown. But it is actually available in a stunning palette of shades from white to brown to red, and to a deep purple black color. Those rice are eaten with their hull or seed coat, taste nuttier than those that are polished and have the exterior hull removed. To an expert, all rice look different, cooked differently, and taste differently.

#### **1.2 Taxonomy and Genetics**

The genus *oryza* belongs to the tribe *Oryzeae* of the family Poaceae. There are 12 genera within the *Oryzeae* tribe (Vaughan 1994). The genus *Oryza* contains approximately 22 species of which 20 are wild species and two, *O. sativa* and *O. glaberrima* are cultivated (Vaughan 1994). *O. sativa* is the most widely grown of the two cultivated species. It is grown worldwide including Asian, North and South American, European Union, Middle Eastern, and African countries. *O. glaberrima*, however, is grown solely in West African countries. Now *O. sativa* and *glaberrima-sativa* hybrids are replacing *O. glaberrima* in many parts of Africa due to higher yield (Linares 2002). Research suggests that the progenitors of *O. sativa* are the Asian species. These are *O. rufipogon* and *O. nivara*, which are perennial and annual, respectively (Vaughan and Morishima 2003). Within the cultivars that have been developed, there are a range of forms bearing more or less

similarity to the wild projenitors. *O. barthii* and *O. longistaminata* are thought to be the progenitors of the African cultivated rice, *O glaberrima* (Vaughan and Morishima 2003). Other wild *Oryza* species are native to Africa, Central and South America, Asia, and Ocenia with overlapping distributions between these regions.

#### Scientific Classification

Kingdom: Plantae Unranked: Angiosperms Unranked: Monocots Unranked: Commelinids Order: Poales Family: Poaceae Genus: *Oryza* Specie: *O. sativa* Binomial name: *O. sativa* (wikipedia)

Most countries cultivate rice from the *Oryza* genus which has around 20 different species. *O. sativa* and *O. glaberrima* are the primary species and are grown in Asia and Africa, respectively. *O. sativa* has three important ecogeographic races: *japonica*, *indica*, and *javanica*. Depending on differences in production conditions, ethnicity, and flavor preference, each country has different cultivar preferences. *Japonica* and *indica* have distinct differences in grain appearance, color, flavor, and chemical composition. For example, *japonica* has medium to short grains (also called round grains) while *indica* grains are long, thin, and flat. In addition, many *indica* cultivars are aromatic, whereas *japonica* cultivars preferred in China, Japan, and Korea, are typically nonaromatic (Chaudhary and Tan 2001).

*Oryza sativa* has a relatively small (430 million base pairs) diploid genome (2n–24). This is the smallest genome of all food crops and approximately 50 % of the genome is composed of repetitive sequences (Chang 2003). Most other *Oryza* species are diploid, however some are tetraploid (4n–48). Species in the *Oryza* genus can be classified into nine groups (with one unclassified species, Table 1.1) on the basis of genome compatibility at meiosis in F1 (first generation) hybrids. These groups along with the geographic locations of each species are shown in Table 1.1 (OECD 1999; Vaughan and Morishima 2003).

Data in table are summarized from (OECD 1999; Vaughan and Morishima 2003):

- 1 Vaughan (Vaughan and Morishima 2003) uses this nomenclature as it is widespread in the literature but recognizes no characteristics to distinguish this species from *O. rufipogon*.
- 2 *O. malampuzhaensis* has recently been distinguished from *O. officinalis* on the basis of morphological characters and its tetraploid genome (Li et al. 2000; Thomas et al. 2001). Its distribution is restricted to the small region of southern India.
- 3 The genome type of *O. schlechteri* has not been determined yet (Vaughan and Morishima 2003).

Orya species	Genome	Africa	Central or South America	Asia	Oceania
O. sativa complex					
O. sativa	AA	1	1	1	1
O. glaberrima	AA	1			
O. barthii	AA	1			
O. glumaepatula1	AA		1		
O. longistaminata	AA	1			
O. meridionalis	AA				1
O. nivara	AA			1	
O. rufipogon	AA		1	1	1
O. officinalis complex					
O. punctata	BB, BBCC	1			
O. malampuzhaensis2	BBCC			1	
O. minuta	BBCC			1	1
O. eichingeri	CC	1		1	
O. officinalis	CC			1	1
O. rhizomatis	CC			1	
O. alta	CCDD		1		
O. grandiglumis	CCDD		1		
O. latifolia	CCDD		1		
O. australiensis	EE				$\checkmark$
O. brachyantha	FF		1		
O. granulata complex					
O. granulata	GG			1	
O. meyeriana	GG			1	
O. redleyi complex					
O. longiglumis	ННЈЈ				1
O. ridleyi	ННЈЈ			1	1
O. schlechteri	??				$\checkmark$

Table 1.1 The distribution and genomic classification of rice species throughout the world

The Biology and ecology of Rice (Oryza sativa L.) in Australia (2005)

#### **1.3 Rice Domestication**

The geographical site of the origin of rice and its domestication is not yet definitely known. The general assumption is that rice domestication occurred independently in China, India, and Indonesia, thereby giving rise to three races of rice: sinica (also known as *japonica*), *indica*, and *javanica* (also known as bulu in Indonesia). It is reported that rice was cultivated in India between 1500 and 2000 B.C. and in Indonesia around 1648 B.C. Archeological findings have shown that tropical or

*indica* rice was being cultivated in Ho-mu-tu, Chekiang Province, China at least 7000 years ago (Chang 1983). Recently, remains of the temperate or sinica (*japonica*) rice of the same age were found at Lou-jia-jiao, also in Chekiang Province (Chang 1985). Rice was rapidly dispersed from its tropical (southern and southeastern Asia) and subtropical (southwestern and southern China) habitats to much higher altitudes and latitudes in Asia, reaching Japan as recently as 2300 years ago (Chang 1983). It was introduced to points as far as West Africa, North America, and Australia within the last six centuries. Rice growing became firmly established in South Carolina in the United States in about 1690 (Adair 1972). Rice was cultivated in Europe from the eighth century in Portugal and Spain and by the ninth to the tenth century in southern Italy (Lu and Chang 1980).

#### **1.4 Conditions of Growth**

Rice is a tropical plant and requires high heat and high humidity for its successful growth and productivity. The optimum temperature should be fairly high at mean monthly of 24 °C during rice crop standing period. It should be 20-22 °C at the time of sowing, 23–25 °C during growth and 25–30 °C at the harvesting time (IRRI 2015). It is the dominant crop in areas of over 200 cm annual rainfall and is still an important crop in areas of 100–200 cm rainfall. The average annual rainfall required by rice is 150 cm. The fields must be flooded under 10–12 cm deep water at the time of sowing and during early stages of growth. Therefore, the fields must be leveled and have low mud walls to retain water. This peculiar requirement of water makes it primarily a crop of plain areas. Rice grown in well-watered low-land plain areas is called wet or lowland rice.

Rice can be grown on a variety of soils including silts, loams, and gravels and can tolerate acidic as well as alkaline soils. However, deep fertile clayey or loamy soils which can be easily puddled into mud and develop cracks on drying are considered ideal for raising this crop. Such soil requirements make it dominantly a crop of river valleys, flood plains, deltas, and coastal plains, and a dominant crop there. High level loams and lighter soils can be used for quick maturing varieties of rice. Thus it is a labor intensive cultivation and requires large supply of cheap labor for its successful cultivation.

#### **1.5 Rice Cropping Seasons**

Rice is grown almost throughout the year in hot and humid regions in the world where two to three crops in a year are common. But the winters are too cold for rice cultivation and only one crop is grown in those areas. Table 1.2 gives the period of sowing and harvesting the rice crop.

Crop	Local name	Sowing	Harvesting
Autumn (Kharif)	Aus or Kar	May–June	Sept-Oct
Winter (Rabi)	Aman, Sali, or Karthika	June-July	Nov-Dec
Summer (Spring)	Boro or Dalua	Nov-Dec	March-April

Table 1.2 Rice cropping seasons in South Asia





#### 1.6 Rice Grain Structure

The rice whole grain is fertilized and fully developed ovary with the hull and awn attached. Caryopsis is the dehulled rice grain commonly known as brown rice. It is enveloped by the pericarp, tegument, and aleurone layers. Residing inside is the endosperm which is the nourishing tissue for the embryo or germ that develops into the next generation of rice plant (Fig. 1.1).

#### 1.7 Types of Rice

More than 40,000 types of rice varieties are available in the world. All varieties of rice are not cultivated yet because only a small number offer the quality acceptable to be grown commercially. Depending on the size (length) and shape of the kernel of rice, it can be classified as long, medium, and short grain rice. The primary



Fig. 1.2 Varieties of rice that differ in bran color, grain morphology, and texture after cooking. IRRI (2015)

differences in these rice is their cooking characteristics and a subtle flavor difference. With respect to their ecology, these rice have different days to heading, days to maturity, and reaction toward diseases like rice leaf blast. From a nutritional standpoint they are equal and indeed can often (with the exception of waxy rice or arborio) be interchanged in recipes (Fig. 1.2).

There is no international standard for brown rice grain size and shape. International Rice Research Institute (IRRI 2015) uses the following scale for size:

extra long => 7.50 mmlong = 6.61 to 7.50 mmmedium = 5.51 to 6.60 mm and short =< 5.50 mm

Grain shape is characterized based on length-to-width ratio:

slender => 3.0medium = 2.1 to 3.0bold = 1.1 to 2.0 and round =< 1.0

Similarly, the Codex Alimentarius Commission committee considering the draft standard for rice proposed the following classification of milled rice based on length-to-width ratio:

long grain =< 3.1 medium grain = 2.1 to 3.0 and short grain =< 2.0 (Codex Alimentarius Commission 1990).



Fig. 1.3 Long grain rice

#### 1.7.1 Long Grain Rice

Long grain rice has a long slender kernel, four to five times longer than its width. Grains are separate, light, and fluffy after cooking. It is perfect and used as a side dish. There are also glutinous long grain rices in Laos and Thailand. Examples are basmati rice from India and Pakistan, jasmine white rice from Thailand, and ferrini from Italy (Fig. 1.3).

#### 1.7.2 Medium Grain Rice

Medium grain rice is in between long and short grain rice, has a shorter, wider kernel (two to three times longer than its width) than long grain rice. Cooked grains are more moist and tender, and have a greater tendency to cling together than long grain rice. Risotto is made with medium grain rice as in paella. Examples are arborio, carnaroli, vialone, nano, etc. (Fig. 1.4).

#### 1.7.3 Short Grain Rice

Short grain rice has a short, plump, only slightly longer than it is wide, looks almost (as long as wide) round kernel (4 to 5 mm long and 2.5 mm wide). Cooked grains are soft and cling together. This rice stays tender even at room temperature. Example is sushi (Fig. 1.5).



Fig. 1.4 Medium grain rice



Fig. 1.5 Short grain rice

#### 1.8 Other Rice Types

Rice can be classified and named in different ways based on its taste, color, site of origin, aroma, etc. Some other rice types are described here.

#### 1.8.1 Sweet Brown Rice

Sweet rice is short and plump with a chalky white, opaque kernel. Sweet rice loses its shape when cooked and is very sticky (Fig. 1.6).

Fig. 1.6 Sweet brown rice



Fig. 1.7 Wehani rice



#### 1.8.2 Wehani Rice

This is a long grain honey red rice and is naturally bred, developed from an Indian basmati type seed. Like other aromatic rices, it has a distinctive nutty fragrance when cooked (Fig. 1.7).

#### 1.8.3 Brown Basmati Rice

Basmati is aromatic, long grain rice with a distinct "popcorn" aroma and is popular in India. The basmati rice, cultivated in India and Pakistan is the best known and

Fig. 1.8 Brown basmati rice



most appreciated. It is indispensable in Hindu cooking and has a light and dry texture and an aromatic taste (Fig. 1.8).

#### 1.8.4 Himalayan Red Rice

This long grain rice has a reddish bran layer and a nutty, complex flavor that adds visual and taste delight to any dish. Red rice has a red bran covering the kernel (Himalayan, Bhoutanais, or Thai). This rice is also imported from India (Fig. 1.9).





Fig. 1.10 Colusari red rice



#### 1.8.5 Colusari Red Rice

Colusari red rice originated in a seed bank in Maryland. When cooked, it adds an upscale burgundy color to the plate. It is a naturally bred rice. It is grown in the Sacramento Valley of the US (Fig. 1.10).

#### 1.8.6 Purple Thai Rice

Purple Thai rice was traditionally used in dessert recipes, but is now turning up in savory dishes too. It is slightly sweeter than some other rices (Fig. 1.11).

Fig. 1.11 Purple Thai rice



Fig. 1.12 Chinese black rice



#### 1.8.7 Chinese Black Rice

Chinese black rice is medium grain rice with white kernels inside the black bran. It takes on a deep purplish color after cooked. This rice has a black thin bran covering a white grain. It comes from Bali, China, Japan, Korea, and Thailand (Fig. 1.12).

#### 1.8.8 Sweet or Waxy Rice

US sweet rice is short and plump with a chalky white, opaque kernel. When cooked, sweet rice loses its shape and become very glutinous. Sweet rice is more often used in commercial product formulations. The starch and flour from sweet rice are used in frozen products as a binder for gravies, sauces, and fillings because it is resistant to breakdown during freezing and thawing, unlike some corn or wheat starches (Fig. 1.13).

#### 1.8.9 Aromatic Rice

Aromatic rices have flavor and aroma similiar to roasted nuts or popcorn. Aromatic rice has more flavor than the other varieties. The most popular domestically grown aromatic rices include della which cooks dry, separate, and fluffy; jasmine which cooks more moist and tends to cling together; and basmati which cooks into very long, slender grains which are dry, separate, and fluffy. Jasmine rice grows in Northeast Thailand (Isarn region) and is also appreciated worldwide (Fig. 1.14).

Fig. 1.13 Sweet or waxy rice



Fig. 1.14 Aromatic rice



#### 1.8.10 US Arborio Rice

US arborio rice is a large, bold rice with a characteristic white dot at the center of the grain. Arborio rice has white and round grain and is considered one of the finest rices because it can absorb a high quantity of liquid while cooking without becoming soggy. By the way of length/width ratio and starch characteristics, it is

#### Fig. 1.15 US Arborio rice



classified as a medium grain rice primarily used in risotto. This rice develops a creamy texture around a chewy center and has exceptional ability to absorb flavors (Fig. 1.15).

#### 1.9 Rice Forms

Rice can be purchased cooked or uncooked, canned or dehydrated and also in frozen forms. Few foods are packaged so extensively and are offered in so many combinations as rice. To meet the many special requirements of packaged foods, rice undergoes varying degrees of processing, including regular milled, parboiled, precooked, and brown.

#### 1.9.1 Rough Rice

Kernels remain attached still within the hull. Before the rice can be packaged or cooked, the outer hull or husk must be removed. Usually rice is stored longer times in rough forms at moisture 10-12 % (Fig. 1.16).

#### Fig. 1.16 Rough rice



#### 1.9.2 Brown Rice

Rice from which only the external and nonedible husk has been removed is the brown rice. The bran layer remains attached with kernel making rice more nutritive than white rice. In Europe, this type of rice is often called "Cargo rice" because of the way it is transported by sea. Brown rice is unmilled and retains the bran and germ that surrounds the kernel, giving it a chewy texture and a flavor often described as nutty. Brown rice may be eaten as it is or milled into white rice. Cooked brown rice has a slightly chewy texture and a nut-like flavor. The light brown color of brown rice is due to the presence of bran layers which are rich in minerals and vitamins, especially the B-complex group. It takes longer time to cook brown rice than white rice. Because of the oil in the bran and germ, rice spoils more easily and so it should be kept refrigerated. It is also known as husked rice (Fig. 1.17).

Fig. 1.17 Brown rice



Fig. 1.18 White rice



#### 1.9.3 White Rice

White rice has its bran and germ milled away. It cooks up tender and delicate and is less nutritious than brown rice. Milled white rice is often referred as "white" or "polished" rice and it is the most common form of rice. White rice is most popular which is consumed widely. The outer husk is removed, and the layers of bran are milled until the grain is white. It contains less niacin, thiamin, magnesium, zinc, iron, and fiber than the brown rice. In some countries, like the United States, white rice might be enriched with iron, niacine, and thiamine so that it can reclaim part of its original nutritive value. White rice may be covered with magnesium silicate or with a mixture consisting of talc and glucose and is also known as "talc-coated rice" (Fig. 1.18).

#### 1.9.4 Paraboiled Rice

Rough rice that has gone through a steam pressure process before milling is the paraboiled rice. This procedure gelatinizes the starch in the grain and ensures a firmer, more separate grain. Parboiled rice is favored by consumers and chefs who desire extra fluffy and separate cooked rice (Fig. 1.19).
#### Fig. 1.19 Paraboiled rice



# 1.9.5 Precooking Rice

White or brown rice has been completely cooked and dehydrated after milling. This process reduces time required for cooking (Fig. credit from 1.3 to 1.20: USA Rice Federation and Lundberg Family Farms) (Fig. 1.20).

Fig. 1.20 Precooked rice



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# Chapter 2 Black Rice

# 2.1 Introduction

Black rice is a type of the rice species Oryza sativa L. which is glutinous, packed with high level of nutrients and mainly cultivated in Asia. The pericarp (outer part) of kernel of this rice colour is black due to a pigment known as anthocyanin, an antioxidant. Black rice is also known as purple rice, forbidden rice, heaven rice, imperial rice, king's rice and prized rice. Many people assume this rice as a panacea of many culinary diseases because of its high nutritive value and curative effect. This rice is supposed to enhance the longivity of life, hence it is also known as long life rice. This rice includes several varieties with a long history of cultivation in Southeast Asian countries such as China, India and Thailand (Kong et al. 2008). There are more than 200 types of black rice varieties in the world. Only China is responsible for 62 % of global production of black rice and it has developed more than 54 modern black rice varieties with high yield characteristics and multiple resistances. China cultivates the most black rice followed by Sri Lanka, Indonesia, India and Philippines etc. Thailand occupies the ninth position to black rice cultivation (Ichikawa et al. 2001; Sompong et al. 2011). Interest in black rice is indicated by the number of accessions held in germplasm collections, e.g. China–359, Sri Lanka-50, Indonesia-42, India-30, the Philippines-25 and Bangladesh-24 (Chaudhary 2003). Nutrients such as protein, minerals (Ca, P, Fe, and Zn) and dietary fiber contents are higher in black rice compared to brown and white rice. Demand for this rice is growing fast in the USA and European countries due to its value as a healthy food and its attractive organic food color. Ichikawa et al. (2001) reported that black rice is efficient, and two fold stronger with respect to antioxidant activities of blueberries. Black rice is surely a special breed of rice that is cultivated on earth. This rice is getting popular in recent years because of its high nutritive value and antioxidative properties.

Black rice is actually heirloom rice, means it is open pollinated, was grown at earlier times in the history, and is not grown on a large scale in modern agriculture.

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The term 'black rice' actually refers to a variety of rice types from the species *Oryza* sativa, and is descriptive of the colour of grain, rather than other properties. Black rice also comes in a number of short grain, long grain and glutinous varieties similar to brown rice. This rice has an incredibly rich history and among its strains has one variety known as "Imperial Rice." Imperial rice was reserved for the emperor's consumption only. Black rice, as one would imagine, is deep black in color and mutates into a regal purple hue when cooked. The purple colour is due to the grain's naturally high anthocyanin content, a trait most typically observed in fruits such as blueberries and blackberries. Black rice has dark purple hues in its outer bran layer that are so intense that the rice appears to be black. Once cooked, the color lightens into that same deep purple/violet found in blueberries. This dark purple color predominantly comes from anthocyanins which are flavonoids that perform as antioxidants in the body.

There exists no other rice with a higher nutritional spectrum near black rice. This rice is free of gluten, free of cholesterol, low in sugar, salt and fat. Black rice is a whole grain, super nutritious type of rice that is high in fiber, anthocyanin, antioxidants, vitamins B and E, iron, thiamine, magnesium, niacin and phosphorous. A huge number of scientific studies show that black rice powder is one of nature's most well balanced superfood and its abilities are truly remarkable. Black rice anthocyanins (BRACs) are a kind of anthocyanins that are extracted from the aleurone layer of black rice which is a major cereal crop existing since ancient times in China and other Eastern Asia countries (Ling et al. 2002). The anthocyanin components in BRACs are about 26.3 %, and cyaniding-3-O-glucoside and peonidin-3-O-glucoside are the main effective constituents accounting for about 90 % (Chang et al. 2010). Among different compounds of black rice, anthocyanin is the one which mop up harmful molecules and help to protect arteries and prevent the DNA damage. Anthocyanins are the flavonoid pigments of black rice and are the source of antioxidants that have the ability to inhibit the formation or to reduce the concentrations of reactive cell damaging free radicals (Adom and Liu 2002). Black rice extracts could scavenge superoxide anions more effectively than hydroxyl radicals (Nam et al. 2006). This rice has long been consumed in Korea, Japan and China (Ryu et al. 2000; Han et al. 2004). Black rice has been eaten throughout Asia for thousands of years and has a significant history of use in China, India and Thailand. Up until modern times, black rice was not easy to come by. It had been highly treasured and protected in Asia for many centuries. But black rice consumption is more common nowadays. This rice is becoming popular among rice consumers and dieticians day by day mainly because of its high nutritive and medicinal value. Therefore, black rice is becoming the new "IT" organic food that everyone is talking about and the attention it is getting is well deserved (Fig. 2.1).

Dictionary meaning of black rice (Segen's Medical Dictionary 2012).

A strain of rice that has currency as both a food and medicine. It owes its dark colour to the high concentration of anthocyanins, which are potent antioxidants. It is regarded as a 'superfood' that may lower the risk of cancer due to its high concentrations of fibre, B vitamins, niacin, vitamin E, calcium, magnesium, iron, and zinc.



Fig. 2.1 A typical kernel of Chinese black rice with attached hull and dehulled

Black rice is typically sold as unmilled rice, means the fiber rich black husks of the rice are not removed. This rice is commonly used as a condiment, dressing, or as a decoration for different types of desserts in many countries around the world. The unusual colour makes it very popular for desserts and the high nutritional value is an added benefit. This rice is often served with fresh fruit such as mangoes and lychees, especially when drizzled with a fruit or rice syrup. Traditionally this rice has been used in China and Taiwan as dessert rice, but it can actually be used in almost any sweet or savory dish where plain rice would be used. Its pitch is black when raw, but it actually turns a beautiful, shiny indigo when cooked. Its flavor is richer and sweeter than white rice and its texture is slightly sticky. This short grain rice has a slightly nutty flavor, and its texture is smooth and firm. It is suitable for making porridge, dessert, traditional Chinese black rice cake or bread. Noodles also have been produced from black rice. Black rice is usually consumed mixed with white rice in Korea. The grain has a similar amount of fiber to brown rice and like brown rice, it has a mild and nutty taste.

Black rice includes many more varieties of dark colour rice like Forbidden rice, Purple rice, Japonica black rice, Chinese black rice, Indonesian black rice and Thai black rice. The reason they are grouped under the term "Black Rice" is the unusual dark/black colour of the grain. Black rice has diversity in terms of colour due to anthocyanin content and other morphological characters. Looking black rice in the morning is an indication that the whole day will be successful. Based on historical record, black rice was only for the kings of China and Indonesia (forbidden rice). This is because black rice has a double function, namely as a source of staple food with good taste, fluffier and fragrance, as well as an efficacious medicine to cure various illnesses (Kristamtini 2009). Black rice is the most popular staple food in Europe even more than Southeast Asia (Simmons and Williams 1997). This rice is not currently grown on a commercial scale in the US, Europe and other parts of the world but it is hoped that its commercial cultivation will cover the world. Black rice is still a niche rice product, but its popularity seems to be growing.

Black rice special and medicinal values are truly stunning even today with all of our medical knowledge and tools. Thus black rice is a kind of food that can make us healthy and save our life. Black rice is a whole grain and nowadays, whole grain is categorized as one of the potent functional food sources since it contains high amounts of phenolic compounds, especially anthocyanins in pericarp (Abdel-Aal et al. 2006; Ryu et al. 1998a, b; Yawadio et al. 2007). Black rice has long been consumed in Japan and China and is considered to be a healthy food because of its antioxidant content that are able to prevent oxidative stress. Oxidative modification of low-density lipoprotein (LDL) may play an important role in the development of atherosclerosis. Chinese black rice is rich in iron and is considered as a blood tonic. This rice is claimed to be good for the kidney, stomach and liver in China. Previous studies show that black rice was an alternative healthy food for diseases treatment as it contains antioxidative agent, such as anthocyanin (Chutipaijit et al. 2011). This rice also contains higher levels of proteins, vitamins and minerals than common white rice (Suzuki et al. 2004). Compared to white rice, black rice is relatively rich in the mineral contents such as Fe, Zn, Mn and P and has higher variability in mineral content that depend upon varieties and soil types of the planting area (Qiu et al. 1993; Liu et al. 1995; Zhang 2000). Black rice has high nutritional value and it contains the highest levels of anthocyanin. Its dark purple color is primarily due to its anthocyanin content, which is higher by weight than that of other coloured grains. Anthocyanins are a group of reddish purple water soluble flavonoids (Shen et al. 2009) located on pericarp, seed coat and aleurone layer (Sompong et al. 2011; Ryu et al. 1998a, b; Takashi et al. 2001). Black glutinous rice has been also shown to accumulate compounds such as anthocyanins (Zhang et al. 2004a, b; Abdel-Aal et al. 2006) and gamma oryzanol (Rong et al. 1997; Qureshi et al. 2000; Van 2004; Juliano et al. 2005).

### 2.1.1 Chinese Black Rice

Among all black rice varieties, Chinese black rice is the one which is most popular and it has its own history and medicinal property. In China, the least known and least used rice is Chinese black rice. It is a short grain glutinous rice. This purple cooking rice is grown on the banks of the Yangzi river. Chinese black rice is different from other rice. It is very short with low fat, and is rarely polished. It tastes nuttier than any other rice after cooking. Special characteristics of Chinese black rice is that it is black on the outside and black on the inside. Not all black rices look like this. Chinese black rice and most glutinous rices are exceptionally high in amylopectin, and if cooked too long they disintegrate. That's why Chinese black rice is never milled and always cooked with seed coat or hull intact. Traditionally, this black rice was steamed in a bamboo tube for two hours. Nowadays, it is boiled for about forty minutes or steamed for an hour. No matter how the rice are cooked, immediately after cooking, it is best to leave this rice sit for about twenty minutes to absorb any remaining water; this allows the grains to separate. Chinese black rice is a food, eaten frequently by many Chinese peoples and people groups, particularly those in and around the Yunnan Province where it is grown mostly. People like this rice because of its sweet flavor. They also like this rice because it remains chewy and has a starchy taste. Some people like it because it does not need to be washed, though many recipes suggest doing so (www.flavorandfortune.com). Those who have celiac disease, their intestinal villae do not allow them to properly absorb gluten, can eat this glutinous black rice. There is no evidence where this glutinous rice acquired its name, the assumption is that it is like gluten when wet and looks very sticky. Wheat flour has lots of gluten, and the gluten in it provides structure to foods when wet and heated. That is why, with a leavening agent, breads rise and when heated (baked) hold their shape. With or without a leavening agent, rice can not make nor can it hold a structure or shape because it has no gluten.

Chinese black rice (Forbidden rice) is medium grained glutinous rice which is different from purple sticky rice. It is not as sweet, although still sweeter than most rice varieties. Black rice can be used in both sweet and savory dishes. It was once known as Emperor's rice because it was only served to Chinese Emperors and was forbidden else where. Black rice is now widely available and even noodles made from black rice are available today. This rice is high in fiber and minerals, more vitamins than regular rice varieties and with nutty flavor makes a great rice pudding and is used in other sweet dishes. This rice is also popular in Indonesia and Thailand and it is not generally used as the main starch in a meal. Black rice is known for its health benefits and was favored by Chinese Emperors as a tonic. Its black colour is not artificial, the plant has distinctive black tops which stand out from traditional rice paddies. There are records of black rice being grown around 150 BC, and it is considered lucky as well as nutritious. Traditionally served in Taiwan and mainland China as a dessert ingredient in the West. This rice is most often found served with main courses. Chinese black rice has a beautiful shiny, indigo colour after cooked.

## 2.1.2 The Forbidden Rice

Chinese black rice is also known as forbidden rice and it is an ancient grain that has even more impressive health benefits than most other closely related rice varieties, though it is less popular than brown or wild rice. It is not only the rice type that is richest in disease fighting antioxidants, but it also contains dietary fiber, anti-inflammatory properties and has the ability to help and stop the development of diabetes, cancer, heart disease and even weight gain. Black rice has been eaten in regions of Asia for thousands of years, infact for centuries it was reserved for only Chinese royalty. Black rice was reserved in ancient China as the most rare, nutritious and tasty of all rice. Chinese referred it as "forbidden rice" only to be eaten by nobility. Unfortunately, it fell out of favor through the centuries and is now used in Asia primarily as food decoration and as a component of noodles, sushi and desserts. It is still a complete rarity in the West, but its new status as a super food that can fight cancer and prevent heart disease may bring it out of hiding and back into the favor of peoples.

## 2.1.3 The Emperor's Rice

Chinese black rice was set aside exclusively for royalty in ancient China. It was thought that consumption of black rice would extend their lives. Royal families and kings used to eat this special rice to retain good health and make themselves fortune. In light of recent scientific studies on the anti aging effects of antioxidants, it seems that this ancient knowledge was on the mark. Black rice is super nutritious when compared to other varieties of rice. This rice is rich in fiber, as well as iron, vitamin E and is a host of other crucial minerals. Black rice has been characterised by the accumulation of phenolic acids, flavonoids and anthocyanins exhibiting antioxidant activities (Kaneda et al. 2006). However, black rice yield is lower than that of white rice which does not contain anthocyanins.

# 2.2 Origin and Domestication of Black Rice

The origin and spread of novel agronomic traits during crop domestication are complex events in plant evolution. Wild rice (Oryza rufipogon) has red grains due to the accumulation of proanthocyanidins, whereas most cultivated rice (Oryza sativa) varieties have white grains induced by a defective allele in the Rc basic helix-loop-helix (bHLH) gene. Although the events surrounding the origin and spread of black rice traits remain unknown, varieties with black grains due to anthocyanin accumulation are distributed in various locations throughout Asia. Black grain trait originated from ectopic expression of the Kala4 bHLH gene due to rearrangement in the promoter region. Both the Rc and Kala4 genes activate upstream flavonol biosynthesis genes, such as chalcone synthase and dihydroflavonol-4-reductase, and downstream genes, such as leucoanthocyanidin reductase and leucoanthocyanidin dioxygenase, to produce the respective specific pigments. Genome analysis of black rice varieties as well as red and white grained landraces demonstrated that black rice arose in tropical *japonica* and its subsequent spread to the *indica* subspecies can be attributed to the causal alleles of Kala4. The relatively small size of genomic fragments of tropical japonica origin in some indica varieties indicates that refined introgression must have occurred by natural crossbreeding in the course of evolution of the black trait in rice (Oikawa et al. 2015). The black (or purple) grain colour has not been observed in any accessions of *O. rufipogon* (http://www.gramene.org). Thus, the black rice trait is most likely newly acquired, incorporated either during or after rice domestication. Compared with the broad distribution of white rice cultivars, black rice cultivars are sporadically distributed throughout Asia. Black rice was used in ancient China before Chinese dynastic times (Newman 2004) and was sometimes called "emperor's rice" (or "forbidden rice") since it was used as a tribute food and prized for its rarity in ancient China. It is completely unknown how this aesthetic trait arose and has been maintained for such a long time.

Oikawa et al. (2015) demonstrated that genetically the Kala4 gene involved in the origin of black rice corresponds to Os04g0557500 gene. Kala4 encodes a bHLH transcription factor, which is a rice homolog of the maize R/B gene, previously reported as the OSB2 gene (Sakamoto et al. 2001). Oikawa and his friends further demonstrated that a structural change in the Kala4 promoter induced ectopic expression of the bHLH protein and the subsequent activation of the anthocyanin biosynthesis pathway. With this structural change and rearrangement in Kala4, rice acquired the brand new black grain colour trait, resulting in the birth of black rice. Oikawa also demonstrated that a relatively small genomic segment around the Kala4 gene that originated from tropical *japonica* was transferred into the *indica* subspecies, probably as a result of refined introgression through multiple natural crossbreeding processes. The results described reveal the process that led to the neo functionalization of Kala4 and subsequent spread of the black rice allele of Kala4 to other subspecies of rice. Since closely related structural changes of the Kala4 promoter were observed in the 21 black rice landraces, it is likely that black rice possessed the red trait in the course of its evolution. Subsequently, several structural changes must have occurred and spread to other varieties with some DNA variations (Oikawa et al. 2015).

#### **Domestication Process**

#### **Description of Fig. 2.2**

The processes involved in the creation of black rice and its spread can be divided into three categories, i.e. domestication (in blue frame), black rice conversion (in black frame) and spread (in orange frame).

## 2.2.1 Domestication Process

Or-IIIa type *O. rufipogon* is the most closely related ancestral varieties of *japonica* cultivars and landraces (Lu et al. 2008, Huang et al. 2012). The two *O. rufipogon* accessions, W1943 and W1963 which belong to Or-IIIa, do not have LINE1 insertion at intron 2 of the *Kala4* gene (data from Gramene database, www. gramene.org). This observation suggests that the LINE1 insertion occurred after



Fig. 2.2 Schematic diagram of a model showing the processes in the origin of black rice and the spread of the Kala4 black allele (Oikawa et al. 2015)

domestication. Moreover, the LINE1 insertion at this position was observed in both *tropical japonica* and *temperate japonica*. Therefore, the LINE1 insertion probably occurred in the common ancestor before the differentiation to the two *japonica* subspecies (step1) (however, in *temperate japonica* subspecies, the LINE1 insertions were surveyed in only two cultivars). There are relatively few LINE1-inserted cultivars in *indica* and *aus* subspecies, suggesting that the LINE1-inserted allele of the *Kala4* locus was transferred from ancient *japonica* or *tropical japonica* to *indica* and *aus* subspecies.

# 2.2.2 Black Conversion Process

The difference of the DNA methylation status at intron 2 of the *Kala4* gene is observed in only few varieties of *tropical japonica* subspecies (however, only 2 white *temperate japonica* and no *indica* varieties were investigated). Higher DNA methylation of the LINE1 element has been thought to be necessary for inactivation of LINE1 transposition. Therefore, DNA methylation of the LINE1 element likely occurred immediately after LINE1 insertion (step 2), and then after the nonsense mutation arose in ORF2, the DNA methylation status of the 5'-upstream region of LINE1 was decreased in the common ancestral varieties of most *temperate* 

*japonica* and *tropical japonica*. However, the higher DNA methylation status 5'-upstream of LINE1 remained in some *tropical japonica*, and the variety was the origin of black rice. In this variety, the duplication of the LINE1 sequence accompanied by part of *Kala4* structure likely occurred (step 3), followed by insertion of the 11.02-kb fragment originated from approximately 83-kb upstream of the *Kala4* ORF (step4). As a consequence of these duplication and insertion events, the *Kala4* expression was upregulated in both transcription and translation, resulting in the creation of the black *Kala4* allele.

## 2.2.3 Spread Process

The black allele of the *Kala4* locus which was generated in *tropical japonica* subspecies, was transferred into *indica* subspecies by introgression (red arrow). The identical alteration of the *Kala4* promoter structure is also observed in black rice of *temperate japonica* subspecies. The black allele of the *Kala4* locus likely was transferred from black *indica* or black *tropical japonica* subspecies by interbreeding (blue arrows).

## 2.3 Genetics of Black Rice

Molecular genetic background of black rice, however, is not well identified. Among the various coloured rice, black rice is the one which is characterized by dark purple pericarps in seeds with high levels of anthocyanins. The name black rice refer to the kernel colour (black) of the rice which is formed by deposits of anthocyanins in different layers of the pericarp, seed coat and aleurone (Chaudhary 2003). During rice seed development, purple pigments of anthocyanin accumulate rapidly in the pericarp, resulting in the characteristic dark purple grains of black rice (Abdel-Aal et al. 2006; Shao et al. 2011). Previous genetic investigations have shown that cyanidin-3-O-glucoside and peonidin-3-O-glucoside are the two primary anthocyanin pigments deposited in the seed pericarps of black rice (Abdel-Aal et al. 2006; Kim et al. 2011; Zhu et al. 2010). The pericarp pigmentation of black rice requires two genes, PURPLE PERICARP A (Pp, Prpa and Prp1) and PURPLE PERICARP B (Pb, Prpb and Prp2) located on chromosomes 1 and 4 respectively (Hu et al. 1996; Oryzabase, www.gramene.org; Wang and Shu 2007; Wang et al. 2009; Yoshimura et al. 1997). The Pp gene acts in a complementary fashion with the Pb gene for the production of purple pericarps in rice (Hsieh and Chang 1964; Wang and Shu 2007). The presence of at least a dominant Pb allele is an essential factor for colour development in rice pericarps. The Pp allele in rice is incompletely dominant to the recessive pp allele; thus, the number of dominant Pp alleles determines the concentration of cyanidin-3-O-glucoside in black rice (Rahman et al. 2013). R/B homolog genes have been reported to be involved in the regulation of anthocyanin biosynthesis (Hu et al. 1996, 2000) in rice. Only two loci for the red rice trait have been reported; Rc, which encodes a bHLH transcription factor, and Rd, which encodes dihydroflavonol-4-reductase (DFR) (Sweeney et al. 2006; Furukawa et al. 2007). Red rice accumulates tannins in pericarp/testa. The loss of function of two genes, Rd and Rc, changed the colour to white during rice domestication. Black rice is likely to be related to the red rice but accumulates anthocyanins. Up-regulation of *LDOX* in pericarp/testa due to a gain of function event in *kala4* played a pivotal role for the creation of black rice, and the origin of the functional allele of *kala4* occurred in tropical *japonica* rice (Oguchi et al. 2012).

A whole genome survey of the introgression line using DNA markers suggested three regions on chromosomes 1, 3 and 4 are associated with black pigmentation. The locus on chromosome 3 has not been identified previously. These loci are named as Kala1, Kala3 and Kala4 (Maeda et al. 2014). Lee et al. (2015) used a 135 K rice microarray to identify genes involved in anthocyanin biosynthesis and metabolism in black rice. Using multi-interaction screening method, they screened 427 expression genes, 754 orthologous genes, and 416 pathway-network related genes associated with anthocyanin biosynthesis. Eight candidate genes were also identified by comparing pathway expression genes including ortholog-ontology genes. Transcription factor is related to anthocyanin pigmentation biosynthesis. All black rice strains carry Wx (b) allele and the duplication of the 23 bp sequence motif in the exon 2 of the wx gene. This evidence is a common phenomenon in glutinous rice (Prathepha 2007). To better understand the functional characterization of anthocyanin gene expression in black rice, Kim et al. (2010) performed a detailed computational examination. They identified 12 unknown and hypothetical genes involved in anthocyanin biosynthesis. These genes likely play either a regulatory role in the anthocyanin production process or are related to anthocyanin metabolism during flavonoid biosynthesis. The sequence of the OSB1 gene in black rice was found to differ from that in red and white rice. The sequence of the Rc gene in red rice also differed from that in white and black rice (Lim and Ha 2013). The Os01g0781600 and Os01g0748150 genes were highly induced during the early heading stage, suggesting that these genes may play a role in anthocyanin production in the early black rice heading stage (Kim et al. 2010). Black rice upregulation genes are Os01g0781600, Os10g0315400, Os01g0633500, Os08g0389700, Os01g0615050, Os01g0959100, and Os01g0748150 and down regulation genes are Os01g0246400, Os02g0113800, actin (Kim et al. 2010).

The genetic mechanism involved in a transition from the black coloured seed hull of the ancestral wild rice (*Oryza rufipogon* and *Oryza nivara*) to the straw white seed hull of cultivated rice (*Oryza sativa*) during grain ripening remains unknown. Zhu et al. (2011) reported that the black hull of *O. rufipogon* was controlled by the Black hull4 (Bh4) gene, which was fine mapped to an 8.8-kb region on rice chromosome 4 using a cross between *O. rufipogon* W1943 (black hull) and *O. sativa indica* cv Guangluai 4 (straw-white hull). Bh4 encodes an amino acid transporter. A 22-bp deletion within exon 3 of the bh4 variant disrupted the Bh4 function, leading to the straw white hull in cultivated rice. Transgenic study indicated that Bh4 could restore the black pigment on hulls in cv Guangluai 4 and

Kasalath. Bh4 sequence alignment of all taxa with the outgroup *Oryza barthii* showed that the wild rice maintained comparable levels of nucleotide diversity that were about 70 times higher than those in the cultivated rice. The results from the maximum likelihood Hudson-Kreitman-Aguade test suggested that the significant reduction in nucleotide diversity in rice cultivars could be caused by artificial selection. Zhu et al. (2011) proposed that the straw-white hull was selected as an important visual phenotype of non shattered grains during rice domestication.

The rice cultivar show great diversity in genetic background, composition, granule size and thermal and rheological properties of starches (Sodhi and Singh 2003; Xia et al. 2006). The starch separated from the rice varies significantly in its composition during rice grain development (Ashoka et al. 1985). Black rice varieties are represented in two categories: grain with purple pigmentation on glumes and various colour shades on the pericarp and grains with yellow straw glumes and purple pericarp. In most cases the purple pericarp colour is associated with purple glumes. Based on two growing condition for black rice, rainfed lowland and rainfed upland, chloroplast DNA type is distinct from each other. All rice strains from rainfed lowland is deletion plastotype, but all other rainfed upland strains are non-deletion types (Prathepha 2007) (Table 2.1, Fig. 2.3).

## 2.4 Health Benefits

American Health Association, the American Cancer Society and the 2005 Dietary Guidelines for Americans recommended an increase in the consumption of black rice to prevent heart disease and certain kinds of cancers (USA Rice Federation 2008). Moreover, the US Food and Drug Administration have recognized black rice as a healthy whole grain capable of reducing the risk of certain diseases. Unfortunately, it is not well accepted among many ones since it is difficult to cook and because of its distinct off taste, dark appearance and hard cooked rice texture. Sprouted brown, red and black rice contain anthocyanins, an antioxidants found in blueberries, grapes and acai that have been linked to a decreased risk of heart disease and cancer (USA rice Federation). Research suggests that plant antioxidants which mop up harmful molecules can help to protect arteries and prevent the DNA damage that leads to cancer (www.independent.co.uk).

Black rice contains many vitamins and minerals, including iron, vitamin A and vitamin B, which are beneficial for overall health and the prevention of heart disease (Chen et al. 2003). The health benefits of black glutinous rice have recently been reported by several investigators. A recent report showed that anthocyanin supplementation in humans improves LDL and HDL levels (Qin et al. 2009) and can delay cancer development in rodents models of carcinogenesis (Thomasset et al. 2009). Black rice may have antiatherogenic activity and may improve certain metabolic pathways associated with diets high in fructose (Guo et al. 2007). These marked health benefits have been attributed to the antioxidant properties of anthocyanin (Wu and Prior 2005). Reactive free radicals contribute to the

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Categories	Proteins	Species	Gene names	Locus IDs	References
Structural genes	Chalcone synthase (CHS)	Maize	Colored aleurone2 (C2) <sup>a</sup>	GRMZM2G151227 (Chr.2)	Wienand et al. (1986)
		Rice	OsCHS1 <sup>b</sup>	Os11g0530600	Reddy et al. (1996)
			OsCHS2 <sup>b</sup>	Os07g0214900	Shih et al. (2008)
	Chalcone isomerase (CHI)	Maize	CHI1 <sup>b</sup>	GRMZM2G155329	Grotewold and Peterson
				(Chr.1)	(1994)
		Rice	OsCHI <sup>b</sup>	Os03g0819600	Druka et al. (2003)
	Flavanone 3-hydroxylase (F3H)	Maize	F3H <sup>b</sup>	GRMZM2G062396 (Chr.2)	Deboo et al. (1995)
		Rice	OsF3H-1 <sup>b</sup>	Os04g0662600	Kim et al. (2007)
			OsF3H-2 <sup>b</sup>	Os10g0536400	
			OsF3H-3 <sup>b</sup>	Os04g0667200	
	Flavanone 3'-hydroxylase (F3'H)	Maize	Purple aleurone1 (Pr1) <sup>a</sup>	GRMZM2G025832 (Chr.5)	Sharma et al. (2011)
		Rice	OsF3'H <sup>b</sup>	Os10g0320100	Shih et al. (2008)
	Dihydroflavonol reductase (DFR)	Maize	Anthocyaninless1 (A1) <sup>a</sup>	GRMZM2G026930 (Chr.3)	O'Reilly et al. (1985)
		Rice	Rd <sup>a</sup>	Os01g0633500	Furukawa et al. (2007)
	Leucoanthocyanidin dioxygenase (LDOX)	Maize	Anthocyaninless2 (A2) <sup>a</sup>	GRMZM2G345717 (Chr.5)	Menssen et al. (1990)
		Rice	OsANS1 <sup>b</sup>	Os01g0372500	Shih et al. (2008)
			OsANS2 <sup>b</sup>	Os06g0626700	
	UDP-glycosyl transferase (UGT)	Maize	Bronze1 (Bz1) <sup>a</sup>	GRMZM2G165390	Dooner and Nelson
				(Chr.9)	(1977)
		Rice	OsUGT <sup>b</sup>	Os06g0192100	Tanaka et al. (2008)
					(continued)

Table 2.1 Anthocyanin biosynthesis-related genes in rice and maize

Categories	Proteins	Species	Gene names	Locus IDs	References
Regular genes	Basic helix-loop-helix transcriptional factor (bHLH)	Maize	Red (R) <sup>a</sup>	GRMZM5G822829 (Chr.10)	Styles et al. (1973)
			Booster (B) <sup>a</sup>	GRMZM2G172795 (Chr.2)	Chandler et al. (1989)
		Rice	OSB1 <sup>b</sup>	Os04g0557800	Sakamoto et al. (2001)
			OSB2 <sup>b</sup>	Os04g0557500	
	R2R3-Myb transcriptional factor (Myb)	Maize	Colored aleurone1	GRMZM2G005066	Paz-Ares et al. (1987)
			$(C1)^{a}$	(Chr.9)	
			Purple plant (Pl) <sup>a</sup>	GRMZM2G701063	Cone et al. (1993)
				(Chr.6)	
		Rice	OsC1 <sup>b</sup>	Os06g0205100	Saitoh et al. (2004)
	WD40 repeat	Maize	Pale aleurone color1	GRMZM2G058292	Carey et al. (2004)
			(FACI)	(CIII.)	
		Rice	Unidentified		
<sup>a</sup> These genes We	ere genetically identified in maize or rice				

Table 2.1 (continued)

<sup>a</sup>These genes were genetically identified in maize or rice <sup>b</sup>These genes were identified based on sequence similarity with other plant species. Although there are many homologous genes annotated in both genomic



#### 2.4 Health Benefits

Fig. 2.3 Schematic representations of anthocyanin and PA biosynthetic pathways in rice. a Pathway for anthocyanin and PA biosynthesis. The first committed step is catalyzed by chalcone synthase (CHS) using malonyl CoA and 4-coumaroyl CoA as substrates. Enzymes are as follows: acetyl CoA carboxylase (ACCase), anthocyanidin synthase (ANS), anthocyanidin reductase (ANR), chalcone isomerase (CHI), dihydroflavonol 4-reductase (DFR), flavanone 3-hydroxylase (F3H), flavonoid 30 hydroxylase (F30H), flavonol synthase (FLS), leucoanthocyanidin reductase (LAR), O-methyltransferase (OMT), rhamnosyl transferase (RT) and UDP glucose: flavonoid-3-O-glucosyltransferase (UFGT). b Proposal for anthocyanin and PA regulation. Either MYB or bHLH transcriptional factors may bind the promoter region and control the expression of anthocyanin and PA biosynthetic genes. Biosynthetic steps that remain unknown are indicated by a question mark (Lim and Ha 2013)

development of chronic inflammatory proliferative diseases (CIPDs) (Ishihara and Hirano 2002), particularly arteriosclerosis and cancer by causing oxidative damage to essential enzymes, cells and tissues (Klaunig and Kamendulis 2004; Namsh et al. 2006). Especially very high anthocyanin content with superior antioxidant in sticky black rice help to prevent the harmful effects of free radicals (Tananuwong and Tewaruth 2010). The anthocyanins in rice act as antioxidants which can inhibit inflammation throughout the body (Tsuda et al. 1996), act as anticancer agents (Kamei et al. 1995; Martin et al. 2003; Hyun and Chung 2004; Zhao et al. 2004; Chen et al. 2006), promote blood circulation, slow damage and aging of tissues, reduce cholesterol and blood sugar levels (Tedesco et al. 2001; Hirunpanich et al. 2005; Rechner and Kroner 2005), affect pituitary gland function, inhibit gastric acid secretion and inhibit platelet aggregation (Butelli et al. 2008). Defa and Meizu (2006) showed the presence of nutrients especially in sticky black rice as fiber, protein, essential amino acids, B vitamins, minerals etc. that stand out from the others are completely beneficial to human health. The use of food products from rice gave up germs that help to prevent headaches, relieve symptoms of constipation, prevent colon cancer, adjust blood sugar levels, prevent heart disease, lower blood pressure and prevent Alzheimer's disease (Kayahara and Tsukahara 2000). Young and Kim (2007) also found that the antioxidant activity of the extract from rice germ is 1.3–1.6 times higher than the regular white rice. It is advised that rice germs have the ability to improve mental health and immunity in women who are breast feeding (Shigeko et al. 2007). A study was conducted in China on the nutritional composition of sticky rice as "A study on special nutrient of purple glutinous rice", and Defa found that the presence of nutrients especially in sticky rice as fiber, protein, B vitamins, minerals (Ca, P, Fe, etc.) are much higher than ordinary rice and completely beneficial to human health (Defa and Meizu 2006). A number of studies have showed black rice compounds can reduce low-density lipoprotein cholesterol (LDL), improve lipid profiles, have anti-inflammatory and antioxidative activities, may help to fight heart disease and prevent diabetes (Guo et al. 2007).

Free radicals are unstable and highly reactive molecules. It is normal for bodies to produce these free radicals in small amounts. However many factors like metabolic stress and UV radiation can increase the formation of these free radicals. Free radicals cause oxidative damage within the body which may eventually result

in DNA and protein damage and even cell death. Antioxidants are able to neutralise these free radicals, and can help to prevent oxidative damage. Studies show that antioxidant supplementation can exert a preventative effect against the development of serious conditions like cancer, and may improve overall health. Antioxidant supplementation has been shown to lower markers of inflammation in the body. Inflammation has been a subject of significant research interest in recent times, because it is heavily involved in the pathology of serious conditions including diabetes and cardiovascular disease. Aside from anthocyanins, black rice is rich in tocopherols, another type of powerful antioxidant which is better known collectively as vitamin E. Recent research has shown that antioxidants may work synergistically, meaning that foods containing two or more types of antioxidant may deliver greater health benefits than the sum of each antioxidant alone. Body builders work very hard and place a lot of stress on their bodies. Unfortunately strenuous exercise is known to generate a lot of free radicals. The antioxidants in black rice may assist in neutralising these damaging oxygen species. This not only has general preventative health benefits, but the high antioxidant levels also have the potential to improve post training recovery through their role in reducing inflammation. Additionally, B vitamins, iron and potassium are essential to muscle building. Additionally, 2-(3,4-dihydroxyphenyl)-4,6-dihydroxybenzofuran-3-carboxylic acid methyl ester and 4-carbomethoxy-6-hydroxy-2-quinolone in black rice display antioxidative activity in a 1,1-diphenyl-2-picrylhydrazyl free-radical scavenging assay (Chung and Woo 2001).

Black rice is one of the most healthy food types of today. It is packed with a wide array of nutrients. Aside from being an extremely rich source of nutrients, black rice is comparetively cheaper and lower in sugar than other superfoods like berries. 21st century food scientists have discovered that black rice has many health benefits. Knowing its health benefits, black rice was first introduced to the United States in 1995. Black rice (*Oryza sativa* L. var. *japonica*) has been used in folk medicine in Asia (Sim et al. 2007). Black rice varieties also have historically been used in Chinese medicine. This rice is high in nutritional value and contains 18 amino acids, iron, zinc, copper, carotene, and several important vitamins. Thus black rice is panacea.

## 2.5 Pecularity of Black Rice

Black rice varieties have been the subject of exploratory research for its potential biomedical applications. This rice contains an antioxidant, the anthocyanins which are also found in dark hued fruits such as acai berries, blueberries, blackberries, dark grapes and dark cherries. That's why black rice is darker in colour than other rices. Anthocyanins are linked with better heart health, cancer prevention, relieving inflammation, and increasing memory. One tablespoon of black rice contains the same or more anthocyanins than the same amount of blueberries. This makes it a stellar addition to the diet in place of other rices. White rice is digested rapidly

because it is fibre free. Even anybody eat a large amount of white rice, he will soon feel hungry again which causes him to snack and which would likely lead to weight gain. But black rice is digested slowly because of its high fibre content and a man feel hungry with longer time. This rice is naturally high in iron that causes the dense purple colour, and it is also high in fiber, since the bran is left on the rice. A research on physicochemical properties of black rice and white rice varieties were investigated; black rice varieties showed a higher amount of minerals, faster hydrolysis rate, and lower blue value than the ordinary white rice. This study illustrated the wide variation in the physicochemical properties of the black rice varieties analyzed (Kang et al. 2011). Frank et al. (2012) reported that black rice exhibited, in particular, higher levels of fatty acid methyl esters, free fatty acids, organic acids and amino acids compared to non-coloured and red rice. Some major features of this rice are as follows:

## 2.5.1 Rich in Iron

Black rice has higher iron content and is excellent for those concerned about getting enough iron on a plant based diet. Iron is essential for healthy red blood cell production, for energy expenditure, and even for digestion.

## 2.5.2 Rich in Protein

Black rice is also higher in protein than other sources of rice. It contains 8.5 grams of protein per cup. It can help keep full, and satisfy rice craving. Getting enough protein is not hard to do on a plant based diet since so many plant based foods contain high quality protein.

#### 2.5.3 A Naturally Gluten Free Grain

Black rice naturally consists of no gluten (protein present in all wheat, rye and barley products). It is approximated that 1 in 7 individuals are responsive to gluten whether they are aware of it or not. But still test negative for celiac disease. After consuming something along with gluten in it, those with a gluten sensitivity suffer most of the same symptoms as individual with ciliac disease (a confirmed allergy to gluten), which includes bloating, constipation, diarrhea, nutrient deficiencies, as well as an increased risk for developing leaky gut syndrome. Consuming black rice might help remove digestive problems related to consuming gluten for most people (www.healthbenefitstimes.com).

# 2.5.4 Black Rice Versus Blueberries

A new healthy rice food on the horizon is the black rice. Emerging research shows that black rice is even richer in antioxidants and is a valuable phytochemicals than blueberries. The special value of the antioxidants in black rice is that they are both water soluble and fat soluble. Vegetables and fruits like blueberries are rich in the water soluble antioxidants (vitamin C), while soybeans contain fat soluble antioxidants (vitamin E). Black rice is exceptional because it contains a rich mixture of both classes of antioxidants (www.circ.ahajournals.org).

# 2.5.5 Power Up

Black rice is rich in antioxidants particularly anthocyanin which can reduce risk of infection, heart attack and cancer. Anthocyanin is concentrated in the kernels of black rice bran outer layer and gives it dark color. A 1/3 cup serving has as much fiber as a full cup of brown rice, so it is great for digestive system. Black rice is rich in vitamin E (which strengthens the immune system and promotes healthy skin and eyes), iron (which helps make red blood cells), manganese (which aids the nervous and reproductive systems) and other minerals.

# 2.5.6 Delicious

Black rice is delicious and much more flavorful than white rice. It has a naturally sweet flavor but does not contain sugar. The benefits of black rice compared to white rice is that the fibers will help slow down the starches in the rice which is much better for blood sugar maintainance and helpful for preventing Type 2 diabetes.

# 2.5.7 Affordable

It is affordable. It is much better to spend 5 dollar more for rice that will keep you full, nourished and healthy than one that's lower in nutrients like processed white minute rice.

# 2.5.8 Easy to Prepare

Black rice can be prepared like any other rice. It do not need any sophisticated ingredinets thus it is easy to prepare.

## 2.5.9 Taste and Smell

Black rice has magical aromas. It has deliciously nutty taste, soft texture and a beautiful deep purple colour that makes it a striking presence in any dish. Black rice is praised globally for their health benefit which makes it the perfect rice selection for the best health return.

## 2.6 Popularity of Black Rice

Within the past 100 years, the nutrient content of foods has been declined significantly. It is due to the depletion of soils caused by chemicals and heavy agricultural methods. The loss of vital minerals of farm soil due to soil depletion has an untold effect on nutrient content of food. Similarly, a move towards hybrid varieties (HYV) that were developed to optimize yields (without concerning nutrient value of food) has diminished the overall nutrition of food. Unhealthy eating habits and an over reliance on heavily processed foods and refined grains has taken a toll on everyone health. The rise in chronic diseases such as heart disease, cancer and diabetes, as well as the alarming increase in obesity rates are all indicators of this negative trend. Scientific research has confirmed a diet rich in whole grains, fruits, vegetables and high quality proteins is an important factor in maintaining overall good health. Either selections of nutrient dense lands whose soils are rich in nutrients than other lands or growing nutrient packed rice landraces in organic way make rice naturally more healthful. Traditional and heirloom varietals are naturally more nutritious than high yielding varietals. Black rice is cherished for its exceptional nutritional value, as well as its sustainability. Other rice variety can not meet nutrient content compared to black rice though it is grown in simple organic land.

Human micronutrient deficiencies are relatively severe in areas where rice is the major staple. Often, calorie demand is met by an increase in rice without a corresponding increase in other foods such as legumes or fish. Increasing the density of provitamin A carotenoid, iron, and zinc in rice can alleviate these deficiencies, especially among the urban and rural poor people who have little access to alternatives such as enriched foods and diversified diets. Research is under way to fortify rice with micronutrients in areas where these are inadequate in the diet. It is still debated whether such micronutrient increases in the endosperm are sufficient to significantly affect human nutrition (Ricepedia). Besides these fortified rice, there is a hope on black rice which is a rich source of many micronutrients and full of antioxidants. Thus black rice is a remedy of fatigue health.

Though the popularity of black rice and knowledge about its health benefits is growing in western nations including the US, it still remains much less popular than white and brown rice. As more people learn about black rice's benefits and demand it, black rice will probably become more widely available at larger chain supermarkets and restaurants. Today this type of rice is picking up popularity and popping up in more health food stores across the US, Australia, and Europe, as people discover the numerous health benefits that whole grain black rice has to offer. Black rice has experienced times when it nearly disappeared because of the popularity of white rice varieties, but resurgence in popularity has brought new life to this ancient and venerable grain. The "forbidden rice" of Imperial China, a scarce grain was said to be permitted only to the royalty. Today it is a guilt free Asian grain that is gaining steam because of its delicately nutty taste and its substantial health benefits. It is less sticky than white rice, less chewy than brown, and loaded with nutrients that can help to grow old and wise like Confucius. Black rice has soared in popularity in recent years as consumers become aware of its high levels of antioxidants and nutrients. The amount of the antioxidant anthocyanin that is in a spoonful of black rice bran (or 10 spoonfuls of cooked black rice) is equal to that in a spoonful of fresh blueberries. When an old grain is new: "It's rich colour, aromatic nutty flavor, and high levels of antioxidants make this rice special" (www. crops.org). Black rice was initially introduced to the US in 1995.

## 2.7 Black Rice Cons, Side Effects and Negatives

There are no real drawbacks to eating black rice. It is a nutrient rich, low fat, source of long lasting carbohydrates and is tasty and versatile. The only negative mounting evidence is that the benefits of antioxidant consumption may be overstated. Antioxidant consumption does not directly increase the level of antioxidant in the blood plasma as was first thought. New research suggest molecules like anthocyanins may still modulate oxidative damage but in different ways. One of the more popular theories is that plant flavonoids like anthocyanins do not act directly, but work as signalling molecules telling certain cells in the body to alter their expression of particular genes. It is the products of these genes which then exert protective effects against free radicals in the body. The inhibitor of tyrosinase activity in black rice bran has been investigated and ethyl acetate extract is found to be the most potent inhibitior against tyrosinase activity by 80.5 % at a concentration of 0.4 mg/mL (Miyazawa et al. 2003).

## 2.8 Ways to Call Black Rice in Different Languages

Africa	swart rys
Albanian	oriz i zi
Arabic	أسود الأرز
Armenian	Սեւ բրինձ
Azerbaijani	qara düyü
Basque	Arroz beltza

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Belarusian	чорны рыс
Bulgarian	черен ориз
Catalan	arròs negre
Chinese	黑米
Croatian	crna riža
Czech	černá rýže
Danish	sorte ris
Dutch	zwarte rijst
Estonian	must riis
Filipino	itim na bigas
Finnish	musta riisi
French	riz noir
Galician	arroz negro
Georgian	შავი ბრინჯით
German	Schwarzer Reis
Greek	Μαύρο ρύζι
Haitian creole	nwa diri
Hebrew	אורז שחור
Hindi	काले चावल
Hungarian	fekete rizs
Icelanding	svartur hrísgrjón
Indonesian	beras hitam
Irish	rís dubh
Italian	riso nero
Japanese	黒米
Korean	검은 쌀
Latvian	melns rīsi
Lithuanian	juodųjų ryžių
Macedonian	црн ориз
Malay	beras hitam
Maltese	iswed ross
Nepali	काले चावल
Norwegian	sort ris
Persian	برنج سياه و سفيد
Polish	czarny ryż
Portugese	arroz preto
Romanian	negru de orez
Russian	черный рис
Serbian	црна рижа
Slovak	čierna ryža
Slovenian	črni riž

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Spanish	arroz negro
Swahili	mchele mweusi
Swedish	svart ris
Thai	ข้าวสีดำ (kao neow dom)
Turkish	siyah pirinç
Ukrainian	чорний рис
Urdu	سياه چاول
Vietnamese	màu đen gạo
Welsh	reis du
Yiddish	שוואַרץ רײַז

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# Chapter 3 History

# 3.1 History of Black Rice

The origin of black rice is not well understood but it is said that black rice might be originated from many Asian countries including China (Mingwei et al. 1995; Hoahua et al. 1996; Gu and Xu 1992), India (Sastry 1978), Japan (Natsumi and Noriko 1994), and Vietnam (Quan 1999). Chaudary and Tran (2001) reported that black rice might be originated from Sri Lanka, the Philippines, Bangladesh, Thailand, Myanmar, and Indonesia. China is the richest country in black rice genetic resources (62 %) followed by Sri Lanka (8.6 %), Indonesia (7.2 %), India (5.1 %), the Philippines (4.3 %), Bangladesh (4.1 %), and few in Malaysia, Thailand, and Myanmar (Chaudhary 2003). Black rice was known long before Chinese dynastic times. During the Warring States Period (476-221 BCE), a different tale of its use was told. One General Sun Bin was caged, reasons were unclear, and he survived because he ate black rice balls. In Hangzhou, where this General came from, they honor him by eating black rice on the first day of Chinese summer (www.flavorandfortune.com). According to China legend, glutinous rice was used as a mortar compound in the construction of the Great Wall of China (www.blackrice.com) (Fig. 3.1).

Black rice is commonly known as "forbidden rice." The name forbidden rice implies consuming this rice without approval from the proper authorities can have life-threatening consequences for those involved. In ancient China, black rice was set specifically for the Emperor or the Royal family. Common people were not allowed anywhere near the stuff. Really incredible fact about black rice is that it contains even more antioxidants (per serving) than blueberries which are famous for their antiaging properties.

The Emperor says, "Hands off the black rice! It's mine."

According to ancient Chinese legend, black rice was so rare, tasty, and nutritious that only the emperors were allowed to eat. Times have changed and now available



**Fig. 3.1** Traditional rice balls prepared from white, red, and black varieties of rice (Oikawa et al. 2015)

to everywhere. Although black rice is still relatively rare, researchers are trying to bring its distinctive flavor and mixture of antioxidants to the masses (www.cnn.com). Emperors reserved this wonderfully nutty rice for their own consumption because it was thought that it would extend their lives. It is highly likely that the emperors down throughout the ages probably shared their black rice stock with their consorts or concubines.

Chinese mythology about the origin of black rice advises that people acquired this rice as a gift from an animal. This tale is of a tail and it took place in an era when many people were starving. The story is that many people saw a dog coming at them through the fields. They noted long seeds hanging from its tail. While some folks did grab its tail and eat the seeds, others though hungry thought ahead and planted some or all of them. It is from these seeds that rice grew, and their forever hunger abated. This myth does not discuss where this happened or when, nor does it indicate what color or kind of rice this was. There is no notion if it looked brown, white, red, purple, or black.

Chinese black rice is sometimes called fortune rice. It is also known as emperor's rice. It gets these names because it was rare and was used as a tribute food. In early dynastic times, it was given to the emperor and stayed popular as a tribute food through the Tang Dynasty which began in 618 CE and into the Sung which started in 960 CE. It was used earlier, but since ordinary people did not, should not and could not eat it. When China was ruled by an emperor, black rice was dubbed as the "Forbidden Rice." In later times, it was known as Lover's rice as the thought was that it sticks two lovers together for life. In early days, even though only the emperor and his court were allowed to eat this black rice, some young folk did sneak and eat it. However, it was not until later when it became generally available to the masses that this rice gained some protocols. For example, it was customary to consume this black rice in the fourth lunar month and on sweeping the Graves Festival Day, which is the eighth day, and on holidays when courting was common practice. It was cultivated in very small amounts because it was only for the emperor's consumption. Although, no such ban is being practiced nowadays. Black rice is still very much produced in low amounts, especially when compared to brown and white rice. Ancient emperors in China hoarded black rice for the reason that they believe it would lengthen their lives and secure their thrones longer. They may have been right, as research now shows that black rice is one of the world's best super foods.

Noted historian William Hesseltine wrote:

Writing the history of the "black rice" is like looking for a needle in a haystack. I have not seen any old manuscript that tells the story of the "black rice."

There are a number of myths about its origin, one of which had been used by a few enterprising resort owners as a come on for sightseeing tours. A garrison for Spanish soldiers was built in Muelle. The Spaniards built watch towers in Dampalitan Point, near Coco Beach to sound off warships patrolling Puerto Galera Bay against Moro pirates. The bay had been used extensively by Spanish galleons and Chinese merchant ships that sought refuge and traded with the natives for food and other resupply necessities. The rugged topography of Puerto Galera made it essential for the Spaniards to build a warehouse for palay (rice) adjacent the barracks. Farmers from farflung villages brought their sacks of palay to the warehouse to be stored until a ship would come to collect them. One night the warehouse caught fire and was burned to the ground along with an unknown number of sacks of palay. The garrison also did not survive the fire. The blackened grains were scattered all over the banks of Muelle and were preserved for more than 200 years and now known as the "Black Rice of Muelle." Similarly, there is another myth. An old woman appeared one day and begged for food from the soldiers manning the garrison. The warehouse was full of palay but the soldiers refused to give the old hag any food. And so, it was said that the old woman uttered a curse against the soldiers and that very night a fire broke out and burned the garrison and the warehouse to the ground along with the Spanish soldiers. The grains remain in Muelle to serve as a grim reminder of the fate of the greedy officials of the past; it can be a warning to abusive officials of the present to mend their ways or suffer the deathly consequence (Lineses 2006).

It is reported that black rice was cultivated by ancient empires in the Far East dating back to 150 B.C. According to legend, only Chinese emperors were allowed to eat this prized rice. Ancient civilizations believed that black rice was rich in nutrients that protected their emperors from illness and increased their longevity. Black rice was grown in small quantities exclusively for the emperors and nobility who even then knew of its health benefits. During China's Ming dynasty, possession of black rice by a common people was a capital crime. Today black rice is still grown in relatively small quantities, mostly on family-owned farms in China, the Philippines and throughout Asia. Emperors believe that it has something "super" that would help them lengthen their lives thus, lengthens their reign. True to its history, it must really have something that preserves the emperor's lives. Recent studies show that black rice is rich in antioxidants, much more than blueberries which are famous for their anti-aging properties. Although its history traces back to various parts of Asia, it has been referred to as "forbidden rice" because according to legend, regular people could be killed if caught with black rice without the approval of the proper authorities (www.Epicurious.com). Black rice is very popular foods with Europeans, who consume more than South Asians (Simmons and Williams 1997). In Vietnam, the local inhabitants probably planted black rice as long as 4000 years ago in the time of the Hung dynasty (Nguyen 2001).

## 3.2 Discovery of Forbidden Rice

Black rice is a highly treasured rice variety originated from Asia. It has been said that in ancient China, black rice was number one foods that were set aside only for the emperor and the royal family due to their quality or health benefits. No one else was allowed to eat these foods without approval from the Emperor. Life-threatening consequences were for those caught eating these foods without permission. Therefore, black rice is commonly referred to as "forbidden rice." All rice varieties are a substantial and important crop in the diet of nearly every Asian population, and have been practiced for thousands of years. Thousands of years ago in ancient China, noble Chinese men took possession of every grain of the black forbidden rice, banning its consumption among anyone who was not royalty or very wealthy. The crop was only grown in very limited quantities, closely monitored, and reserved for only the highest elite class. The common Chinese people were not allowed to grow or consume this black forbidden rice which led to its unique name that it still carries with it today.

Black rice was first introduced to the United States in the 1995s, although it has been enjoyed in other parts of the world for many years. Today it is no longer forbidden, but still is grown in relatively small amounts compared to other types of widely available rice varieties. Sometimes it is identified on the bulk bins of speciality food markets as Indonesian black rice, black glutinous, or sweet rice and is widely consumed in many of the Indonesian islands. Many Southeast Asian countries also cultivate and consume their fair share of this tasty whole grain. Royals and the regulars can now enjoy its health benefits without one being hanged or put to death. Even Chinese people themselves do not eat much of it nowadays, and only eat it on very rare occasions. Black rice's popularity swelled after TV personality Mehmet Oz, MD, who praised this rice to his national audience. Based on historical record, black rice was only for the Kings of China and Indonesia. This is because black rice has a double function, namely as a source of staple food with good taste, fluffier and fragrance, as well as an efficacious medicine to cure various illnesses (Kristamtini 2009).

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# Chapter 4 Nutrition Profiles of Black Rice

Black rice is truly remarkable in its abilities which are loaded with antioxidants, vitamin E, fiber, and valuable anti-inflammatory properties. Black rice anthocyanins are extracted from the aleurone layer of rice seed which is a major cereal crop existing since ancient times in China and other Eastern Asian countries (Ling et al. 2002). Little is known about the phytochemical profiles and antioxidant activities of different black rice varieties. Research shows that there are significant differences in phytochemical content and antioxidant activity among the different black rice varieties (Zhang et al. 2010). One half cup serving of cooked or about one-fourth cup uncooked black rice contains approximately (in daily recommended values) (www.blackrice.com).

- 160 cal (669 kJ) carbohydrates,
- 1.5 g of fat,
- 34 g of carbohydrates,
- 2 g of fiber, and
- 7.5 g of protein.

Black rice has a number of nutritional advantages over common white rice such as higher protein content, higher vitamins and minerals, although the letter varies with cultivar, production location and production technique (Suzuk et al. 2004). One serving of black rice contains only around 160 cal, but offers a high amount of flavonoid phyto nutrients, a good source of important fiber, substantial mineral content including iron and copper, and even a good source of plant based protein. This rice has the highest levels of anthocyanin, an antioxidants found in any food. Black rice is a healthy source of minerals especially iron. It contains vitamin E and is lower in sugar than berries that have similar phytochemical qualities. Black rice is fiber rich and nutritionally similar but not identical to whole grain brown rice. It contains 18 amino acids, minerals and vitamins like copper, iron, zinc, and carotene.

It is estimated that 50 g of black rice provides about 35 % of the recommended dietary allowance of Se, Cu, Zn, and Mn per day.

"one spoonful of black rice bran contains more anthocyanin antioxidants than a spoonful of blueberries and better yet, black rice offers more fiber and vitamin E antioxidants, but less sugar." 240th National Meeting of the American Chemical Society (ACS).

## 4.1 Nutrition of Different Rice Types

Different rice types are available today and the most common one is the white rice. Out of all the types, black rice is the one that contains the highest amount of nutrition. A comparison of different rice types that differ in terms of nutrient content when compared with 100 g serving of each kind (Table 4.1) (www.naturalnews.com).

The antioxidant activity of all rice bran extracts indicates high antioxidant efficiency in the following order: red>black>white color rice brans (Muntana and Prasong 2010). The Fe, Zn, Cu, and Mn concentration of the colored rice is higher than that of the white rice, and the four mineral concentrations in brown rice is Zn>Fe>Cu>Mn. In addition, Fe and Zn concentration of colored rice are affected by pigment content in pericarp of it, Cu and Mn of color rice are not influenced by pigment content in pericarp of it. Fe concentration of the colored rice is black rice>green rice>brown rice>red rice>yellow rice; Zn concentration of the colored rice is green rice>red rice>black rice>brown rice>yellow rice; Cu concentration of the colored rice is black rice>brown rice>red rice>yellow rice>green rice and Mn concentration of the colored rice is brown rice>black rice>red rice>yellow rice>green rice. There are some differences among Fe, Zn, Cu, and Mn average concentrations in brown rice. Of the different colored rice, black rice and brown rice are rich on Fe, Zn, Cu, and Mn; green rice are rich on Fe and Zn and red rice is rich on Zn and Cu. Similarly, Fe, Zn, Cu, and Mn concentrations of yellow rice are lower than that of the other colored rice (Guo 2011) (Table 4.2).

Polished white rice	6.8 proteins, 1.2 irons, 0.5 zinc, and 0.6 fibers
Brown rice	7.9 protein, 2.2 iron, 0.5 zinc, and 2.8 fiber
Purple rice	8.3 proteins, 3.9 irons, 2.2 zinc, and 1.4 fibers
Red rice	7.0 proteins, 5.5 irons, 3.3 zinc, and 2.0 fibers
Black rice	8.5 proteins, 3.5 irons, 0.0 zinc, and 4.9 fibers

Table 4.1 Nutritional composition of different rice types
Crop types	TPC	TAC	DPPH	IC50
Red rice	$0.10 \pm 0.01^{d}$	$0.05 \pm 0.01^{\rm f}$	$1.63 \pm 0.15^{\rm c}$	>1000
Purple rice	$4.62 \pm 0.18^{b}$	$1.22 \pm 0.08^{\circ}$	$30.92 \pm 1.58^{b}$	475.14 ± 25.46
Black rice	$8.58\pm0.56^{\rm a}$	$3.83 \pm 0.04^{a}$	$73.47 \pm 4.63^{a}$	$13.56 \pm 1.2$
Purple corn	$1.11 \pm 0.09^{\circ}$	$0.31 \pm 0.01^{d}$	$1.68 \pm 0.19^{\rm c}$	833.33 ± 56.31
Black barley	$0.46 \pm 0.04^{c,d}$	$0.27 \pm 0.05^{d,e}$	$2.21 \pm 0.37^{\circ}$	>1000
Black soyabean	$0.75 \pm 0.06^{c,d}$	$0.19 \pm 0.02^{\rm e}$	$4.59 \pm 0.27^{\rm c}$	>1000
Black soyabean coat	$5.26 \pm 0.42^{b}$	$1.63 \pm 0.03^{b}$	$13.94 \pm 4.86^{b}$	$111.11 \pm 21.24$

**Table 4.2** Total Phenolic Content (TPC), Total Anthocyanin Content (TAC), Anti-DPPH Radical Activity and R-Glucosidase Inhibitory of red, purple, and black rice, purple corn, black barley, black soybean, and black soybean coata (Yao et al. 2009)

<sup>a</sup>Data are expressed as mean (SD of triplicate samples. TPC is expressed as grams of GAE/100 g. TAC is expressed as milligrams of anthocyanin/gram. The anti-DPPH capacity is expressed as micromolar TE/gram. Values in the same column sharing different letters are expressed as significantly different (p < 0.05). a IC50 was expressed as mg/mL

#### 4.2 Nutritional Facts of Black Rice

Black rice provides the richest nutritional value, a higher level of vitamins, minerals, and fiber as well as a comprehensive range of amino acids, proteins, vegetable fats, and essential trace elements compared to other rices needed by the body. Finocchiaro et al. (2010) reported that on average, the pigmented rices had a TAC four times higher than the white ones.

A typical nutritional data of black rice is reported here (www.blackrice.com, www.chinese-black-rice.com) (Table 4.3).

#### 4.3 Factors Affecting Rice Nutrition

A detailed analysis of nutrient content of rice suggests that the nutrition value of rice varies based on a number of factors such as sunlight, water, soil nutrient content, organic matter content, variety, and method of rice milling etc. It also depends on the strain of rice that is between white, brown, black, red, and purple varieties of rice prevalent in different parts of the world. It also depends on nutrient quality of the soil where rice is grown, whether and how the rice is polished or processed, the manner it is enriched, and how it is prepared before consumption. Comparative nutrition studies on red, black, and white varieties of rice suggest that pigments in red and black rice varieties may offer nutritional benefits over white rice. Red or black rice consumption is found to reduce or retard the progression of atherosclerotic plaque development, induced by dietary cholesterol in mammals. White rice consumption offers no similar benefits (Table 4.4).

NutrientAmountAlanine0.437 gArginine0.569 gAsh1.27 gAspartic acid0.702 gCalcium, Ca33 mgCarbohydrate, by difference76.17 gCopper, Cu0.277 mgCystine0.001 gEnergy1515 kJEnergy362 kcalFatty acids, total monusaturated0.959 gFatty acids, total saturated0.536 gFiber, total dietary3.4 gFolate, food20 mcgGlutamic acid1.528 gGlycine0.369 gHistidine0.190 gIron, Fe1.80 mgIsoleucine0.318 gLeucine0.286 gManganese, Mn3.743 mgMathenia1.493 mgPhosphorus, P264 mgPhosphorus, P264 mgPotosinu, K268 mgProline0.352 gProline0.352 gProline0.352 gProline7.50 gRibofavin0.043 mgThron, Fianna4.13 mgThron, P264 mgProtein7.50 gRibofavin0.275 gTotal lipid (fat)2.68 gTryptophan0.096 g		
Alanine         0.437 g           Arginine         0.569 g           Ash         1.27 g           Aspartic acid         0.702 g           Calcium, Ca         33 mg           Carbohydrate, by difference         76.17 g           Copper, Cu         0.277 mg           Cystine         0.091 g           Energy         1515 kJ           Energy         362 kcal           Fatty acids, total polyunsaturated         0.971 g           Fatty acids, total polyunsaturated         0.536 g           Fiber, total dietary         3.4 g           Folate, DFE         20 mcg_DFE           Folate, food         20 mcg           Glutamic acid         1.528 g           Glutamic acid         1.528 g           Glycine         0.369 g           Histidine         0.190 g           Iron, Fe         1.80 mg           Isoleucine         0.620 g           Lysine         0.286 g           Magnesium, Mg         143 mg           Magnesium, Mg         143 mg           Magnesium, Mg         143 mg           Photsphorus, P         264 mg           Potasium, K         266 mg           Proline	Nutrient	Amount
Arginine         0.569 g           Ash         1.27 g           Aspartic acid         0.702 g           Calcium, Ca         33 mg           Carbohydrate, by difference         76.17 g           Copper, Cu         0.277 mg           Cystine         0.091 g           Energy         1515 kJ           Energy         362 kcal           Fatty acids, total monounsaturated         0.971 g           Fatty acids, total polyunsaturated         0.959 g           Fatty acids, total aburated         0.536 g           Fiber, total dietary         3.4 g           Folate, DFE         20 mcg_DFE           Folate, food         20 mcg           Glycine         0.369 g           Histidine         1.190 g           Iron, Fe         1.80 mg           Isoleucine         0.286 g           Magnesium, Mg         143 mg           Mangarese, Mn         3.743 mg           Methionine         0.169 g           Niacin         4.308 mg           Patoshenic acid         1.493 mg           Phenylalanine         0.387 g           Phosphorus, P         264 mg           Potine         0.352 g           <	Alanine	0.437 g
Ash         1.27 g           Aspartic acid         0.702 g           Calcium, Ca         33 mg           Carbohydrate, by difference         76.17 g           Copper, Cu         0.277 mg           Cystine         0.091 g           Energy         1515 kJ           Energy         362 kcal           Fatty acids, total monounsaturated         0.971 g           Fatty acids, total polyunsaturated         0.959 g           Fatty acids, total saturated         0.536 g           Fibre, total dietary         3.4 g           Folate, food         20 mcg_DFE           Folate, food         20 mcg           Glutamic acid         1.528 g           Glycine         0.369 g           Histidine         0.190 g           Iron, Fe         1.80 mg           Isoleucine         0.620 g           Lysine         0.286 g           Magnese, Mn         3.743 mg           Methionine         0.169 g           Niacin         4.308 mg           Phosphorus, P         264 mg           Proline         0.352 g           Proline         0.352 g           Proline         0.352 g           Protine	Arginine	0.569 g
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Fatty acids, total saturated0.536 gFiber, total dietary3.4 gFolate, DFE20 mcg_DFEFolate, food20 mcgFolate, total20 mcgGlutamic acid1.528 gGlycine0.369 gHistdine0.190 gIron, Fe1.80 mgIsoleucine0.318 gLeucine0.620 gLysine0.286 gMagnesium, Mg143 mgManganese, Mn3.743 mgMethionine0.169 gNiacin4.308 mgPantothenic acid1.493 mgPhosphorus, P264 mgPotassium, K268 mgProtein7.50 gRiboflavin0.043 mgSerine0.388 gSodium, Na4 mgThraonine0.275 gTotal lipid (fat)2.68 gTryptophan0.096 g	Fatty acids, total polyunsaturated	0.959 g
Fiber, total dietary         3.4 g           Folate, DFE         20 mcg_DFE           Folate, food         20 mcg           Folate, total         20 mcg           Glutamic acid         1.528 g           Glycine         0.369 g           Histidine         0.190 g           Iron, Fe         1.80 mg           Isoleucine         0.318 g           Leucine         0.620 g           Lysine         0.286 g           Magnesium, Mg         143 mg           Manganese, Mn         3.743 mg           Naicin         4.308 mg           Pantothenic acid         1.493 mg           Phenylalanine         0.387 g           Phosphorus, P         264 mg           Potassium, K         268 mg           Proline         0.352 g           Protein         7.50 g           Riboflavin         0.043 mg           Serine         0.388 g           Sodium, Na         4 mg           Thiamin         0.413 mg           Threonine         0.275 g           Total lipid (fat)         2.68 g           Tryptophan         0.096 g	Fatty acids, total saturated	0.536 g
Folate, DFE         20 mcg_DFE           Folate, food         20 mcg           Folate, total         20 mcg           Glutamic acid         1.528 g           Glycine         0.369 g           Histidine         0.190 g           Iron, Fe         1.80 mg           Isoleucine         0.318 g           Leucine         0.620 g           Lysine         0.286 g           Magnesium, Mg         143 mg           Manganese, Mn         3.743 mg           Naicin         4.308 mg           Pantothenic acid         1.493 mg           Phenylalanine         0.387 g           Phosphorus, P         264 mg           Potsinu, K         268 mg           Proline         0.352 g           Protein         7.50 g           Riboflavin         0.043 mg           Serine         0.388 g           Sodium, Na         4 mg           Thiamin         0.413 mg           Threonine         0.275 g           Total lipid (fat)         2.68 g           Tryptophan         0.096 g	Fiber, total dietary	3.4 g
Folate, food         20 mcg           Folate, total         20 mcg           Glutamic acid         1.528 g           Glycine         0.369 g           Histidine         0.190 g           Iron, Fe         1.80 mg           Isoleucine         0.318 g           Leucine         0.620 g           Lysine         0.286 g           Magnesium, Mg         143 mg           Manganese, Mn         3.743 mg           Methionine         0.169 g           Niacin         4.308 mg           Pantothenic acid         1.493 mg           Phenylalanine         0.387 g           Phosphorus, P         264 mg           Potassium, K         268 mg           Proline         0.352 g           Protein         7.50 g           Riboflavin         0.043 mg           Serine         0.388 g           Sodium, Na         4 mg           Thiamin         0.413 mg           Threonine         0.275 g           Total lipid (fat)         2.68 g           Tryptophan         0.096 g	Folate, DFE	20 mcg_DFE
Folate, total         20 mcg           Glutamic acid         1.528 g           Glycine         0.369 g           Histidine         0.190 g           Iron, Fe         1.80 mg           Isoleucine         0.318 g           Leucine         0.620 g           Lysine         0.286 g           Magnesium, Mg         143 mg           Manganese, Mn         3.743 mg           Methionine         0.169 g           Niacin         4.308 mg           Pantothenic acid         1.493 mg           Phenylalanine         0.387 g           Phosphorus, P         264 mg           Potassium, K         268 mg           Proline         0.352 g           Protein         7.50 g           Riboflavin         0.043 mg           Serine         0.388 g           Sodium, Na         4 mg           Thiamin         0.413 mg           Threonine         0.275 g           Total lipid (fat)         2.68 g           Tryptophan         0.096 g	Folate, food	20 mcg
Glutamic acid       1.528 g         Glycine       0.369 g         Histidine       0.190 g         Iron, Fe       1.80 mg         Isoleucine       0.318 g         Leucine       0.620 g         Lysine       0.286 g         Magnesium, Mg       143 mg         Manganese, Mn       3.743 mg         Methionine       0.169 g         Niacin       4.308 mg         Pantothenic acid       1.493 mg         Phenylalanine       0.387 g         Phosphorus, P       264 mg         Potassium, K       268 mg         Proline       0.352 g         Protein       7.50 g         Riboflavin       0.043 mg         Serine       0.388 g         Sodium, Na       4 mg         Thiamin       0.413 mg         Threonine       0.275 g         Total lipid (fat)       2.68 g         Tryptophan       0.096 g	Folate, total	20 mcg
Glycine         0.369 g           Histidine         0.190 g           Iron, Fe         1.80 mg           Isoleucine         0.318 g           Leucine         0.620 g           Lysine         0.286 g           Magnesium, Mg         143 mg           Manganese, Mn         3.743 mg           Methionine         0.169 g           Niacin         4.308 mg           Panothenic acid         1.493 mg           Phenylalanine         0.387 g           Phosphorus, P         264 mg           Potassium, K         268 mg           Proline         0.352 g           Protein         7.50 g           Riboflavin         0.043 mg           Serine         0.388 g           Sodium, Na         4 mg           Thiamin         0.413 mg           Threonine         0.275 g           Total lipid (fat)         2.68 g           Tryptophan         0.096 g	Glutamic acid	1.528 g
Histidine       0.190 g         Iron, Fe       1.80 mg         Isoleucine       0.318 g         Leucine       0.620 g         Lysine       0.286 g         Magnesium, Mg       143 mg         Manganese, Mn       3.743 mg         Methionine       0.169 g         Niacin       4.308 mg         Pantothenic acid       1.493 mg         Phenylalanine       0.387 g         Phosphorus, P       264 mg         Potassium, K       268 mg         Proline       0.352 g         Protein       7.50 g         Riboflavin       0.043 mg         Serine       0.388 g         Sodium, Na       4 mg         Thiamin       0.413 mg         Threonine       0.275 g         Total lipid (fat)       2.68 g         Tryptophan       0.096 g	Glycine	0.369 g
Iron, Fe       1.80 mg         Isoleucine       0.318 g         Leucine       0.620 g         Lysine       0.286 g         Magnesium, Mg       143 mg         Manganese, Mn       3.743 mg         Methionine       0.169 g         Niacin       4.308 mg         Pantothenic acid       1.493 mg         Phenylalanine       0.387 g         Phosphorus, P       264 mg         Potassium, K       268 mg         Proline       0.352 g         Protein       7.50 g         Riboflavin       0.043 mg         Serine       0.388 g         Sodium, Na       4 mg         Thiamin       0.413 mg         Threonine       0.275 g         Total lipid (fat)       2.68 g         Tryptophan       0.096 g	Histidine	0.190 g
Isoleucine         0.318 g           Leucine         0.620 g           Lysine         0.286 g           Magnesium, Mg         143 mg           Manganese, Mn         3.743 mg           Methionine         0.169 g           Niacin         4.308 mg           Pantothenic acid         1.493 mg           Phenylalanine         0.387 g           Phosphorus, P         264 mg           Potassium, K         268 mg           Proline         0.352 g           Protein         7.50 g           Riboflavin         0.043 mg           Serine         0.388 g           Sodium, Na         4 mg           Thiamin         0.413 mg           Threonine         0.275 g           Total lipid (fat)         2.68 g           Tryptophan         0.096 g	Iron, Fe	1.80 mg
Leucine         0.620 g           Lysine         0.286 g           Magnesium, Mg         143 mg           Manganese, Mn         3.743 mg           Methionine         0.169 g           Niacin         4.308 mg           Pantothenic acid         1.493 mg           Phenylalanine         0.387 g           Phosphorus, P         264 mg           Potassium, K         268 mg           Proline         0.352 g           Protein         7.50 g           Riboflavin         0.043 mg           Serine         0.388 g           Sodium, Na         4 mg           Thiamin         0.413 mg           Threonine         0.275 g           Total lipid (fat)         2.68 g           Tryptophan         0.096 g	Isoleucine	0.318 g
Lysine         0.286 g           Magnesium, Mg         143 mg           Manganese, Mn         3.743 mg           Methionine         0.169 g           Niacin         4.308 mg           Pantothenic acid         1.493 mg           Phenylalanine         0.387 g           Phosphorus, P         264 mg           Potassium, K         268 mg           Proline         0.352 g           Protein         7.50 g           Riboflavin         0.043 mg           Serine         0.388 g           Sodium, Na         4 mg           Thiamin         0.413 mg           Threonine         0.275 g           Total lipid (fat)         2.68 g           Tryptophan         0.096 g	Leucine	0.620 g
Magnesium, Mg143 mgManganese, Mn3.743 mgMethionine0.169 gNiacin4.308 mgPantothenic acid1.493 mgPhenylalanine0.387 gPhosphorus, P264 mgPotassium, K268 mgProline0.352 gProtein7.50 gRiboflavin0.043 mgSerine0.388 gSodium, Na4 mgThiamin0.413 mgThreonine0.275 gTotal lipid (fat)2.68 gTryptophan0.096 g	Lysine	0.286 g
Manganese, Mn3.743 mgMethionine0.169 gNiacin4.308 mgPantothenic acid1.493 mgPhenylalanine0.387 gPhosphorus, P264 mgPotassium, K268 mgProline0.352 gProtein7.50 gRiboflavin0.043 mgSerine0.388 gSodium, Na4 mgThiamin0.413 mgThreonine0.275 gTotal lipid (fat)2.68 gTryptophan0.096 g	Magnesium, Mg	143 mg
Methionine0.169 gNiacin4.308 mgPantothenic acid1.493 mgPhenylalanine0.387 gPhosphorus, P264 mgPotassium, K268 mgProline0.352 gProtein7.50 gRiboflavin0.043 mgSerine0.388 gSodium, Na4 mgThiamin0.413 mgThreonine0.275 gTotal lipid (fat)2.68 gTryptophan0.096 g	Manganese, Mn	3.743 mg
Niacin4.308 mgPantothenic acid1.493 mgPhenylalanine0.387 gPhosphorus, P264 mgPotassium, K268 mgProline0.352 gProtein7.50 gRiboflavin0.043 mgSerine0.388 gSodium, Na4 mgThiamin0.413 mgThreonine0.275 gTotal lipid (fat)2.68 gTryptophan0.096 g	Methionine	0.169 g
Pantothenic acid1.493 mgPhenylalanine0.387 gPhosphorus, P264 mgPotassium, K268 mgProline0.352 gProtein7.50 gRiboflavin0.043 mgSerine0.388 gSodium, Na4 mgThiamin0.413 mgThreonine0.275 gTotal lipid (fat)2.68 gTryptophan0.096 g	Niacin	4.308 mg
Phenylalanine0.387 gPhosphorus, P264 mgPotassium, K268 mgProline0.352 gProtein7.50 gRiboflavin0.043 mgSerine0.388 gSodium, Na4 mgThiamin0.413 mgThreonine0.275 gTotal lipid (fat)2.68 gTryptophan0.096 g	Pantothenic acid	1.493 mg
Phosphorus, P264 mgPotassium, K268 mgProline0.352 gProtein7.50 gRiboflavin0.043 mgSerine0.388 gSodium, Na4 mgThiamin0.413 mgThreonine0.275 gTotal lipid (fat)2.68 gTryptophan0.096 g	Phenylalanine	0.387 g
Potassium, K         268 mg           Proline         0.352 g           Protein         7.50 g           Riboflavin         0.043 mg           Serine         0.388 g           Sodium, Na         4 mg           Thiamin         0.413 mg           Threonine         0.275 g           Total lipid (fat)         2.68 g           Tryptophan         0.096 g	Phosphorus, P	264 mg
Proline         0.352 g           Protein         7.50 g           Riboflavin         0.043 mg           Serine         0.388 g           Sodium, Na         4 mg           Thiamin         0.413 mg           Threonine         0.275 g           Total lipid (fat)         2.68 g           Tryptophan         0.096 g	Potassium, K	268 mg
Protein7.50 gRiboflavin0.043 mgSerine0.388 gSodium, Na4 mgThiamin0.413 mgThreonine0.275 gTotal lipid (fat)2.68 gTryptophan0.096 g	Proline	0.352 g
Riboflavin0.043 mgSerine0.388 gSodium, Na4 mgThiamin0.413 mgThreonine0.275 gTotal lipid (fat)2.68 gTryptophan0.096 g	Protein	7.50 g
Serine0.388 gSodium, Na4 mgThiamin0.413 mgThreonine0.275 gTotal lipid (fat)2.68 gTryptophan0.096 g	Riboflavin	0.043 mg
Sodium, Na4 mgThiamin0.413 mgThreonine0.275 gTotal lipid (fat)2.68 gTryptophan0.096 g	Serine	0.388 g
Thiamin0.413 mgThreonine0.275 gTotal lipid (fat)2.68 gTryptophan0.096 g	Sodium, Na	4 mg
Threonine0.275 gTotal lipid (fat)2.68 gTryptophan0.096 g	Thiamin	0.413 mg
Total lipid (fat)2.68 gTryptophan0.096 g	Threonine	0.275 g
Tryptophan 0.096 g	Total lipid (fat)	2.68 g
	Tryptophan	0.096 g

 Table 4.3 Nutritional data of black rice per 100 g

(continued)

Nutrient	Amount
Tyrosine	0.281 g
Valine	0.440 g
Vitamin B-6	0.509 mg
Water	12.37 g
Zinc, Zn	2.02 mg

Table 4.3 (continued)

Table 4.4 Test results of quality raw materials of sticky black rice (by the volume of dry substance) (Minh 2014)

Unit	Result
%	11.7
%	8.9
%	3.3
%	75.5
%	13.18
%	0.49
(g/1000	25.1
grains)	
%	2.12
%	0
%	95.4
	Specific colored rice
	Black purple
	Characteristic of rice, no strange smells, does not smell musty
	Unit % % % % % (g/1000 grains) % % %

# 4.4 Chemicals Present in Black Rice

Black rice is an excellent source of minerals such as iron, zinc, manganese, selenium, and magnesium. It contains substantial amounts of protein, dietary fiber, vitamin E, B vitamins, unsaturated fats,  $\gamma$ -oryzanol,  $\gamma$ -amino butyric acid (GABA), antioxidants, phenolic compounds, and other phytochemicals (Roohinejad et al. 2009). The color of black glutinous rice is caused by anthocyanins which are a group of reddish purple water-soluble flavonoids (Shen et al. 2009) located on pericarp, seed coat, and aleurone layer (Sompong et al. 2011). Anthocyanins are the main antioxidants in black glutinous rice and are composed of anthocyanidin and sugar. Six anthocyanin pigments are identified and the predominant anthocyanins are cyanidin-3-glucoside (572.47 µg/g, 91.13 % of total) and peonidin-3-glucoside (29.78 µg/g; 4.74 % of total). Minor constituents include three cyanidin-dihexoside isomers and one cyanidin hexoside (Hiemori et al. 2009). Several research show that only six types of anthocyanins, cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, delphinidin, cyanidin, pelargonidin, and malvidin are present in raw black rice bran (BRB) and black rice bran colorant powder (BCP). Zhang et al. (2006) reported four main anthocyanidins in black rice are malvidin, pelargonidin-3,5 glucoside, cyaniding-3-gluconside, and cyaniding-3,5-diglucoside. Cyaniding-3-glucoside and pelargonidin-3-glucoside show aldose reductase inhibitory activities, and therefore, they would be beneficial for the prevention of diabetes (Yodmanee et al. 2011). Cyanidin 3-glucoside (Cy-3-G) was found in the highest amounts in unprocessed and extruded rice bran (Ti et al. 2015). Cvanidin-3-O-glycoside has been shown to have antioxidant activity 3.5 times more potent than Trolox, a vitamin E analog (Zuo et al. 2012). Notably, a black rice variety that contains the highest amount of anthocyanins possesses the lowest antioxidant activity and total phenolic content. This indicates that the overall phenolic components rather than the anthocyanin pigments contribute to the antioxidative capacity of black rice brans (Pitija et al. 2013). Eleven flavonoids are detected and six of them are found for the first time in black rice bran are taxifolin-7-O-glucoside, myricetin-7-O-glucoside, isorhamnetin-3-O-acetylglucoside, isorhamnetin-7-O-rutinoside, 5,6,3',4',5'-pentahydroxyflavone-7-O-glucoside, and 5,3',4',5'-tetrahydroxyflavanone-7-O-glucoside. The quantitative results reveal that different rice varieties possess flavonoids in different concentrations. The most abundant glycoside derivative of flavonoids that widely distributed among the rice varieties is monoglucoside (quercetin-3-O-glucoside, isorhamnetin-3-O-glucoside and myricetin-7-O-glucoside) (Sriseadka et al. 2012). The black rice bran contains most of the antioxidant compounds including phytic acid,  $\gamma$ -oryzanol, anthocyanins, and vitamin E homologues (Kong and Lee 2010). The thermal degradation of both the visual color and the anthocyanin content in the BCP follow a first-order kinetic reaction model. The temperature-dependent degradation is adequately fit to the Arrhenius equation (Loypimai et al. 2015).

The phenolic constituents of rice are mainly distributed in rice pericarp which accounts for 2–3 % of rice caryopsis, and can be separated in three different groups: phenolic acids, flavonoids, and proanthocyanidins. The simple phenolic acids and flavonoids are the most common phenolic compounds and they generally occur as soluble conjugated (glycosides) and insoluble forms (bound) (Nardini and Ghiselli 2004). Free and soluble conjugated phenolic forms are absorbed in the stomach and small intestine, the dietary intake of bound phenolics presents a chemopreventive activity against colon cancer (Acosta et al. 2014). Anthocyanins (cyanidin-3-O-βglucoside and peonidin-3-O- $\beta$ -glucoside) and tocopherols are identified in black rice which prove that they have aldose reductase inhibitory activity (Yawadio et al. 2007). Polar extractions of rice bran are high in antioxidative compounds and activities than nonpolar extractions (Pengkumsri et al. 2015). Interestingly, the phenolics, flavonoids, and anthocyanins of black rice bran are mainly present in free form. Nonfermented black rice bran extract (NFBE) show greater antioxidant activities than fermented black rice bran extract (FBE). This result suggests that the cytotoxic activity of black rice bran improve through fermentation while antioxidant activity gets reduced (Yoon et al. 2014).



**Fig. 4.1** Identification of anthocyanin species in black rice crude extract. **a** Appearance of black rice. **b** Black rice section stained with toluidine blue O. Scale bar: 1.0 mm. **c** Enlarged image of the dorsal region of the longitudinal black rice section. **d** Mass spectrum of the black rice crude extracts (Yoshimura et al. 2012)

Currently, 250 anthocyanins have been identified; however, only six of them pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin are commonly found in fruits and cereal grains (Escribano et al. 2004). These compounds have been recognized as health enhancing substances, owing to their antioxidant activity, anti inflammatory properties, and hypoglycaemic effects (Nam et al. 2006; Philpott et al. 2004). They also show other biological effects including antimutagenic and anticarcinogenic activities (Hyun and Chung 2004) (Fig. 4.1).

# 4.4.1 Anthocyanin Profile of Black Rice

Anthocyanins are naturally occurring plant pigments belong to the flavonoid family. They are widely distributed in nature and are responsible for the attractive red (rasp berries), purple (grasses and purple cabbage), and blue (blue berries) colors of many flowers, fruits, and vegetables (Jackman et al. 1987), and when ingested they protect cells against oxidative damage from free radicals and are widely used for their antioxidant and pharmacological properties (Guo 2008). The accumulation of anthocyanins in leaves, shoots, and roots is stimulated by various environmental cues such as temperature, light, and nutrients. The six common anthocyanidins are delphinidin, cyanidin, peonidin, pelargonidin, petunidin, and malvidin (Table 4.5).

Anthocyanins have been shown to be beneficial to health due to their high antioxidant capacity. Scientists says that one reason of fruits and vegetables are so

Anthocyanidins	Substitution pattern	Molecular weight
Delphinidin	3,5,7,3',4',5'-ОН	303
Cyanidin	3,5,7,3',4'-ОН	287
Pelagonidin	3,5,7,4'-OH	271
Petunidin	3,5,7,3,4,5'-OH; 3'-OMe	317
Peonidin	3,5,7,3,4'-OH; 3'-OMe	301
Malvidin	3,5,7,4'-OH; 3,5-OMe	333

 Table 4.5
 Common anthocyanidins present in nature

healthy is that they are rich in the vitamins and phytochemicals that help to defend against this damage. Though the best known antioxidants are vitamin C, vitamin E, selenium, zinc, and beta-carotene; hundreds or thousands of other similar healthy compounds (flavonoids, anthocyanins, polyphenols, and catechins) are found in foods like nuts, berries, beans, and tea. Antioxidants have been shown to have an important role in fighting a number of diseases including heart disease and cancer. Anthocyanins have many biological properties such as prevention of inflammation, reduction of DNA cleavage, antimutagenicity in bacterial model, and gastric protective effects. Black rice containing cyanidin and peonidin suppresses oxidative modification of human low-density lipoprotein and reduces the formation of nitric oxide (Hu et al. 2003). Hyun and Chung (2004) reported that cyanidin and malvidin mediate cytotoxicity through the arrest of the G2/M phase of the cell cycle and by induction of apoptosis. Furthermore, Ling et al. (2001) showed that reduction of atherosclerotic plaque formation is due to anthocyanins present in red and black rice. The antioxidants in black rice may help fight heart disease and reduce blood levels of "bad" cholesterol which is low-density lipoprotein or LDL. Anthocyanin antioxidants deliver multiple powerful health benefits such as

- 1. Eliminating harmful free radical molecules
- 2. Protecting arteries from plaque buildup
- 3. Protecting against DNA damage that causes cancer.

Anthocyanins are the pigments responsible for the bran color of black rice (Escribano et al. 2004) which has been proven to have 96 times more anthocyanin content than pigmented (blue, pink, purple and red) corn, wheat, and barley with the values reaching as high as  $3276 \ \mu g/g$  (Abdel et al. 2006). Meanwhile, proanthocyanidins are the pigments responsible for red rice color (Oki et al. 2002), although according to several studies (Abdel et al. 2006; Yoshinaga et al. 1986; Morimitsu et al. 2002), anthocyanins can also be found in red rice in lower concentration up to 35 times less than that of black rice. Black rice has high anthocyanin content located in the pericarp layers and is a good source of fiber, minerals, and several important amino acids. Black glutinous rice contains many nutritious substances such as essential amino acids, vitamins, minerals, and especially effective antioxidants (Minh 2014). Black waxy rice is one of the most potential plant sources of dark purple color of anthocyanin pigment. The bran layer of the rice kernel contains

high level of bioactive compounds such as oryzanol, anthocyanins, and phenolic compounds. In some varieties of black rice, anthocyanins are present in the stem and leaves as well as the kernels in others only the grains are pigmented.

Only little is known about the phytochemical profiles and antioxidant activities of different black rice varieties. Total anthocyanin content in the black rice extract is 43.2 %; other components of the extract are carbohydrate, 21.6 %; protein, 4.9 %; flavonoids, 16.6 %; water, 5.5 %; and others, 8.2 % (Xia et al. 2006). The spectroscopy analysis indicated that the four active components of the antioxidative extract of black rice are four anthocyanin compounds of malvidin, pelargonidin-3,5-diglucoside, cyaniding-3-glucoside, and cyaniding-3,5-diglucoside. Thus the anthocyanin compounds are the most important substantial foundations for antioxidation (Zhang et al. 2006). Several works were done on rice to either determine the anthocyanin profile (Ichikawa et al. 2001; Yawadio et al. 2007) the antioxidant activity of rice (Choi et al. 2007) or more recently with selected fractions there of, e.g., rice bran (Chotimarkorn et al. 2008; Lai et al. 2009) (Table 4.6).

An experiment on the phytochemical profiles and antioxidant activity of rice bran samples from 12 diverse varieties of black rice were determined. The average values of total phenolic contents of black rice bran were six times higher than those of white rice bran respectively (p < 0.05). The percentage contribution of free anthocyanins to the total ranged from 99.5 to 99.9 %. Cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-glucoside were detected in black rice bran samples and ranged from 736.6 to 2557, from 22.70 to 96.62, and from 100.7 to 534.2 mg/100 g of DW, respectively. The percentage contribution of free antioxidant activity to the total ranged from 88.7 to 96.0 %. The average values of total antioxidant activity of black rice bran were six times higher than those of white rice bran respectively (p < 0.05). These results indicate that there are significant differences in phytochemical content and antioxidant activity among different black rice varieties. Interestingly, the phenolics, flavonoids, and anthocyanins of black

Table 4.6Anthocyanincontent and visual color ofraw black rice bran(BRB) and black rice brancolorant powder(BCP) (Loypimai et al. 2015)

Characteristics	BRB	BCP
Anthocyanins (µg/g)	$1042.4 \pm 10.5$	$7235.5 \pm 18.3$
Cyanidin-3-O-glucoside	$27.3\pm3.19$	$93.25 \pm 10.2$
Cyanidin-3-O-rutinoside	$451.3\pm7.33$	$723.8 \pm 19.7$
Delphinidin	$184.3\pm8.35$	$638.4 \pm 23.3$
Cyanidin	$334.9 \pm 10.2$	$1654.2 \pm 54.2$
Pelargonidin	$51.6\pm3.98$	$101.8 \pm 11.2$
Malvidin	$2947.3 \pm 22.5$	$12,540.8 \pm 85.9$
Total contents		
Color value		
L <sup>a</sup>	$37.2\pm0.84$	$39.4 \pm 0.95$
$C^{\mathrm{a}}$	$15.2\pm0.38$	$17.2 \pm 0.48$
$h^{\circ}$	$310.9 \pm 6.61$	$348.8 \pm 6.74$

<sup>a</sup>Values are means  $\pm$  SD of triplicate samples (n = 3) (on a wet weight basis)

rice bran are mainly present in free form. The total antioxidant activity of black rice bran was correlated to the content of total phenolics, total flavonoids, and total anthocyanins and also was significantly correlated to the contents of cyanidin-3-glucoside, cyanidin-3-rutinoside, and peonidin-3-glucoside. Knowing the phytochemical profile and antioxidant activity of black rice bran gives insights to its potential application to promote health (Zhang et al. 2010) (Fig. 4 2).

Yoshimura et al. (2012) identified seven species of anthocyanin monoglycosides and two species of anthocyanin diglycosides in crude extracts from black rice by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) analysis. They also analyzed black rice sections by MALDI-IMS and found two additional species of anthocyanin pentosides and revealed different localization patterns of anthocyanin species composed of different sugar moieties. Anthocyanin species composed of a pentose moiety (cyanidin-3-O-pentoside and petunidin-3-O-pentoside) were localized in the entire pericarp, whereas anthocyanin species composed of a hexose moiety (cyanidin-3-O-hexoside and peonidin-3-O-hexoside) were focally localized in the dorsal pericarp. These results indicate that anthocyanin species composed of different sugar moieties exhibit different localization patterns in the pericarp of black rice.

Anthocyanin types and total content vary among different rice cultivars. The antioxidant capacity of colored rice is mainly found in the seed capsule. Colored rice cultivars show stronger antioxidant activities than white rice. Antioxidant



**Fig. 4.2** MALDI-IMS analysis of anthocyanins in black rice sections. **a** Black rice section stained with toluidine blue O after MALDI-IMS analysis of the ions at m/z 449. **b** Localization pattern of the ions at m/z 449. **c** Localization pattern of the ions at m/z 822 corresponding to PC (diacyl C36:3), which marks the nucellus epidermis/aleurone layer. **d** Localization pattern of the ions at m/z 496 corresponding to LPC (C16:0), which marks the endosperm. **e** Merged ion image of m/z 449 (*red*), m/z 822 (*green*) and m/z 496 (*white*). **f** *Black* rice section stained with toluidine blue O after MALDI-IMS analysis of the ions at m/z 463. **g** Localization pattern of the ions at m/z 463. **h** Localization pattern of the ions at m/z 822 corresponding to PC (diacyl C36:3). **i** Localization pattern of the ions at m/z 822 corresponding to LPC (C16:0). **j** Merged ion image of m/z 463 (*red*), m/z 822 (*green*) and m/z 496 (*white*). Scale bar: 1.0 mm (Yoshimura et al. 2012)

capacity is mainly due to the other phenolic compound instead of anthocyanin content. Colored rice particularly black rice has been shown to possess bioactive properties and rice bran contains high levels of several antioxidant compounds. The black rice bran contains most of the antioxidant compounds including phytic acid,  $\gamma$ -oryzanol, anthocyanins, and vitamin E homologues (Kong and Lee 2010). The spectroscopy analysis indicates that four active components of the antioxidative extract of black rice are cyanidin-3-glucoside, peonidin-3-glucoside, cyanidin-3,5-diglucoside, and cyanidin-3-rutinoside (Hou et al. 2013). The predominant anthocyanins are cyanidin-3-glucoside (572.47 µg/g, 91.13 % of total) and peonidin-3-glucoside (29.78 µg/g, 4.74 % of total). Minor constituents include three cyanidin-dihexoside isomers and one cyanidin hexoside. It is concluded that the anthocyanin compounds are the most important substantial foundations for antioxidation (Zhang et al. 2006). Zhang et al. (2015) identified the breeding black rice line YF53 has the highest total phenolic content (23.3 mg ferulic acid equiv./g), total anthocyanin content (2.07 mg cyanidin-3-glu equiv./g), and antioxidant activities; total anthocyanin content and antioxidant activity of black rice is significantly higher in germinated rice (Sutharut and Sudarat 2012). Phonsakhan and Ngern (2014) reported that the accumulation of anthocyanins in black glutinous rice varieties range from 0.262 to 2.539 mg/g.

Colored rice has red or purple pigments in its bran. The anthocyanins contain approximately 95 % of cyanidin-3-O-glucoside and 5 % of peonidin-3-O-glucoside. The 100 g/ml concentration of the anthocyanin extract exhibits 88.83 % inhibition on the peroxidation of linoleic acid, 55.20 % DPPH free radical scavenging activity, 54.96 % superoxide anion radical scavenging activity, and 72.67 % hydrogen peroxide scavenging activity, and it also shows high ferrous ion reducing capability. These data suggest that the anthocyanin extracted from black rice may be utilized as a possible antioxidant agent against ROS (J. Microbiol. Biotechnol, 01/2008). The total anthocyanin content varies greatly among black rice cultivars (79.5-473.7 mg/100 g), but is lower in red rice (7.9-34.4 mg/100 g). Total phenolic content is similar between red (460.32-725.69 mg/100 g) and black (417.11-687.24 mg/100 g) rice. The oxygen radical absorbing capacity is ranked as follows: red (69.91-130.32 µmol Trolox/g)>black (55.49-64.85 µmol Trolox/g)>green (35.32 µmol Trolox/g)>white (21.81 µmol Trolox/g) rice. The antioxidant capacity results mainly from the seed capsule, not the endosperm. The anthocyanin pigments contributed little to the total antioxidant capacity of red (0.03-0.1 %) and black (0.5-2.5 %) rice cultivars. Hence, the antioxidant capacity is derived mainly from other phenolic compounds (Chen et al. 2012). Anthocyanin can be a key factor in functional property of black rice and "Heugjinjubyeo" a black rice variety may be very important source concerning nutritional value (Lee 2010). A new 2-aryl benzofuran, 2-(3,4-dihydroxyphenyl)-4,6-dihydroxybenzofuran-3-carboxylic acid methyl ester, oryzafuran (1), has been isolated from the black colored rice bran of Oryza sativa cv. Heugijnjubyeo. This compound showed strong antioxidative activity in a 1,1-diphenyl-2-picrylhydrazyl free radical scavenging assay (Han et al. 2004).

Different methods of anthocyanin extraction from black rice are available nowadays. Studies show that high-performance countercurrent chromatography (HPCCC) coupled with reversed-phase medium pressure liquid chromatography RP-MPLC method is more rapid and efficient than multi-step conventional column chromatography for the separation of anthocyanins (Jeon et al. 2015). Similarly, the VIS/NIRS method would be applicable only for rapid determination of cyanidin-3-glucose content in black rice flour samples (Sig et al. 2012) (Fig. 4.3).

Blue characters indicate biosynthetic enzymes of each steps of the pathway. Enzyme names are abbreviated as follows: CHS, chalcone synthase; CHI, chalcone isomerase; F3H, Flavanone 3-hydroxylase; F3'H, Flavanone 3'-hydroxylase; F3'5' H, Flavanone 3'5'-hydroxylase; FLS, Flavonol synthase; DFR, Dihydroflavonol reductase; LAR, leucoanthocyanidin reductase; LDOX, leucoanthocyanidin dioxygenase; ANR, anthocyanidin reductase; UGTs, UDP-glucosyl transferases. This figure was redrawn from data described by Petroni and Tonelli (2011).

Antioxidant activity was studied for anthocyanins extracted from purple black rice (PBR) by a 3 % aqueous trifluoroacetic acid solution (TFA), as well as for anthocyanins extracted from blueberry (Bluetta, high bush type). Capillary zone electrophoresis revealed that the PBR extract contained almost exclusively a single anthocyanin, which was identified as cyanidin 3-O- $\beta$ -D-glucoside (Cy 3-Glc) after purification by polyvinyl pyrrolidone column chromatography. In contrast, 11 anthocyanins were identified in the blueberry extract. PBR extract showed slightly weaker superoxide scavenging and crocin bleaching activities than blueberry



Fig. 4.3 Schematic diagram of the anthocyanin and proanthocyanidin biosynthesis pathway in plants

extract did. Both PBR and blueberry extracts, however, showed 10-25 times stronger activity than the same concentration of Trolox used as a reference antioxidant. It was further noted that the purified Cy 3-Glc from PBR extract retained approximately 74 % of the antioxidant activity (both crocin bleaching and superoxide scavenging) observed in the original TFA extract. The hydroxyl radical scavenging activity of both extracts was several times weaker than that of the same concentration of Trolox, although the PBR extract showed approximately two times stronger activity than blueberry extract did. The hydroxyl radical scavenging activity of the purified Cy 3-Glc from PBR, however, decreased to approximately 20 % of that of the original PBR extract. These results indicate that the anthocyanin Cy 3-Glc contributes to the antioxidant activity of PBR through its strong superoxide radical but not hydroxyl radical scavenging activity (Ichikawa et al. 2001). A new compound of 2-aryl benzofuran, 2-(3,4-dihydroxy phenyl)-4,6-dihydroxy benzofuran-3-carboxylic acid methyl ester, oryzafuran (1), was isolated which showed strong antioxidative activity in a 1,1-diphenyl-2-picrylhydrazyl free radical scavenging assay (Han et al. 2004).

# 4.4.2 Cyanidin-3-Glucoside (C3G)

The most abundant anthocyanins are cyanidin 3-glucoside in black and red rices and in blue, purple, and red corns, pelargonidin 3-glucoside in pink corn, and delphinidin 3-glucoside in blue wheat (Abdel et al. 2006). Cyanidin 3-glucoside and peonoidin 3-glucoside are confirmed as the dominant anthocyanins in black rice varieties with contents ranging from 19.4 to 140.8 mg/100 g DM and 11.1–12.8 mg/100 g DM, respectively (Muntana and Prasong 2010). Park et al. (2008) reported the anthocyanins in black rice include cyanidin 3-O-glucoside, peonidin 3-O-glucoside, malvidin 3-O-glucoside, pelagonidin 3-O-glucoside, and delphinidin 3-O-glucoside. Similarly, four anthocyanins are detected in black rice are identified as cyanidin-3-glucoside (91.01 %), peonidin-3-glucoside (7.13 %), cyanidin-3,5-diglucoside (0.92 %), and cyanidin-3-rutinoside (0.94 %). The main anthocyanins are cyanidin-3-glucoside and peonidin-3-glucoside (Hou et al. 2013) (Table 4.7).

Cyanidin-3-O- $\beta$ -d-glucoside (C3G), the best known and most investigated anthocyanin, has many pharmacological properties and black rice bran is an outstanding source of C3G due to its high C3G content and a simple anthocyanin

	Thailand		China
	Niaw Dam Pleuak	Niaw Dam Pleuak	China black
	Khao (PK)	Dam (PD)	Rice (CNB)
Anthocyanins			
Cyanidin 3-glucoside	137.41 ± 16.66	$19.39 \pm 0.09$	$140.83 \pm 2.0$
Peonidin 3-glucoside	$11.07 \pm 0.97$	$12.75 \pm 0.51$	$11.1 \pm 0.16$

 Table 4.7
 Anthocyanin profile in black rice varieties (Sompong et al. 2011)

composition. 1.43 g of pure C3G (purity 94.38 %) was obtained from 3.0 g of anthocyanin enriched extract by reversed-phase  $C_{18}$  column chromatography (Li et al. 2012). Two novel oxidized products are isolated from radical oxidation of cyanidin 3-O-glucoside and the products obtain from the anthocyanin oxidation are dependent on the nature of the solvent (Kamiya et al. 2014). Cyanidin 3-glucoside, peonidin 3-glucoside, and cyanidin occurs in black and some light purple rice samples. The average anthocyanins content in black rice bran is over 3.5 mg/g and vitamin E content is between 0.01 and 0.05 %, oryzanol is 0.1–0.3 % and the fiber content is 7–11 %. A large-scale isolation of C3G from the crude black rice bran extract is obtained by combining high-speed countercurrent chromatography (HSCCC) and reversed-phase C18 column chromatography. The method is time-saving, low risk of sample denaturation, high sample recovery, and so is suitable for large-scale preparation of C3G for further studies of bioactivities (Du et al. 2012) (Fig. 4.4).

Black purple rice is becoming popular among health-conscious food consumers. A study was conducted where the secondary metabolites in dehulled black purple rice cv. Asamurasaki were analyzed using HPLC–PDA–MS<sup>2</sup>. The results showed that seeds contains a high concentration of seven anthocyanins (1400  $\mu$ g/g fresh weight) with cyanidin-3-O-glucoside and peonidin-3-O-glucoside predominating. Five flavo-nol glycosides, principally quercetin-3-O-glucoside and quercetin-3-O-rutinoside and



Fig. 4.4 Chemical structure of different anthocyanin compounds

flavones. The seeds also contained 3.9  $\mu$ g/g of carotenoids consisting of lutein, zeaxanthin, lycopene, and  $\beta$ -carotene.  $\gamma$ -Oryzanol (279  $\mu$ g/g) was also present as a mixture of 24-methylenecycloartenol ferulate, campesterol ferulate, cycloartenol ferulate, and  $\beta$ -sitosterol ferulate (Caro et al. 2013).

# 4.5 Rice Preparation for Consumption and Its Effects on Nutrition

# 4.5.1 Washing or Rinsing Rice Before Cooking

This is a common practice in places where rice packaging and storage methods leave rice exposed to dust and other contaminants. Rinsing rice, sometimes multiple times results in the loss of water-soluble nutrients, including starch, protein, vitamins, minerals, and fats. Use of clean packaged rice reduces or removes the need for washing and thus prevents the loss of nutrients (FAO). Similar nutrient loss would also occur, however, if rice is presoaked and then drained before cooking, or if rice is cooked in excess water that is drained away before consumption (Ricepedia). Washing of milled rice prior to cooking is a common practice in Asia to remove bran, dust, and dirt from the food, since rice is often retained in open bins and thus exposed to contamination. During washing, some water-soluble nutrients are leached out and removed.

Boiling in excess water results in leaching out of water-soluble nutrients including starch and their loss when the cooking liquor is discarded. For example, 0.8 % of the starch is removed on two washings of three milled rices, but 14.3 % of the starch by weight is in the rice gruel after cooking for about 20 min in 10 weights of water (Perez et al. 1987) Protein removal is 0.4 % during washing and 0.5 % during cooking (Table 4.8).

# 4.5.2 Cooking

Cooking milled (polished/white) rice by boiling in water (without washing) results in the loss of up to 7 % of protein, 36–58 % of crude fat, 16–25 % of crude ash, 21 % of calcium, 47–52 % of thiamine, 35–43 % of riboflavin, and 45–55 % of niacin (Ricepedia).

### 4.5.3 Wet Milling

Water-soluble nutrients are also lost during wet milling of rice flour (a process used for making rice noodles, egg roll wrappers, and some rice flourcakes etc.), in the

Nutrient	Washing	a		Washing and cooking <sup>b</sup>	Cooking	without w	vashing <sup>c</sup>
	Raw milled rice	Brown rice	Parboiled milled rice	Milled rice	Milled rice	Brown rice	Parboiled milled rice
Weight	1–3	0.3-0.4		5–9	2-6	1-2	3
Protein	2–7	0-1		2	0–7	4-6	0
Crude tat	25-65			50	36–58	2-10	27–51
Crude fiber	30						
Crude ash	49				16–25	19	29–38
Free sugars	60			40			
Total polysaccharides	1–2			10			
Free amino acids	15			15			
Calcium	18-26	4-5		1-25	21		
Total phosphorus	20–47	4		5			
Phytin phosphos	44						
Iron	18-47	1-10		23			
Zinc	11	1					
Magnesim	7–70	1	1				
Potassium	20-41	5	15				
Thiamine	22–59	1-21	7–15	11	47–52		
Riboflavin	11–26	2-8	12–15	10	35-43		
Niacin	20-60	3–13	10–13	13	45-55		

Table 4.8 Percent nutrient losses during washing and cooking in excess water (Hayakawa and Igaue 1979; Perez et al. 1987)

filtration step. This includes vitamins, minerals, free sugars and amino acids, water-soluble polysaccharides, protein (albumin), fat and starch (Ricepedia).

# 4.5.4 Parboiling

Parboiling rough rice before milling, as is common in India and Bangladesh, allows a portion of the vitamins and minerals in the bran to permeate the endosperm and be retained in the polished rice. This treatment also lowers protein loss during milling and increases whole grain recovery (Ricepedia). Parboiling reduced the TSPCs concentration in the grains due to the loss of part of them in the processing water, thermal decomposition and, possibly, interaction with other components. This reduction is related to the lower AOA in these grains. In a similar way, cooking also reduced the concentration of TSPCs, especially in brown and polished grains (Walter et al. 2013).

# 4.5.5 Fermentation

Fermentation of rice is another popular preparation method. Reportedly, protein content of fermented rice decreases from 1.54 % after one day of fermentation to 1.14 % after 3 days at 70 % moisture (Ricepedia).

# 4.6 Rice Cooking Methods and Anthocyanin Content

All cooking methods cause significant (P < 0.001) decreases in the anthocyanins. Pressure cooking results in the greatest loss of cyanidin-3-glucoside (79.8 %) followed by the rice cooker (74.2 %) and gas range (65.4 %). Conversely, levels of protocatechuic acid increase from 2.7 to 3.4 times in response to all cooking methods. These findings indicate that cooking black rice results in the thermal degradation of cyanidin-3-glucoside and concomitant production of protocatechuic acid (Hiemori et al. 2009). Proximate analysis and light micrographs revealed higher migration of red rice proteins than black rice proteins to the endosperm as a result of parboiling. Parboiling reduces the ash content of red rice while no difference is determined in black rice. Polishing removes more than 90 % of free phenolics while parboiling allow the partial preservation of free phenolics content in polished rice. Parboiling induces an increase in the cooking time of red rice, but a decrease in the cooking time of black rice (Paiva et al. 2015). Roasting result in the greatest decrease (94 %) in anthocyanins, followed by steaming (88 %), pan-frying (86 %), and boiling (77 %). The contents of phenolic compounds decrease drastically after cooking, with significantly lower retention in the black rice cultivar that had higher amylose content. DPPH radical scavenging activity of black rice decrease after cooking compared to the raw ones. In contrast, metal chelating activities significantly increase after cooking. The contents of anthocyanins shows a high positive correlation with total phenolic compounds whereas a significant negative correlation with metal chelating activity. These results indicate that cooking degrades anthocyanins and other phenolic compounds, but concomitant increase in phenolics from possible degradation of the anthocyanins which results in the enhancement of metal chelating activity (Surh and Koh 2014) (Fig. 4.5).

Antioxidants in black rice bran tend to leach out into the water during cooking as rice is usually cooked in excess water which is discarded after cooking. The percentages of antioxidant extractability from pigmented rice into the cooking water are 88.42 and 103.26 %, respectively, for red rice and black rice. However, red rice drink possess significantly (p < 0.05) higher antioxidant activity than black rice



drink, except for its total monomeric anthocyanin content. Black rice and red rice cooking water have the potential of being new antioxidant drinks (Handayani et al. 2014). All processed black rice exhibit significantly (p < 0.05) lower TPC, TFC, CTC, MAC, Cy3glc, Pn3glc, and antioxidants as compared to the raw rice. Different processing methods significantly degrade the content and activities of antioxidants of both waxy and nonwaxy black rice. Under the same cooking time, black rice porridge retain more active substances than that of cooked rice by rice cooker. Therefore, to maintain bioavailability of active components, black rice porridge may gain more health promoting effects (Tang et al. 2015). The risotto cooking allows a complete absorption of water, could be considered a recommended cooking method to retain phenolic compounds and total antioxidant capacity and, in the case of the black rice. Consumption of whole meal rice particularly the black rice should be recommended as a good source of phenolic compounds in a diet, especially when it is cooked with the complete absorption of water (Zaupa et al. 2015) (Table 4.9).

Rice samples	Min cooking	Elongation	Cooked l/b	Water	Cruel soild
	time (min)		(mm)	uptake ratio	loss (%)
White local	$10.00 \pm 0.0^{a}$	$1.43 \pm 0.0^{b}$	$3.27\pm0.1^{b}$	$2.44\pm0.3^a$	$6.43 \pm 0.0^{f}$
Brown	$31.67 \pm 0.6^{d}$	$1.68 \pm 0.3^{d}$	$2.25\pm0.4^a$	$3.95 \pm 0.3^{d}$	$3.37 \pm 0.6^{b}$
Bario	$12.67 \pm 0.6^{b}$	$1.37\pm0.2^{a}$	$3.07\pm0.2^{b}$	$2.75 \pm 0.2^{b}$	$5.46 \pm 0.3^{d}$
Black (imported)	$22.66 \pm 0.6^{\circ}$	$1.57 \pm 0.1^{c}$	$3.45 \pm 0.3^{c}$	$2.73 \pm 0.1^{b}$	$4.22 \pm 0.4^{c}$
Glutinous	$22.67 \pm 0.0^{\circ}$	$1.41 \pm 0.1^{b}$	$3.17\pm0.4^{b}$	$2.33\pm0.8^a$	$5.76 \pm 0.2^{e}$
Basmati	$22.00 \pm 0.6^{c}$	$1.77\pm0.0^{\rm d}$	$4.18\pm0.1^{d}$	$3.77 \pm 0.6^{c}$	$3.17 \pm 0.2^{a}$

**Table 4.9** Cooking properties of rice varieties ( $n = 3 \pm s.d.$ ) (Thomas et al. 2013)

Same letter in the same column are not significantly different from each other at p < 0.05



	Raw	Risotto	Boiled
Cyanidin 3-O-glucoside	$255.7\pm4.2^a$	184.6 ± 13.7 <sup>b</sup> (-27.8 %)	$96.2 \pm 0.6^{\circ} \ (-62.4 \ \%)$
Peonidin 3-O-glucoside	$50.8\pm0.7^{\rm a}$	$28.2 \pm 0.6^{\rm b} \ (-44.4 \ \%)$	$15.5 \pm 0.9^{\circ} \ (-69.5 \ \%)$
Cyanidin 3-O-rutinoside	$7.5 \pm 0.1^{a}$	$6.5 \pm 1.0^{a} \ (-13.0 \ \%)$	$1.9 \pm 0.4^{\rm b} \; (-74.7 \; \%)$
Peonidin 3-O-rutinoside	$1.3 \pm 0.1^{a}$	$0.8 \pm 0.2^{b} \; (-38.5 \; \%)$	$0.5 \pm 0.0^{\rm c} \ (-61.5 \ \%)$
Cyanidin-O-diglucoside	$18.6 \pm 0.3^{a}$	$14.4 \pm 0.7^{b} (-22.6 \%)$	$5.4 \pm 0.3^{\circ} (-71.0 \%)$
Total	$333.9 \pm 4.4^{a}$	234.5 ± 14.1 <sup>b</sup> (-29.8 %)	$119.5 \pm 1.2^{\circ}$ (-64.2 %)

 Table 4.10
 Anthocyanin content of raw and cooked black rice and percentage of losses by cooking (Zaupa et al. 2015)

<sup>a</sup>Peonidin-3-O-glucoside is quantified as cyanidin 3-O-glucoside equivalents. Peonidin 3-O-rutinoside and cyanidin O-diglucoside are quantified as cyanidin 3-O-rutinoside equivalents <sup>b</sup>Values are presented as mean  $\pm$  SD, n = 3 and expressed as lg/g dry weight. Different letters in the same row correspond to significantly (p < 0.05) different samples. Values in parentheses represent the percentage of loss with cooking with respect to raw rice

<sup>c</sup>Cyanidin O-diglucoside content is the sum of the three peaks (r.t. 4.22, 4.61 and 4.86 min)

Hou et al. (2013) presented a study and provided detail information regarding the changes in kinetic stability of the four anthocyanins in black rice at temperature (80 °C, 90 °C, and 100 °C) at different pH levels (1.0–6.0). The data showed that degradation of anthocyanins followed first-order reaction kinetics. The four anthocyanins degraded more quickly with increasing heating temperature and pH values, especially, as heating temperature increasing to 100 °C and pH value to 5.0. Thus higher stability of anthocyanins is achieved by using lower temperature, lower pH values, and short time. This confirms the stability of anthocyanins depend on temperature and pH (Table 4.10).

Cooking and physiochemical properties of rice are strongly dependent on amylose content. Black rice variety has the higher protein content (8.16 %) with lower fat content (0.07 %) (Thomas et al. 2013). Variations in composition and cooking quality of rice mainly depend on the genetic as well as surrounding



environmental factors where they are grown (Giri and Vijaya 2000; Singh et al. 2005). Rice grain quality is influenced by various physicochemical characteristics that determine the cooking behavior as well as the cooked rice texture (Bocevska et al. 2009). Physicochemical properties and cooking characteristics of rice depend on amylose content (Saikia et al. 2012) (Fig. 4.6).

# 4.7 Nutrient Content and Degree of Milling

Black rice shows a thicker bran layer than red rice. Around 80 and 65 % of the ash content of red and black rice is distributed in the bran layer, respectively. 4 % degree of milling (DOM) reduce 47 % of the fat content in red rice, while in order to reduce similiar fat content in black rice, a 7 % DOM is necessary. The total free phenolics are around 6 and 7 fold higher than bound phenolics for black and red rice respectively. Although the nonmilled black rice presents higher free and bound phenolics contents than nonmilled red rice, the red rice shows higher DPPH and ABTS + antioxidant activities (Paiva et al. 2014). Milling for 20 s is sufficient to remove most of the bran layer of the black rice sample, but 10 s of milling retains higher contents of nutritional components and rice antioxidants (Laokuldilok et al. 2013). Thus the relationship between milling time and DOM show a nonlinear curve. After milling, the amount of bran left on milled rice kernels is measured and is termed as Degree of Milling (DOM) (Fig. 4.7).

Glutinous black rice flour appears to have significantly higher gelatinization temperature and pasting viscosities including peak, trough, breakdown, and final



Fig. 4.7 Dehulled black rice and red rice samples subjected to different degrees of milling (Paiva et al. 2014)

viscosities. Compared to dry milled black rice flour, wet milled black rice flour show lower peak viscosity and higher final viscosity resulting in increase setback value (Lee 2013).

# 4.8 Aromatic Chemistry of Black Rice

Black rice (Oryza sativa L.) is a special aromatic rice popular in Asia. Characteristic flavor of black rice varieties plays an important role in the rice's reputation and its acceptance by customers. It has a unique flavor, but the volatile chemistry has not been reported in detail yet. Thirty five volatile compounds have been identified in black rice by gas chromatography mass spectrometry. Aldehydes and aromatics have been found quantitatively in the greatest abundance accounting for 80.1 % of total relative concentration of volatiles. The concentration of 2-acetyl-1-pyrroline (2-AP) is high, exceeded only by hexanal, nonanal, and 2-pentylfuran. But 2-AP, guaiacol, indole, and *p*-xylene largely influence the difference between the aroma in cooked black and white rice. 2-AP and guaiacol are major contributors to the unique character of black rice based on odor thresholds, relative concentrations, and olfactometry (Yang et al. 2008). Similarly, 21 and 23 odorants have been detected using GC-O for cooked samples of premium quality, waxy, and black pigmented rice cultivars, respectively. Hexanal is the main odorant in premium quality and waxy cultivars; however, waxy cultivars have 16 times higher hexanal odor activity values (OAVs) than premium quality cultivars indicating that the premium quality rice has a less-pronounced overall aroma. 2-Acetyl-1-pyrroline was found to be the main contributor to overall aroma in black pigmented rice followed by guaiacol. Six odor-active compounds (2-acetyl-1-pyrroline, guaiacol, hexanal, (E)-2-nonenal, octanal, and heptanal) contributed the most in discriminating the three types of specialty rice (Yang et al. 2010). Ajarayasiri and Chaiseri (2008) reported that benzaldehyde was found in the highest concentration (1,203.7 ng/kg) but 4-vinyl-2-methoxyphenol (sweet, spicy and smoky odor) had the highest odor active value (OAV = 133) in black glutinous rice. However, the results from gas chromatography-olfactometry (GCO) indicated that there were four aroma active compounds in black glutinous rice. The most prominent odorants were (E,E)nona-2,4-dienal (sweet, fatty) and 2-acetyl-1-pyrroline (pandan) that had a flavor dilution (FD) factor of 3. Two other compounds that had an FD factor of 1 were 2,3-butanediol (sweet, balsamic) and 1-octen-3-ol that had straw and earthy-aroma characteristics.

A research on red and black rice oil chemistry was conducted. The red rice bran essential oils yield was 0.031 %, and its major constituents were (E)- $\beta$ -ocimene (3.12 %), nonanal (11.32 %), (2E, 4E)-decadienal (2.54 %), myristic acid (41.32 %), geranyactone (2.41 %), and methyl oleate (2.46 %). The black rice bran essential oils yield was 0.053 %, and its major constituents were nonanal (8.31 %), acrylic acid (3.21 %), 2-hydroxy-6-methylbenzaldehyde (2.81 %), pelargonic acid (4.21 %), and myrisitc acid (28.07 %) (Chung et al. 2011). Trans- $\beta$ -carotene,

quercetin, and isorhamnetin were present in the bran of all black rice cultivars within the range of 33.60–41, 1.08–2.85, and 0.05–0.83  $\mu$ g/g, respectively (Nakornriab et al. 2008). Similarly, Sukhonthara et al. (2009) reported one hundred twenty nine (129) volatile compounds in red and black rice bran. He said

Myristic acid, nonanal, (E)-beta-ocimene and 6,10,14-trimethyl-2-pentadecanone are main compounds in red rice bran, whereas myristic acid, nonanal, caproic acid, pentadecanal, and pelargonic acid are main compounds in black rice bran.

Guaiacol, presented at 0.81 mg/100 g in black rice bran is responsible for the characteristic component in black rice (Sukhonthara et al. 2009). The main constituents of the volatile compounds are nonanal, butylated hydroxytoluene, 1-hexanol, naphthalene, and dodecamethyl-cyclohexasiloxane. The changes of variety and content of volatile compounds from raw and processed materials could be attributed to the soaking and heating during the processing which might induce some chemical reactions and promote the extraction of some compounds. Ninety four of volatile compounds have been identified in black rice. And processing can significantly change the volatile compounds of black rice (Wu et al. 2013). Names of 94 volatile compounds are listed here:

- 1. Hexamethyl-cyclotrisiloxane
- 2. Methyl-pyrazine
- 3. Ethylbenzene
- 4. p-Xylene
- 5. 1-Hexanol
- 6. Styrene
- 7. 1,3-Dimethyl-benzene
- 8. 5-(1-Methylet hylidene)-1,3-cyclopentadiene
- 9. 1,3-Dimethyl-benzene
- 10. 2-Chloro-pentane
- 11. 2-Amino-5-methylbenzoic acid
- 12. 2,5-Dimethyl-pyrazine
- 13. Ethyl-pyrazine
- 14. Morpholine
- 15. 6-Propyltetrahydro-2H-thiopyran-2-one
- 16. (Z)-2-Heptenal
- 17. Benzaldehyde
- 18. Benzoyl bromide
- 19. N-Methyl-3-nitro-N-(2-phenylethyl)-benzeneethanamine
- 20. 1,2,4-Trimethyl-benzene
- 21. N-methyl-3-nitro-N-(2-phenylethyl)-benzeneethanamine
- 22. 1-Butanol
- 23. 1-Octen-3-ol
- 24. 6-Methyl-5hepten-2-one
- 25. 1-Butyl-cyclohexene
- 26. 2-Pentyl-furan

- 27. 2-Ethyl-6-methyl-pyrazine
- 28. Hexanoic acid, ethyl ester
- 29. 1-Methyl-2-pyrrolidinone
- 30. Octamethyl-cyclotetrasiloxane
- 31. 1,4-Dichloro-benzene
- 32. 2-Ethenyl-6-methyl-pyrazine
- 33. 2-Ethyl-1-hexanol
- 34. Indane
- 35. Benzeneacetaldehyde
- 36. (E)-2-Octenal
- 37. Acetophenone
- 38. 1-Heptanol
- 39. 3-Ethyl-2,5-dimethyl-pyrazine
- 40. 2-(Cyclopropylmethyl)-4,5-dimethoxy-benzenamine
- 41. Hexamethyl-cyclotrisiloxane
- 42. 1-Ethyl-2,4-dimethyl-benzene
- 43. 2-(1-Methylvinyl)thiophene
- 44. 1-Methyl-azetidine
- 45. Nonanal
- 46. 1-Ethyl-2,3-dimethyl
- 47. 4-Hydroxy-benzonitrile
- 48. 1,2,3,5-Tetramethyl-benzene
- 49. 1,7,7-Trimethyl-(1R)-Bicyclo[2.2.1]heptan-2-one
- 50. 1,2,3,4,5-Tetramethyl-benzene
- 51. 1-Ethyl-2,3-dimethyl-benzene
- 52. (E)-2-Nonenal
- 53. Octyl-oxirane
- 54. 3,4-Dimethylcyclohexanol
- 55. Naphthalene
- 56. Decanal
- 57. 2,3-Dihydro-benzofuran
- 58. Benzaldehyde,4-methyl-oxime,(Z)-
- 59. Isoquinnoline
- 60. 6-Methyl-tridecane
- 61. Ether, hexyl pentyl
- 62. 2-Methyl-naphthalene
- 63. Hexadecane
- 64. Oxalic acid, isobutyl pentyl ester
- 65. 1-Methyl-naphthalene
- 66. 2-Methoxy-4-vinylphenol
- 67. Docosane
- 68. Hexadecane
- 69. 1-Iodo-nonane
- 70. (Aminomethyl) cyclopropane
- 71. Dodecamethyl-cyclohexasiloxane

- 72. 5-Butyl-5-ethyl-1,3-bis(trimethylsilyl)-2,4,6- (1H,3H,5H)-pyrimidinetrione
- 73. Heptacosane
- 74. 2,3,6-Trimethyl-decane
- 75. Heptadecane, 2,6,10,14-tetramethyl
- 76. Propanoic acid,2-methyl-,hexyl ester
- 77. Biphenyl
- 78. Tetradecane
- 79. 2,2-Dimethyl-butane
- 80. 1-Phenyl-3-methylpenta-1,2,4-trien
- 81. 1,3-Dimethyl-naphthalene
- 82. 5,9-Undecadien-2-one,6,10-dimethyl-,(Z)-
- 83. Heptadecane
- 84. Pentadecane
- 85. Tetradecamethyl-cycloheptasiloxane
- 86. Butylated Hydroxytoluene
- 87. Fluorene
- 88. 2,3,4-Trimethyl-hexane
- 89. Phenanthrene
- 90. Carbon dioxide
- 91. Phthalic acid, isobutyl octadecyl ester
- 92. Hexadecanoic acid, methyl ester
- 93. 4,5-Nonadiene
- 94. Cyclohexanemethanol.

Fragrance is the most important trait among the domesticated characteristics of rice (*O. sativa* L.). It is highly valued trait and known to be primarily associated with 2-acetyl-1-pyrroline. It has been previously determined that the fragrance gene is located on chromosome 8 that controls the level of aromatic compound 2-acetyl-1pyroline (Bradbury et al. 2005). The recessive fragrance gene on chromosome 8 is associated with rice fragrance. The structure of fragrance gene (fgr) comprises 15 exons interrupted by 14 introns. Fragrance is a recessive trait, the alleles from fragrance varieties show the presence of mutations portion (i.e., the 8 bp deletion in exon 7), resulting in the loss of function of the fragrance gene product. This allele is responsible for rice fragrance. Interestingly, the concentration of 2-acetyl-1-pyroline (2-AP) is high in cooked black rice (Yang et al. 2008; Prathepha 2008).

Black nonglutinous rice contains the greater number of monoterpenoids. An herbaceous odorant, myrcene, occurs in the bran of all black rice varieties but not in the bran of white rice. This monoterpene could, therefore, be considered as a characteristic odorant of black rice (Chumpolsri et al. 2015). Terpenoids found in black glutinous rice (Adams 2001; Mottaram 2004) are as follows:

#### Compounds name—Odor description

- 1. α-Pinene-Green, pine-like
- 2. Camphene-Herbal, woody

- 3. Sabinene-Fresh, citrus note
- 4. β-Pinene-Sweet, green
- 5. Myrcene-Herbaceous, metallic
- 6. 1,4-Cineol-Eucalyptol-like
- 7. β-Cymene-Citrus, woody
- 8. Limonene-Green, citrus-like
- 9. 1,8-Cineol-Spicy, camphor-like
- 10. Trans-β- ocimene-Tropical, green
- 11. γ-Terpinene-Herbaceous, fruity
- 12. Trans-linalool oxide-Sweet, floral
- 13. Cis-linalool oxide-Sweet, floral
- 14. Linalool-Fruity, green
- 15. Camphor-Camphor-like
- 16. Terpinen-4-ol-Sweet, herbaceous
- 17. Fenchyl acetate-Coniferous, herbaceous
- 18. 10-(Acetylmethyl)-3-carene
- 19. Carveol-Citrus-like, fruity
- 20. α-Copaene-Woody, honey
- 21. α-Ylangene
- 22.  $\alpha$ -Elemene-Herbal, fresh
- 23. α-Gurjunene-Wood, balsam
- 24. Trans-caryophyllene-Spicy, dry
- 25. α-Neo-clovene
- 26. Trans-cadina-1(6),4-diene
- 27. β-Bisabolene-Balsamic, woody
- 28. 7-Epi-α-selinene-Amber.

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# Chapter 5 Black, Brown, and Red Rices

# 5.1 Major Differences Among Different Rice

Rice (Oryza sativa L.) exists in different colors such as white, purple, black, red, and brown. The most common rice consumed by human being is white rice followed by brown rice. However, rice genotypes with red, purple, or black bran layer have been cultivated for a long time in Asia (Ahuja et al. 2007). Although white rice is the most widely consumed rice, pigmented rice is considered as enriched rice for taste and health benefits due to the presence of anthocyanins (Ryu et al. 1998). Colored rice possess unique color and flavor, therefore they are used as an ingredient in many dishes (Rhee et al. 2000). However, due to the limitation in term of hard texture of cooked colored rice, they are not popular for consumption even though it has been long known about the beneficial effects of pigment in these groups of rice. There are naturally occurring color substances in pigmented rice that belong to the flavonoid group called anthocyanins. Positive health effects of the pigments present in the bran layer of rice have been reported by many scientists. A commonly found anthocyanin in colored rice is acetylated procyanidins which is reported to possess a free radical scavenging activity (Oki et al. 2002). Pigmented rice has become increasingly interested for its antioxidants, mainly due to that it is a good source of bioactive compounds such as  $\gamma$ -oryzanol,  $\alpha$ -tocopherols, and phenolic compounds. The phenolic compounds in pigmented rice have been reported to contain anthocyanins cyanidin-3-glucoside as a major in black rice (Osawa 1999); proanthocyanidins is a major in red rice (Nawa and Ohtani 1992) and other phenolics (Yawadio et al. 2007). Antioxidant activities of the color pigment in aleurone layer of rice have been already demonstrated by Hu et al. (2003); Ichikawa et al. (2001) and Oki et al. (2002). Colored rice varieties are rich sources of fat-soluble bioactive components, in particular, c-oryzanols, vitamin E isomers, and carotenoids. In addition, it provides a structural basis for studying the biological functions of these bioactive components at molecular levels. Most consumers are already aware that conventional brown rice is nutritionally superior to white rice in

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the way of fiber and beneficial vitamins because its outer layer (also known as a husk or chaff) and bran layers remain intact during processing. Abundant saturated fatty acid in these colored rice varieties are palmitic acid (c16:0) followed by stearic acid (c18:0) (Minatel et al. 2014). The colored varieties have better antioxidant properties than noncolored varieties. Thus it can be concluded that colored varieties could be used as a natural antioxidant source (Moko et al. 2014). Recent studies have demonstrated that pigmented rice has a wide range of biological activities, including amelioration of iron deficiency anemia of the body, antioxidant, anticarcinogenic, antiatherosclerosis, and antiallergic activities (Deng et al. 2013). Rice genotypes with pigmented caryopses have now received increased attention because of their antioxidant properties. Previous works evidenced that the kernel of red rice is characterized by the presence of proanthocyanidins, whereas black rice is characterized by the presence of anthocyanins. Surprisingly, the rice grain has no vitamin D, or vitamin C (FAO 1954) (Table 5.1).

#### 5.1.1 White, Long-Grain Rice

Raw, long-grain white rice is a relatively good source of energy, carbohydrates, calcium, iron, thiamin, pantothenic acid, folate and vitamin E, compared to maize, wheat and potatoes. It contains no vitamin C, vitamin A, beta-carotene, or lutein +zeaxanthin, and is notably low in fiber (Ricepedia<sup>1</sup>) (Fig. 5.1).

# 5.1.2 Colored Rice

Brown rice retains the bran layer (containing many vitamins and minerals as well as fiber), as this has not been polished off to produce white rice. Red rice is known to be rich in iron and zinc, while black and purple rices are especially high in protein, fat, and crude fiber. Red, black, and purple rices get their color from anthocyanin pigments, which are known to have free radical scavenging and antioxidant capacities, as well as other health benefits (Ricepedia) (Table 5.2).

Pigmented rice has a long history for human consumptions, especially in Southeast Asia (Hu et al. 2003). Antioxidant activities of paddy varieties containing color pigments such as red Thai, black rice, red brown, and dark purple had been intensively studied by Muntana and Prasong (2010) and Yodmanee et al. (2011), and they reported that rice with noncolor pigments contain lower phenolic content and antioxidant activities. Many studies have reported that black rice contains anthocyanin and other polyphenolic compounds more abundantly than white rice (Ryu et al. 1998; Zhang et al. 2006). Previous research about antioxidant properties

<sup>&</sup>lt;sup>1</sup>Ricepedia www.ricepedia.org Retrived 5 July 2015.

Table 5.1	Approximate com	position of rc	ugh rice and	l its milling 1	fractions at 14 % m	oisture (Juliano 15	85; Pederse	n and Eggu	m 1983)	
Rice	Crude protein	Crude fat	Crude	Crude ash	Available	Neutral	Energy	(hcal)	Density	Bulk
fraction	$(g N \times 5.95)$	(g)	fiber (g)	(g)	carbohydrates (g)	detergent fiber (g)	content (kJ)		(g/ml)	density (g/ml)
Rough rice	5.8-7.7	1.5–2.3	7.2–10.4	2.9–5.2	64–73	16.4–19.2	1580	378	1.17–1.23	0.56–0.64
Brown rice	7.1–8.3	1.6–2.8	0.6–1.0	1.0–1.5	73–87	2.9–3.9	1520–1 610	363–385	1.31	0.68
Milled rice	6.3-7.1	0.3–0.5	0.2-0.5	0.3–0.8	77–89	0.7–2.3	1460–1 560	349–373	1.44–1.46	0.78-0.85
Rice bran	11.3–14.9	15.0–19.7	7.0–11.4	6.6–9.9	34–62	24–29	670–1 990	399–476	1.16–1.29	0.20-0.40
Rice hull	2.0–2.8	0.3–0.8	34.5-45.9	13.2–21.0	22–34	66–74	1110–1 390	265-332	0.67–0.74	0.10-0.16



Fig. 5.1 Basic structure of a grain of rice, form the inside out, can be divided into five layers sequences. www.worldgranary.com Retrived 23 October 2015

of colored rice bran indicates that rice bran with certain color contains anthocyanin that has a reductase enzyme inhibitory and antidiabetic activity (Yawadio et al. 2007; Kim et al. 2008). Moreover, antioxidants in pigmented rice are able to reduce atherosclerotic plague formation, and some metabolic abnormalities associated with high fructose (Tananuwong and Tewaruth 2010) (Table 5.3).

The distribution of phenolic acids and anthocyanins in endosperm, embryo, and bran of white, red, and black rice grains was studied. It is found that the total phenolic content (TPC) was highest in the bran averaging 7.35 mg GAE/g and contributing 60, 86, and 84 % of phenolics in white, red, and black rices. The average TPC of the embryo and endosperm were 2.79 and 0.11 mg GAE/g accounting for 17 and 23 %, 4 and 10 %, and 7 and 9 % in white, red, and black rices, respectively. *Cis-p*-coumaric was detected in bound form in bran while *cis*-sinapic acid was detected in the free/conjugated form in embryo and bran. Cyanidin-3-O-glucoside and peonidin-3-O-glucoside were identified mainly in black rice bran as the total anthocyanins. Cyanidin-3-O-rutinoside was also detected in black rice bran (Shao et al. 2014a, b). Black rice bran has higher content of phenolics, flavonoids, and anthocyanins and has higher antioxidant activity compared to white rice bran (Table 5.4).

Table 5.2 Vi	tamin and miners	al content of roug	h rice and its n	nilling fractions at 14	% moisture (Ju	ıliano 1985; Pede	ersen and Eggu	um 1983)	
Rice	Thiamine	Riboflavin	Niacin	a - Tocopherol	Calcium	Phosphorus	Phytin P	Iron	Zinc
fraction	(mg)	(mg)	(mg)	(mg)	(mg)	(g)	(g)	(mg)	(mg)
Rough	0.26-0.33	0.06 - 0.11	2.9-5.6	0.90–2.00	Oct-80	0.17 - 0.39	0.18-0.21	1.4-6.0	1.7 - 3.1
rice									
Brown	0.29-0.61	0.04-0.14	3.5-5.3	0.90-2.50	Oct-50	0.17 - 0.43	0.13-0.27	0.2-5.2	0.6 - 2.8
rice									
Milled	0.02 - 0.11	0.02-0.06	1.3-2.4	75-0.30	30-Oct	0.08-0.15	0.02-0.07	0.2 - 2.8	0.6-2.3
rice									
Rice bran	1.20-2.40	0.18-0.43	26.7-49.9	2.60-13.3	30-120	1.1–2.5	0.9–2.2	8.6-43.0	4.3-25.8
Rice hull	0.09-0.21	0.05-0.07	1.6-4.2	0	60-130	0.03-0.07	0	3.9–9.5	0.9-4.0

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Rice fraction	Histi	Isoleucine	Leucine	Lysine + cyste	Methio	Phenyl	Threo	Tryptophan	Valine	Amino acid score <sup>a</sup>
	dine			ine	nine + tyrosin e	alanine	nine			
Rough rice	1.5-2.8	3.0-4.8	6.9-8.8	3.2-4.7	4.5-6.2	9.3-10.8	3.0-4.5	1.2-2.0	4.6-7.0	55-81
Brown rice	2.3-2.5	3.4-4.4	7.9–8.5	3.7-4.1	4.4-4.6	8.6-9.3	3.7-3.8	1.2-1.4	4.8-6.3	64-71
Milled rice	2.2-2.6	3.5-4.6	8.0-8.2	3.2-4.0	4.3-5.0	9.3 - 10.4	3.5-3.7	1.2-1.7	4.7-6.5	55-69
Rice bran	2.7-3.3	2.7-4.1	6.9–7.6	4.8-5.4	4.2-4.8	7.7-8.0	3.8-4.2	0.6–1.2	4.9–6.0	83-93
Rice hull	1.6 - 2.0	3.2-4.0	8.0-8.2	3.8-5.4	3.5-3.7	6.6-7.3	4.2-5.0	0.6	5.5-7.5	66–93
<sup>a</sup> Based on 5.8 §	tysine pe	r 16 g N as 1	H/V) % 001	IO, 1985)						

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Phenolic and Cinnamic acids	Anthocyanis, flavonoids	Steroial compounds	Polymeric carbohydrates
Caffeic acids	Anthocyanin monome	ers, dimers, and polymers	Arabinoxylan
Coumaric acid	Apigenin	Acetylated steryl glucosides	Glucans
Cathecins	Cyanidin glucoside	Cycloartenol ferulate	Hemicellulose
Ferulic acid	Cyanidinrutinoside	Campesterol ferulate	
Gallic acid	Eriodtyol	24-methylenecycloartenol ferulate	
Hydroxybenzoic acid	Hermnetins	r-oryzanol	
Methoxycinnamic acid	Hesperetin	b-sitosterol ferulate	
Sinopic acid	Isorhamnetins	Tocopherols	
Syringic acid	Luteolin	Tocotrienol	
Vanillic acid	Peanidin		
	Glucoside		
	Tricin		

 Table 5.4
 Bioactive compounds present in rice bran (Friedman 2013)

# 5.2 Brown Rice

Brown rice is the most widely produced rice variety worldwide. The bran of brown rice contains a higher level of gamma-tocotrienol (vitamin E compounds) and gamma-oryzanol (an antioxidant) which are lipid-soluble antioxidants. Numerous studies showed that these antioxidants can reduce blood levels of low density lipoprotein (LDL) cholesterol so-called "bad" cholesterol and may help fight heart disease. Temple University scientists have found a specific natural compound in brown rice that can reduce high blood pressure and protect blood vessels. Similarly, Harvard University research suggests consuming brown rice may prevent type-2 diabetes. The overall amylolytic activity of germinated black rice is observed to be higher than that of brown rice (Lee et al. 2013). A higher priority may be given to the development of rice varieties that contain high amounts of various bioactive compounds without altering their agronomic performance as well as preserving the cultural and socially acceptable organoleptic qualities. Brown rice seeds are rich in more nutritional components, such as dietary fibers, vitamins B and E, gamma-oryzanol, and amino butyric acid (GABA) than the ordinary milled rice grains. GABA or 4-aminobutyrate is a well-known non-protein-based amino acid is one of the major inhibitory neurotransmitters in the sympathetic nervous system. The changes of blood cholesterol can be modulated by using brown rice varieties instead of polished rice in human diet. Brown rice varieties are capable to show the hypercholesterolemic effect (Roohinezad et al. 2009). Rice millers remove only the outer husks, or chaff, from each rice grain to produce brown rice. If they process

Table 5.5         Nutrient content	Energy	1,527 kJ (365 kcal)
of long-grain white raw rice. Nutritional value per 100 g (3.5 oz) (USDA Nutrient Database)	Carbohydrates	80 g
	Sugars	0.12 g
	Dietary fiber	1.3 g
	Fat	0.66 g
	Protein	7.13 g
	Vitamins	
	Thiamine (B1)	0.0701 mg (6 %)
	Riboflavin (B2)	0.0149 mg (1 %)
	Niacin (B3)	1.62 mg (11 %)
	Pantothenic acid (B5)	0.164 mg (20 %)
	Vitamin B6	0.164 mg (13 %)
	Minerals	
	Calcium	28 mg (3 %)
	Iron	0.80 mg (6 %)
	Magnesium	25 mg (7 %)
	Manganese	1.088 mg (52 %)
	Phosphorus	115 mg (16 %)
	Potassium	115 mg (2 %)
	Zinc	1.09 mg (11 %)
	Other constituents	
	water	11.61 g

Percentages are roughly approximated using US recommendations for adults.  $\mu g$  = micrograms; mg = milligrams; IU = International units

the rice further, removing the underlying nutrient rich "bran" it becomes white rice. Consumers must have heard that brown rice is more nutritious than white rice. The reason is that the bran of brown rice contains higher level of gamma-tocotrienol (vitamin E compounds), and gamma-oryzanol antioxidants which are lipid-soluble antioxidants (Table 5.5).

Brown rice is a nutrition power house compared to white rice. Brown rice is rich in fiber, vitamin E, and cholesterol. Both brown and black rice are low in fat and are good source of healthy carbohydrates. Laboratory research conducted jointly at Temple University School of Medicine in Philadelphia and the Nagaoka National College of Technology in Japan attributes the cardio-protective effects of brown rice to a thin layer of tissue known as the sub aleurone layer that is rich in oligosaccharides and dietary fibers that is stripped away when brown rice is polished to make white rice. The researchers believe that missing layer may work against angiotensin II, an endocrine protein which contributes to the development of high blood pressure and atherosclerosis. According to these scientists, this could help to explain why fewer people die of cardiovascular disease in Japan compared to the US. In Japan most people eat at least one rice-based dish per day but in the US rice is not a mainstay of the daily diet. Brown rice is produced by removing
<b>Table 5.6</b> Chemical analysis         and phenolic compounds of         Black rice (IAC 600) and         whole rice in dry matter         (Salgado et al. 2008)	Analysis	Black rice	Whole rice
	Humidity (%)	87.95	73.09
	Lipids (%)	3.87	0.9
	Ash (%)	1.98	0.46
	Protein (%)	11.9	2.58
	Dietetic fiber (%)	5.67	1.8
	Carbohydrates (%)	63.45	22.96
	Phenolic compounds (mg = g)	23.78	2.45

only the outermost layer, the hull of the rice kernel keeping most of its nutritional value intact. But when the rice kernel is milled and polished to make it white, it gets destroyed. Along with it, all of the dietary fiber, vitamin B3, vitamin B1, vitamin B6, manganese, phosphorus, irons and all of the essential fatty acids get destroyed. Black rice offers the same health benefits of brown rice but it is also packed with some serious antioxidants. Because of its dark color, black rice bran contains the same anthocyanin antioxidants found in blueberries or blackberries. While brown rice is not a good source of anthocyanin, it is a source of vitamin E which is also an important antioxidant that might offer protection against chronic illness (Table 5.6).

# 5.3 Red Rice

In red rice varieties, the major phenolic acids in the free form are ferulic, protocatechuic and vanillic acid, whereas in black varieties protocatechuic acids are dominant followed by vanillic and ferulic acid. Antioxidant capacity of rice varieties range within 0.9-8.1 mmol Fe(II)/100. g DM for FRAP (Sompong et al. 2011). It is found that the total phenolic content of white, red, and black rice bran extract are in the range of 0.8931-0.9884, 1.0103-1.0494, and 1.0810-1.2239 mg gallic acid equivalent (GAE mg (-1)), respectively. However, the antioxidant activity of all rice bran extracts shows high antioxidant efficiency in the following order: red > black > white color rice brans (Muntana and Prasong 2010). Angrraini et al. (2015) reported that the non polished colored rice have higher antioxidant activity than white rice. The total phenolic content (TPC) and antioxidant capacity are highest at maturity stage in black rice (56.5–82.0) whereas in white (14.6–33.4) and red rice (66.8–422.2) highest accumulation is found 1 week after flowering. The total anthocyanin, cyanidin-3-glucoside, and peonidin-3-glucoside contents of black rice at second and third weeks of development after flowering are significantly higher than at other stages. While several phenolic acids are detected in the bound fraction, with ferulic as the dominant acid, red and black rice show high levels at first week development and at maturity (Shao et al. 2014a, b). It has also been reported that black rice has a scavenging activities higher than red rice variety, while noncolored rice has phenolic content and antioxidant activities which are lower than the colored rice variety (Muntana and Prasong 2010; Yodmanee et al. 2011). Despite its less anthocyanin content, red rice contains higher antioxidant activity compared to black rice (Muntana and Prasong 2010) due to its proanthocyanidin content (Finocchiaro et al. 2007). In a study, purple bran exhibited a minor effect on leukemia and cervical cancer cells, and the red bran exhibited strong inhibitory effects on leukemia, cervical and stomach cancer cells. Chemical analyses suggested that proanthocyanidins might be the major compounds in red bran extract attributed to the anti cancer bioactivity. Red bran has the potential to serve as a functional food supplement for human consumption (Chen et al. 2012).

Red rice cultivars contain malvidin. The total anthocyanin content varies greatly among black rice cultivars (79.5–473.7 mg/100 g), but is lower in red rice cultivars (7.9–34.4 mg/100 g). Total phenolic contents are similar between red (460.32–725.69 mg/100 g) and black (417.11–687.24 mg/100 g) rice. The oxygen radical absorbing capacity is ranked as follows: red (69.91–130.32 µmol Trolox/g) > black (55.49–64.85 µmol Trolox/g) > green (35.32 µmol Trolox/g) > white (21.81 µmol Trolox/g) rice. The antioxidant capacity results mainly from the seed capsule not from the endosperm. The anthocyanin pigments contribute little to the total antioxidant capacity of red (0.03–0.1 %) and black (0.5–2.5 %) rice cultivars. Hence, the antioxidant capacity is derived mainly from other phenolic compounds (Chen et al. 2012). Cells treated with red bran extract (RBE) showed higher protective effect compared to cells treated with white grain extract (WGE) against oxidative insult. According to "Consumer Reports *ShopSmart*" of April 2011 issue.

Any whole grain rice including black, brown, purple, red, wild, and half milled contains more fiber, iron and vitamins than white rice. White rice loses much of its nutritional value in the refining process that strips it of its germ and outer bran layer. Recent research has linked as yet unnamed compound in that layer to reduced blood pressure and a lower risk of clogged arteries. And black rice in particular contains a high level of anthocyanins, a class of disease fighting antioxidants.

#### 5.4 Black Rice

Black rice contains higher levels of anthocyanins than white rice, mainly composed of cyanidin 3-O-glucoside and peonidin 3-O-glucoside (Lee et al. 2014). Black rice contains more nutritional components such as dietary fibers, phytic acid, vitamin E, and vitamin B, than the ordinary milled rice (Banchuen et al. 2010). Salgado et al. (2008) reported that anthocyanin from black rice found higher antioxidant activity than red rice berry. From the nutritional point of view, black rice is the most famous one and generally used as an ingredient is snack and desserts (Tananuwong and Tewaruth 2010). In addition, rough rice retains higher levels of anthocyanin and antioxidant activity after germination than that of rice prepared from dehulled. Therefore, rice with husk intact should be employed for the preparation of germinated pigmented rice to protect anthocyanin and its antioxidant

activity loss during germination process (Sutharut and Sudarat 2012). In northern Philippines, a black rice variety, locally known as Ballatinao rice, is consumed widely in the Mountain Province, Benguet and other neighboring provinces. Chemical analysis of Ballatinao rice showed that it has the highest levels of anthocyanin, vitamin B, crude protein, total phenolics, and fatty acids when compared to red (Chochoros) and non-pigmented (NSIC Rc 160) rice varieties (Romero et al. 2012).

The lipid-soluble antioxidants found in black rice bran possess higher level of anthocyanins which are water-soluble antioxidants. Thus black rice bran may be even healthier than brown rice bran. The ethanolic extracts from pigmented rice cultivars show greater antioxidant activity than that of the normal white rice. The black rice exhibit the highest free radical scavenging activity, ferrous chelating ability, and total phenolic and flavonoid contents (Kang et al. 2013). Previous studies have also demonstrated that black rice bran could exert greater antioxidative, anticancer, anti-endotoxemia, antihepatic steatosis and anti inflammatory in animal models effects compared with white rice (Choi et al. 2010; Jang et al. 2012). It has been suggested that these properties of black rice are due to its high content of total protein (approx. 9.7-10.6 %) and crude fiber, as well as dark pigment ingredients (Hong and Oh 1996). Black rice shows greater effect against oxidative stress as compared to common rice. However, black rice bran is difficult to digest and shows slower absorption in the gastrointestinal system. Meng et al. (2005) have reported that black rice contains iron, zinc, calcium, copper and manganese higher than those in red rice. Dark purple grain has higher iron content, polyphenol content and antioxidant capacities than red brown grain (Yodmanee et al. 2011). Whole cereal grains have been received increasingly attention by consumers due to their potential health benefits because of their antioxidant capacity, which is probably derived from their high contents of phenolics, flavonoids, and other phytochemicals. Black rice bran has higher content of phenolics and anthocyanins, and has higher antioxidant activity when compared to white rice bran (Zhang et al. 2010; Goffman and Bergman 2004).

According to Dr. Zhimin Xu of Louisiana State University Agricultural Center as quoted by the American Chemical Society:

10 spoonfuls of cooked black rice is the equivalent of one spoonful of black rice bran, the exterior of the rice has as much anthocyanin as a spoonful of blueberries. Anthocyanins are water-soluble, unlike other antioxidants in black rice which are fat-soluble. This means the antioxidants in black rice can reach many different parts of our body. I think the black rice bran has an advantage over blueberries, because blueberries still contain a high level of sugar.

Black rice is rich in anthocyanin antioxidants, substances that show promise for fighting heart disease, cancer, and other diseases. Some antioxidants in black (and brown) rice are fat-soluble, while anthocyanins are water-soluble and can therefore reach different areas of the body

Says Joe Vinson, PhD, a Professor of Chemistry at the University of Scranton in Pennsylvania. White rice has been stripped of the healthful anthocyanin rich bran that makes black rice so nutritive. The bran of brown rice has been shown to contain higher concentrations of gamma-oryzanol antioxidants that lower LDL "bad" cholesterol and help prevent heart disease.

Black rice contains biologically many active compounds. Black rice extracts attenuated oxidative insult by inhibiting cellular ROS and malondialdehyde MDA increase and by modulating antioxidant enzyme activities in HepG2 cells (Lee et al. 2014). Tang et al. (2015) reported that greater phenolics and antioxidant capacities are detected in non-waxy rice rather than waxy one. The black variety shows the highest antioxidant capacity and phenolic content among the analyzed varieties in terms of quantity and type of molecules containing anthocyanins, flavonols and phenolic acids (Zaupa et al. 2015).

### 5.5 Difference in Black and Brown Rice

Some major differences in black and brown rice are described here in detail.

## 5.5.1 Difference in Calories

A 1/3-cup serving of dry black rice contains 200 calories while the same serving of brown rice contains 226 calories. Twenty six calories may not seem like much of a difference but consuming an extra 26 calories a day over one year can lead to a 2.7 pound weight gain. One cup of cooked black rice (1 cup = 201 g = 7.1 oz) contain 200 calories.<sup>2</sup> The calorie content of one cup of cooked rice varies from a high of 241.8 kcals for medium- or short-grain white rice to 218.4 kcals for medium grain brown rice, 216.5 kcals for long-grain brown rice, 205.4 kcals for regular long-grain white rice to a low of 165.6 kcals for wild rice (Ricepedia).

## 5.5.2 Differences in the Carbohydrates, Protein and Fat

Black rice is lower in carbohydrates but higher in fiber, and a better source of protein than brown rice. A 1/3-cup serving of dry black rice contains 43 g of carbohydrates, 3 g of fiber, 6 g of protein, and 2 g of fat while the same serving of brown rice contains 47 g of carbohydrates, 2 g of fiber, 5 g of protein, and 2 g of fat.

<sup>&</sup>lt;sup>2</sup>www.blackrice.com Retrived 23 June 2015.

#### 5.5.3 Difference at the Minerals

The mineral content between both black and brown rice is very similar. A serving of either rice meets 8 % of the daily value for zinc and 20 % of the daily value for phosphorus. But the black rice is a slightly better source of iron meeting 6 % of the daily value compared to 5 % of the daily value in a serving of brown rice. Zinc is a mineral that supports immune health, phosphorus is needed for the formation of teeth and bones and iron helps transport oxygen throughout the body.

#### 5.5.4 Difference in Antioxidant Power

A major difference between the black rice and brown rice is its color. The color of black rice also makes it a better source of antioxidants according to the American Chemical Society. Anthocyanin, a pigment found in the rice grain creates its dark hue is an antioxidant that may aid in fight against heart disease and cancer. Cyanidin-3-glucoside and peonoidin-3-glucoside are confirmed as the dominant anthocyanins in black rice varieties with contents ranging from 19.4 to 140.8 mg/100 g DM and 11.1–12.8 mg/100 g DM respectively (Sompong et al. 2010). The predominant anthocyanins are cyanidin-3-glucoside (572.47  $\mu$ g/g, 91.13 % of total) and peonidin-3-glucoside isomers and one cyanidin hexoside. The antioxidant activity of all rice bran extracts indicates high antioxidant efficiency in the following order: red > black > white color rice brans (Pakistan Journal of Biological Sciences 2010, 13: 170–174).

One spoonful of black rice bran or 10 spoonfuls of cooked black rice contains the same amount of anthocyanin as a spoonful of fresh blueberries

According to a new study presented at the American Chemical Society in Boston.

Black rice bran possesses strong scavenging activities for reactive oxygen species (ROS). Identified candidate scavengers are cyanidin-3-glucoside (Cy-3-glu) and cyanidin. Although ferulic acid is known to be an antioxidative component of bran in currently available common white rice varieties but it is not found in the black rice bran extracts. These anthocyanin compounds are found to possess both strong ROS scavenging activities and to suppress cell-damaging effects of UVB, indicating that both Cy-3-glu and cyanidin are the active components involved in the antioxidative activity of black rice bran extracts (Kaneda et al. 2006). The Boston based Whole Grains Council refers on its site that a team of researchers at Cornell University found antioxidants are about six times higher in black rice than in common brown and white rice. The researchers looked 12 varieties of black rice and analyzed the phenolic content and antioxidant activity also.

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# Chapter 6 Economic Importance

Black rice is mainly used in Asia for food decoration, making noodles, sushi, and pudding. It is also used in place of brown or white rice and is often featured in dessert recipes particularly rice puddings because its natural flavor is rich and sweet. Food manufacturers could potentially use black rice bran or bran extracts to make breakfast, cereals, beverages, cakes, biscuits, and other foods healthier (www. independent.co.uk). Scientists also demonstrated that pigments in black rice bran extracts can produce a variety of different colours ranging from pink to black and may therefore provide a healthier alternative to artificial food colorants. Some studies have linked artificial food colorants badly act as behavioural problems in children and other health problems including cancer. Thus, black rice bran is one of the best natural alternative food colourants. Black rice bran is also used in local medicines because of its therapeutic value. It is gaining popularity in foodie circles around the world for its unusual taste and lovely colour. Thus, Black rice should be developed in food applications as health products, food industry and pharmaceutical to add value to rice (Saenkod et al. 2013).

Tananuwong and Tewaruth (2010) suggest that the rice extracts with anthocyanins could be used as both antioxidant and colorant in food and beverages. BRB can be an excellent ingredient to increase the nutritional value and antioxidant properties of noodles (Kong et al. 2012). Black rice bran (BRB), a waste product from the rice milling process, has gained recent attention for its potential use as a functional food because it contains high levels of polyphenols, especially anthocyanins which are mainly found in the pericarp and aleurone layers of the bran fraction removed from the rice during the milling process (Jang and Xu 2009; Yawadio et al. 2007). Moreover, the bran is a rich source of bioactive substances such as tocopherols, tocotrienols, and  $\gamma$ -oryzanol (Loypimai et al. 2015; Ryynanen et al. 2004), which are well known as beneficial compounds for human health. Among all of the studied coloured grains, black rice possess the highest TPC, which is 86 times greater than that of red rice. In addition, black rice has the highest total anthocyanin contents and R-glucosidase inhibitory activity. A significant positive correlation of the antioxidant activity and R-glucosidase inhibitory activity with

101

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total anthocyanin content and TPC is observed. Black rice possesses the highest antioxidant activity and R-glucosidase inhibitory among all of the coloured grains tested and can be further explored as a functional food (Yang et al. 2009).

The black rice with highly concentrated anthocyanins has the potential to replace a wide range of artificial colorants in foods and dietary supplements. Extracts of pigmented rice have great potential to be new sources of natural pigments for applications in the food and pharmaceutical industries on the basis of their anthocyanin content and composition. Black rice varieties contain a rich heterogeneous mixture of phytochemicals which may provide a basis for the potential health benefits, and highlights the rice as a functional food (Bordiga et al. 2014). Recent reports indicates the various potential health benefits of anthocyanin that has led to increased interest in the use of this pigment in common food sources (Zhang et al. 2004). Therefore, the presence of anthocyanin in food could become a major factor considered by consumers when making a purchasing decision and, as such, could ultimately influence human behavior (Robert and Naofumi 2007). The black purple rice in the dehulled form in which it is consumed by humans contains a rich heterogeneous mixture of phytochemicals which may provide a basis for the potential health benefits and highlights the possible use of the rice as functional food (Caro et al. 2013). Knowing the phytochemical profile and antioxidant activity, black rice bran gives insights to its potential application to promote health (Zhang et al. 2010).

Black rice bran powder has potential medical uses and functionality and it is an effective ingredient for increasing the consumer acceptability and functionality of cookies (Joo and Choi 2012). Sebnem and Tuncay (2014) results demonstrated that methanol extract from black rice show strong DPPH (1, 1-diphenyl-2-picrylhydrazyl radical) scavenging effect and antioxidant ability. This result could help rice producers to promote black rice. Black rice generally used as an ingredient in snacks and desserts in some regions. In Turkey, as in other countries, black rice is used in food industry due to its unusual colour and sweet nutty flavor. Typically black rice grains are aromatic and its fragrance is an important feature of premium value rice which commands higher prices in domestic and international markets. This rice should be used in food industry, cosmetics, and health products, pharmaceutical and medicinal applications. As extrusion increases the free phenolics, anthocyanins and oxygen radical absorbance capacity (ORAC) and decreased the bound forms. Thus these results provide the basis for the development of different milled fractions of extruded black rice with balanced nutritional characteristics for today's functional food markets (Ti et al. 2015).

Black rice is popular in Asian countries where it is mixed with white rice prior to cooking to enhance the flavor, colour and nutritional value (Yang et al. 2008). Historically, black rice has been reserved for use in festival foods and desserts in Asian countries. Typically, black rice grains are aromatic and because grain fragrance is an important feature of premium value rice, it commands higher prices in domestic and international markets. In Southeast Asia, especially in Lao PDR and north and northeastern regions of Thiland, black rice serves as the staple food; Khao kam is the traditional name of black rice in these regions (Xia et al. 2006). The black rices of Manipur, India have their own importance as glutinous or sticky rice and are used for the community feast as well as ceremonial purposes as delicacy. It is sold in the local markets at about Rs. 325 per kg of rice. The black aromatic rice of Manipur is served in standard hotels as a top rated variety. Rice flake prepared from black rice is becoming popular. Though the black glutinous aromatic rices of Manipur are poor yielders (about 2,500 kg/ha), these rices cover less than 10 % of the rainfed wetland area under local cultivars, however, there is a premium in the price of these rices. In Nepal, this rice is sold Rs 475–550 per kg in supermarkets and high class people are attracted to consume this rice.

Besides its application in food industry, black rice is also used as construction materials. Incorporation of Black rice husk ash (BRHA) decrease compressive strength at all ages. And addition of 1.5 % nano TiO<sub>2</sub> enhances mechanical properties of BRHA mortars. Nano TiO2 incorporation improves microstructure of BRHA mortars (Noorvand et al. 2013). Black rice pigment sensitized TiO<sub>2</sub> thin film is used to determine the biogenic amines generated by pork during storage. The developed films have good sensitivity to analogous gases such as putrscine, and cadaverine that will increase during storage (Yanxiao et al. 2014). BRHA also provides a positive effect on the autogenous shrinkage and weight loss of concretes exposed to hydrochloric and sulfuric acid attacks. In addition, the resistance to acid attack directly varied with the (SiO\n 2 + Al\n 2O\n 3 + Fe\n 2O\n 3)/CaO ratio. Results show that ground BRHA can be applied as a pozzolanic material and also improve the durability of concrete (Chatveera and Lertwattanaruk 2011). Similarly, Chatveera and Lertwattanaruk (2009) reported that BRHA can be applied as a pozzolanic material to concrete and also improve resistance to sodium sulfate attack, but it can impair resistance to magnesium sulfate attack.

Hom Dang (black rice) seemed to be the most potent antioxidative stress and could be considered as a candidate extract for cosmetic preparation (Butchan et al. 2014). Black rice and red rice cooking water have the potential of being new antioxidant drinks (Handayani et al. 2014). Similarly, A 16S rDNA homology research tells that the soil microorganism with the ability to modify the structure of black rice anthocyanin is an *Enterobacter aerogenes* strain and this discovery has industrial applications too (Saigusa et al. 2014).

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# Chapter 7 Black Rice Applications

Food color is a significant part of food products; not only for its appearance but also for its attractive consumer acceptability. Black rice powder extracted from the rice bran could be used as a healthful food coloring dye in sodas and other products. The effective extraction of active phenolic compounds from pigmented rice can be utilized for developing functional foods, cosmetics and in nutraceutical, pharmaceutical, and other health products. Study of black and red rice bran shows high antioxidant activities and they contain high amount of phenolic compounds too. Indeed, black and red rice bran could be better raw materials for manufacturing the food with high antioxidant activity (Jang et al. 2012). Black Rice Bran (BRB) can be an excellent ingredient to increase the nutritional value and antioxidant properties of noodles (Kong et al. 2012). Bran is the hard outer layer of rice.

Black sticky rice is eaten most often as a sweet pudding in Thailand. The rice is boiled in water until cooked, drained, and served soaked with sweetened coconut milk. A thicker pudding of sweetened black rice cut into squares, is often sold in markets in the South. In Central Thailand, black rice is combined with white sticky rice, sweetened coconut milk, black beans, and roasted inside hollow sections of bamboo cane. Black rice is also fermented with Thai yeast powder which mildly alcoholic and eaten as a snack. This rice can be served as a savory dish, combined with steamed white sticky rice or even as a basis for a rice salad. Black sticky rice is also used primarily in sweet snacks and desserts in Asia. Black rice grain is often used to make Chinese desserts, though it is also popular in many other Asian nations, all of which have their own special names for the product. It tends not to be very glutinous, although it is sometimes treated that it would be less sticky. Researchers claimed that black waxy rice is one of the most potential plant sources of dark purple color of anthocyanins which is used for making different products and among them some are listed here:

Some of the popular products of black rice:

- Black Rice Bran
- Black Rice Flour
- Black Rice Noodles
- Black Rice Pasta
- Black Rice Vinegar
- Black Rice Beer
- Black Rice Wine
- Black Rice Liquor
- Black Rice Punch
- Black Rice Chocolate
- Black Rice Tea
- Black Rice Milk
- Black Rice Sushi
- Black Rice Yeast
- Black Rice Bread
- Black Rice Cakes
- Black Rice Cookies
- Black Rice Crackers
- Black Rice Crispies
- Black Rice Chips
- Black Rice Muesli
- Black Rice Cereal
- Black Rice Porridge
- Black Rice Congee
- Black Rice Kheer
- Black Rice Extract
- Black Rice Pigment
- Black Rice Soap
- Black Rice Scrubs
- Black Rice Skin Cream

# 7.1 Natural Food Coloring Dye

Black rice extracts is an excellent natural food coloring dye. It may offer a healthy alternative to the artificial food colorings whereby some manufacturers add to beverages and foods. The pigments in black rice extracts can produce a variety of

colors from black to pink which might make the powder of black rice bran a good source for natural food coloring. The 2011 "New York Times" article, "F.D.A. Panel to Consider Warnings for Artificial Food Colorings," discussed possible connections between artificial food colourings and health conditions such as cancer, allergies and children's physiologically based behavioral problems. The use of natural anthocyanin pigments as coloring agents in food products is receiving increasing attention as they are attractive to consumers and have positive health benefits (Chou et al. 2007). Anthocyanin pigments are permitted as natural food colorants in the USA under the category of fruits (21 CFR 73.250) and vegetables (21 CFR 73.260), and the EU classification number is E163 (Lipman 1996; Wrolstad 2000). Pigments in black rice bran extracts can produce a variety of colors, from pink to black, and may be a healthier alternative to artificial food colorants that manufacturers now add to some foods and beverages. Black rice bran could be used to boost the health value of foods, such as snacks, cakes, and breakfast cereals.

## 7.2 Future Uses of Black Rice

Increasing attention has been paid to the important nutrients and antioxidants of black rice and black rice bran. Researchers from Louisiana State University in 2010 suggested that food manufacturers began including black rice bran in certain foods including cereals, cakes, cookies and drinks in order to boost nutrients and bring health-related improvements. Aside from its use as a dietary or grain staple, Chinese black rice is used to make black vinegars, particularly from Zhejiang variety from that region of China. Vinegar when not transliterated into pinyin; it is spelled Chekiang. This type of black vinegar has been compared with balsamic vinegar. Chinese black rice is also used to make different kinds of wine. Almost all of these wines are delicately scented. They are excellent for drinking and for cooking purposes (www.Epicurious.com).

Black waxy rice is one of the most potential plant sources of dark purple color of anthocyanins. The colorant powder contains higher concentration of phytochemicals than untreated black rice bran. The successful application of colorant powder has been achieved in terms of providing a purplish pink color to yogurt, achieving a good stability of color, and increasing phytochemicals contributed by black waxy rice bran. This makes the colorant powder possible to apply in food products as a functional food colorant. However, for further applications of this functional food colorant to other food products, more quality evaluations are required such as sensory and safety evaluation. Jun et al. (2015) reported that black rice bran

contains phenolic compounds of a high antioxidant activity. Jun and their colleague finding identified ferulic acid as a major phenolic compound in black rice bran, and supported the potential use of black rice bran as a natural source of antioxidant. Particularly, the ethyl acetate fraction and subfraction two have potential for use as functional food additives in breakfast, cereals, snacks, breads, beverages, cakes, cookies, and other foods because they have stronger antioxidant activity than butylated hydroxytoluene (BHT). Black rice bran contains gallic, hydroxybenzoic, and protocatechuic acids in higher contents than red rice bran and normal rice bran. Furthermore, the addition of 5 % black rice bran to wheat flour used for making bread produced a marked increase in the free radical scavenging and antioxidant activity compared to control bread (Laokuldilok et al. 2011).

The wide variation in the physicochemical properties of the black rice varieties have been analyzed and these results could serve as baseline information for food processors in evaluating the quality of black rice suitable for specialty food processing (Kang et al. 2011). Kim et al. (2010) said that addition of optimum amounts of blueberry and black rice powders enhanced the overall quality of the Korean traditional rice wine takju. The DPPH radical scavenging activity of the mead made from the black rice grains was higher than that of beverages made from polished rice. The inhibitory activity of lipid peroxidation of the mead made from black rice grains was also higher (Katoh et al. 2011). Similarly, the anthocyanin content of

**Fig. 7.1** Instant sticky black rice



beverages made from uncooked black rice was higher than that of the beverages made from the cooked black rice. The antioxidative activity of alcoholic beverages made from uncooked black rice was also higher than that of beverages made from cooked black rice (Teramoto et al. 2011). Thus, now black rice (*Oryza sativa* L.) and its products are becoming increasingly popular and they are widely consumed in China, Japan, Korea, and other East Asian countries such as Thailand (Caro et al. 2013; Hou et al. 2013) (Figs. 7.1–7.9, Minh 2014) (Figs. 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8 and 7.9).

Fig. 7.2 Instant dried sticky black rice





Fig. 7.3 Sticky black yogurt

Fig. 7.4 Sticky black wine





Fig. 7.5 Red glutinous dairy rice flour

Fig. 7.6 Black sticky rice ice bar



Fig. 7.7 Sticky black bread





Fig. 7.8 Black glutinous rice flour

Fig. 7.9 Black sticky rice wine



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# Chapter 8 Black Rice Cultivation

Cultivating black rice is similar to the general rice cultivation practices. But one should follow some farming rules during cultivation of this forbidden rice. This rice should be grown in organic way in natural environment to maintain its quality. A research trial conducted at the farm of Agriculture Botany Division, Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal on Chinese black rice in 2014 showed that this variety is earlier in maturity than other popular Nepalese rice varieties. It takes about 115–120 days to mature and has medium days to maturity according to Nepalese rice cropping pattern. Thus this rice is 30–40 days earlier in maturity than the locally grown rice. Therefore, farmers can take additional crop benefit like legume or vegetables from the same land using year-round cropping pattern (Kushwaha and Khatiwada 2015). The price of this rice is five to ten times as expensive as normal white rice but the cost of black rice production is equal for both black and white rice. For bumper production and improved seed quality, still research and extension program are needed to continue. Black rice productions in organic farming system do not meet the demand of local customers because of its low productivity. Thus better crop management practices and high yielding varieties are needed to meet the demand. Production of this rice can be increased up to maximum limit with proper crop care and management (Table 8.1). Black rice growth is influenced by water quantities, soil, weed communities, and cultivating systems with predictive relevance value reaching 92.83 %. Water quantities in paddy field are key factors which directly and indirectly determine the growth and productivity of black rice (Budiman and Arisoesilaningsih 2015). China has already developed many high-yielding black rice varieties (of many types coarse, fine, and medium fine grain) still many works have to do.

An experiment conducted in Agriculture Botany Division, Khumaltar, Lalitpur, Nepal showed that black rice can escape cold stress during its reproductive stage. It is because black rice is earlier in heading compared to other popular rice varieties. Thus the transplanting time should be adjusted so that rice heading initiates before cold starts (Kushwaha and Khatiwada 2015). This enthusiastic results show that

Black rice	Spacing	Clumps number (ha)	Dry seed weight (ton/ha)	Vegetative biomass (ton/ha)
Woja Laka	25 cm × 32 cm	125		
Max. growing			5.96	7.39
Min. growing			4.79	5.72
Average			5.37	6.56
Laka	22 cm × 22 cm	206.612		
Max. growing			7.83	19.9
Min. growing			7.06	15.66
Average			7.44	17.78

Table 8.1 Black rice productivity per ha in organic paddy field (Budiman and Wibowo 2012)

black rice can be cultivated from tropical to temperate regions. For Nepal, this rice can be cultivated in terai, i.e., plain regions, mid hill, and high hill regions.

The production of Woja Laka black rice variety which was cultivated in irrigated organic paddy field only reached 6 t/ha especially in dry seasons (Table 8.1). The production might be fewer in rainy seasons. This condition was related to sensitivity of black rice populations to natural enemies such as birds and rats and their responses to environment conditions (Budiman and Wibowo 2012). Previous researches reported by Skerman and Riveros (1990), Leesawatwong (2005), Rao et al. (2006), Sanusan et al. (2010), and Zhu et al. (2010) implied that altitude, microclimates (temperatures, precipitations, and radiation), soil conditions (textures, organic matters, pH, bulk density and nutrition), water availability, and weeds density significantly influence the growth of rice. In nature, environmental factors interact simultaneously with each other and affect the growth of black rice either directly or indirectly. Naharia et al. (2005) reported that paddy field which was flooded produced highest number of tillers and spikes than that with boggy and drought conditions. It is also reported that drought conditions at paddy field have decreased rice production up to 60 %. It was caused by the rate of grain filling which was decreasing in drought conditions (Pringgohandoko 2004). Flooded conditions at paddy field increase soil pH and conductivity and also decrease weed biomass which in turn increase clump height and the number of spikes of black rice. Cultivation system with wider spacing should be applied because it is able to increase black rice growth and productivity (Budiman and Arisoesilaningsih 2015).

## 8.1 Black Rice Varieties

Sticky black rice is a type of rice grown in many Asian countries: Laos, Vietnam, China, Indonesia, Malaysia, Japan, Korea, Philippines, Bangladesh, Thailand, and Cambodia. In Thailand, several pigmented rice varieties have been cultivated and consumed especially black and red rice. Hom Nil black rice (HN), black glutinous rice, and Munpu red rice (MP) are well known as commercial pigmented rice cultivar and possess many active compounds with antioxidant activities. Recently, many attempts have been made to develop better rice varieties which are rich in certain functional compounds exhibiting antioxidant activities, so that some type of rice varieties may be bred which has not only higher yield but also better quality containing increased levels of bioactive compounds. The content of antioxidative substances, i.e., polyphenol in rice grain, is affected by genotype and environment (Goffman and Bergman 2004). For example, C3GHi, a novel black rice variety developed by conventional breeding, has more high contents of cyanidin-3-glucoside (C3G) and a more strong antioxidant than normal black rice (Kim et al. 2010).

Rice cultivars that possess higher degrees of salt tolerance display more enhanced activity of catalase (CAT), a smaller increase in anthocyanin, hydrogen peroxide, and proline content but a smaller drop in the K+/Na+ ratio and chlorophyll accumulation (Chunthaburee et al. 2015). Black scented rice (Chakhao) has a defensive mechanism which can protect it against some of the diseases and pests as a result of the high anthocyanins and phenolic content. The anthocyanin pigment benefits the human health which is also involved in the plant's defensive mechanisms. The research on the importance of these cultivars should encourage the agricultural scientists to include them in the crop improvement programs, and research could increase productivity without losing grain quality characteristics and result in pharmaceutical applications as well as increasing our knowledge of plant defense mechanisms (Asem et al. 2015). The rice grains with extremely small size or low 100-grain weight generally has higher phenolic content, flavonoid content, and antioxidant capacity than grains with normal or large size. Phenolic content and antioxidant capacity of rice grain are significantly correlated with each other. The phenolic content could be indirectly predicted by grain length and 100-grain weight. Therefore, new rice varieties high in antioxidant levels could be achieved by breeding for extremely small grain rice (Yafang et al. 2011). Kushwaha and Khatiwada (2015) found that sufficient soil moisture and optimum temperature during grain filling period of crop increased the anthocyanin content of black rice grain in mid-hill region of Nepal. Kushwaha also reported that anthocyanin content of black rice increases if same rice is grown in cooler climate than warm environment provided sufficient soil moisture during crop growing period. The anthocyanin content also increases as altitude increases from mean sea level. This may be because increase in altitude reduces temperature of environment. Black rice gets more days to mature and anthocyanin is filled completely.

The antioxidant capacity of newly developed and highly popular pigmented rice cultivars (black rice, Galsaekchalmi, Jeoktomi, Hongchalmi, and Nogwonmi) in South Korea was analyzed. The reducing power and phytic acid content were found to be highest in Hongchalmi cultivar. The inhibition of lipid peroxidation was markedly higher in Jeoktomi compared with the other rice samples. The Nogwonmi rice showed the lowest antioxidant activity among the pigmented varieties analyzed. These findings provide valuable information on the antioxidant potential of newly developed pigmented rice varieties and may assist plant breeders in the



Fig. 8.1 Chinese black rice in different growth stages, i.e., tillering, flowering, and maturity stage

selection of cultivars for the development of new lines of rice with enhanced functional quality (Kang et al. 2013a, b) (Fig. 8.1).

## 8.1.1 Typical Characteristics of Black Rice

The special characteristics of black glutinous rice is that they have small and slender culms, narrow short leaves, purple leaf margins, purple leaf tips, and purple stripes on leaf blades and sheathes (Bounphanousay 2008). Black rice could more accurately be described as purple rice as it has a deep, dark vermillion hue when cooked. The name covers a range of rice types including Thai Jasmine black rice and Indonesian black rice. In comparison with ordinary Thai black-colored rice varieties, the newly developed black rices possess longer and more translucent grains which retain Jasmine's cooking qualities of softness, good taste, and good smell. Additionally, some of these new black rice varieties offer an advantage of being fast growing and capable of three crops per year (Pitija et al. 2013). Sticky, sweet, and glutinous black rice is a type of short-grained black rice that is especially sticky when cooked. It is called glutinous in the sense of being glue like (hence, the name glutinous) or sticky, and not in the sense of containing gluten. Two black rice varieties based on waxiness are "black non-waxy" and "black waxy rice." Black rice has diversity in terms of color due to anthocyanin content and other morphological characters. Black glutinous rice is natural rice with grains are unevenly colored and that look like wild rice when dry. Its rich nutty flavor is distinctly

different from the more subtle delicateness of white glutinous rice. Most studies about black rice are done on varieties cultivated in China (Zuo et al. 2012), Thailand (Sangkitikomol et al. 2010), and to some extent, Korea (Seo et al. 2013). Local black rice varieties have low threshing quality. Farmer's feel difficult to thresh grains from its panicles. Black rice varieties have been the subject of exploratory research for its potential biomedical applications (Sangkitikomol et al. 2010) (Table 8.2).

## 8.2 Method of Cultivation

Review of rice production methods has shown that rice cultivation practices range from very primitive to highly mechanized (Luh 1980; Yoshida 1981). In Asia, animals (buffalo, ox, and carabao) are still used for plowing and harrowing. Land preparation may be carried out while the soil is dry or wet, depending on the water supply. For irrigated rice, the soil is prepared wet or puddled in Asia, but puddling is not generally practiced in America, Europe, and Africa. In areas without a hard pan, where animals and tractors sink in the mud, the soil is prepared with hand hoes. Regardless of whether the land is prepared wet or dry, the water is always held on the lowland fields by bunds (FAO).

Ideally, water is maintained in the rice field to suppress weed growth during the growing season. Herbicides are also economical and effective. Fertilization is normally practiced for increased yield, particularly with the modern, semi-dwarf, or high-yielding varieties which respond well to fertilizer without lodging. Both inorganic and organic fertilizers are used, including green manures such as the leguminous shrub *Sesbania* spp. and the water plants *Azolla* and *Anabaena* spp. Modern rice varieties increase in grain yield by 6 kg per kg of applied fertilizer in the wet season and by 9 kg per kg of applied fertilizer in the dry season. Total fertilizer nutrients range from 10 to 100 kg/ha in tropical Asia and from 200 to 350 kg/ha in Japan, Taiwan, and the Republic of Korea (Barker et al. 1985).

Following methods of rice cultivation are practised in south Asia.

## 8.2.1 Broadcasting Method

Seeds are broadcasted by hand. It is the easiest method requiring minimum input but its yields are also minimum. The field is prepared and puddled in the same manner as in the case of transplanted rice. About 100 kg seed is required for one hectare of land. Seeds should be soaked in water and wait for pregermination before broadcast. Pregermination may take few hours to few days depending on the type of variety and seed dormancy. Instead of transplanting the seedlings in the puddled field, the sprouted seeds with radicle length one to two millimeters are uniformly

Name of rice	Seed color	Origin of black rice	Sub species
Melik	Black	Kedon-Ganjuran-Bantul Yogyakarta	
Jlitheng	Black	Sleman–Yogyakarta	
Cempo Ireng	Black	Seyegan-Sleman–Yogyakarta	
Pari Ireng	Black	Padasan-Pakembinangun-Sleman Yogyakarta	
Padi Hitam NTT	Black	Alor-NTT	
Padi hitam Bantul	Black	Njayan—Imogiri—Bantul	
Padi Hitam Magelang (hairy)	Black	Sawangan—Magelang—Central Java	
Padi Hitam Magelang (hairless)	Black	Sawangan—Magelang—Central Java	
Padi Hitam Sragen	Black	Sragen—Central Java	
Padi Hitam Wonosobo	Black	Wonosobo—Central Java	
Padi Hitam Banjarnegara	Black	Banjarnegara—Central Java	
Niaw Dam Pleuak Khao (PK)	Black		
Niaw Dam Pleuak Dam (PD)	Black		
China Black Rice (CNB)	Black	China	Indica
Thai Glutinous Black rice (kao niow dam)	Black	Thiland	
Homnin black rice (HN)	Black	Thiland	
Munpu red rice (MP)	Black	Thiland	
Khaohom Maephayatongdam	Black	Thiland	
Ballatinao Black Rice	Black	Thiland, Phillipines	
Artemide	Black	Piedmont	
Venere	Black	Piedmont	
Nerone	Black	Piedmont	
Heugkwangbyeo	Black		
Heugjinjubyeo	Black		
Asamurasaki	Black		
Okunomurasaki	Black		
Chinakuromai	Black		
Thai Jasmine Rice	Black	Thiland	
Indonesian black rice	Black	Indonesia	
Hom Nil black rice	Black		
Sticky black rice	Black		
Sweet black rice	Black		
Glutinous black rice	Black		

 Table 8.2
 Some popular black rice varieties (Kristamtini et al. 2012; Oikawa et al. 2015)

(continued)

## Table 8.2 (continued)

Name of rice	Seed color	Origin of black rice	Sub species
Hong Xie Nuo	Black	China	Indica
Toketsumochi	Black	China	Indica
Yunan Shiping	Black	China	Indica
Puluik Arang	Black	Indonesia	Indica
KH. Kam	Black	Laos	Indica
Kurogome	Black	Japan	Indica
Yayoimurasaki	Black	Japan	Indica
Akamai	Black	Japan	Temperate japonica
Kuromai	Black	Japan	Temperate japonica
Asamurasaki	Black	Japan	Temperate japonica
Shiho	Black	Japan	Temperate japonica
Xiang Xie Nuo	Black	China	Temperate japonica
Ladang	Black	Indonesia	Tropical japonica
Alus	Black	Malaysia	Tropical japonica
Hitam Pulut	Black	Malaysia	Tropical japonica
Galo	Black	Philippines	Tropical japonica
Kinangdang Itim	Black	Philippines	Tropical japonica
Mitak	Black	Indonesia	Tropical japonica
Ketan Hitam	Black	Indonesia	Tropical japonica
Khao Kam (JP#80521)	Black	Laos	Tropical japonica
Khao Kam (JP#80522)	Black	Laos	Tropical japonica
KH Chepheum	Black	Laos	Tropical japonica
Zhejiang	Black	China	
Woja Laka	Black	Thiland	
Laka	Black	Thiland	
Chakhao	Black	India	
Waxy Black rice	Black		

(continued)

Name of rice	Seed color	Origin of black rice	Sub species
Non waxy black rice	Black		
Kamklaing	Black	Thiland	
Galsaekchalmi	Black	South Korea	
Jeoktomi	Black	South Korea	
Hongchalmi	Black	South Korea	
Nogwonmi	Black	South Korea	
IC 60	Black	South Korea	
Heimi	Black	China	
Jing Nian	Black	China	
Nang Dum	Black	Thiland	

Table 8.2 (continued)

broadcasted by hand. It is a cost-effective method as the number of labor is decreased in this method.

# 8.2.2 Drilling Method

Plowing of land and sowing of seeds is done by two persons. This method is mostly practiced where there is a lack of irrigation facility and or in upland areas.

# 8.2.3 Transplantation Method

This method is practised in areas of fertile soil, abundant rainfall and plentiful supply of labor. To begin with, seeds are sown in nursery and seedlings are prepared. After 4–5 weeks the seedlings are uprooted and planted in the field which has already been prepared for the purpose. The entire process is done by hand. It is, therefore, a very tedious method and requires heavy inputs. But at the same time it gives the highest yields.

# 8.2.4 Japanese Method

This method includes the use of high-yielding varieties of seeds, sowing the seeds in a raised nursery bed, and transplanting the seedlings in rows so as to make weeding and fertilizing easy. It also involves the use of a heavy dose of fertilizers so that very high yields are obtained. Similar to other rice varieties, black rice is also grown mainly in two types of lands, i.e., uplands and lowlands. The system of rice cultivation depends on different factors such as situation of the land, type of soil, nutrient content of the soil, irrigation resources, availability of laborers, type of rice variety, intensity and distribution of rainfall, etc. The following are the principal systems of rice cultivation (Singh 2001).

## 8.2.5 Upland Cultivation

Generally, black rice is not cultivated in dry or semi-dry upland conditions. It is because black rice is sensitive to water, and inadequate supply of water to the rice field affects its nutritional quality and its yield attributes. In this method, the crop depends entirely on rains. Farmers usually grow short duration (up to 100 days) indigenous varieties with little inputs in such areas. Using improved dwarf varieties and new technology the farmers can take higher yields.

## 8.2.6 Lowland Cultivation

This system of rice culture is practiced with an assured irrigation facility. In this system, sprouted seeds may be directly sown in puddled field or the crop may be transplanted with seedlings raised in a nursery. Generally, black rice cultivation needs assured irrigation facilities to maintain its grain quality, and thus here only lowland cultivation practices of rice are described.

## 8.2.7 Transplanting Methods in Detail

About 25–30-day-old seedlings are transplanted to the well-prepared field. Robust and healthy seedlings and good nursery boost crop yield. The success of this system depends on seedlings, fertilizer applied, and crop care and management.

#### 8.2.7.1 Nursery Raising

For seeding and nursery raising, a fertile, well-drained upland field near the source of irrigation or wet low land rainfed field is selected to transplanting one hectare land. About 500 m<sup>2</sup> area is sufficient for nursery raising. Seed should always be true to the type and it should be healthy, viable, clean, and of high germination percentage (85 %). Seeds should be pregerminated by soaking seeds in water before

seeding so that they will start to grow quickly in the field or seedbed. Dry seed can also be seeded directly to the prepared field.

#### 8.2.7.2 Methods of Raising Seedlings

Different methods of seedlings raising are practiced in different rice growing countries of the world. Among them, the most practiced and familiar are wet bed and dry bed methods which are discussed here.

#### 8.2.7.3 Wet Bed Method

Wet nurseries are preferred under irrigated condition. The soil should be plowed two to three times and puddled by two to three runs of puddler. Apply fertilizers as per recommendation. Before fertilizer application, perform soil test to measure its nutrient content. Uniformly broadcast about two to three handfuls of seed on a square meter of seed bed. Keep the seed beds saturated with water for first five days and then increase gradually the level of water up to 5 cm as the seedlings grow. Drain the excess water in periods of heavy rains during the first week of sowing. Adopt the suitable disease and pest control measures. Seedlings would be ready for transplanting at an age of 20–25 days.

#### 8.2.7.4 Dry Bed Method

This method is practised in those areas where there is insufficient irrigation facility to grow seedlings in wet nurseries. Plow the field three to four times till the soil is thoroughly pulverized. Prepare beds of the same size as in wet nurseries but 15 cm high soil raised with channels (30 cm wide) between them. Sow the seed in rows 10 cm apart in dry or moist condition of soil. Use the same seed rate and fertilizer as in wet nurseries. Cover the sown seeds immediately with a layer of soil. Seed covering is needed to protect the seeds from birds and other seed-eating animals like rats. Special care should be taken for water management in these beds. Allow the water to run in channels first and then raise the level of water slowly to saturate the soil of beds. Do not flood water in beds. Keep the seed bed saturated with water to maintain a thin film of water if possible after 5 days of sowing. Follow all the operations as described for wet nurseries (Singh 2001).

#### 8.2.7.5 Field Preparation

Before start of transplanting, field should be plowed 20–25 cm deep by mold board plow and it should be kept flooded or saturated with water for about one fortnight before transplanting. This helps in decomposition of chaff and straw of previous

crops. Puddling should begin about two weeks ahead of transplanting. Before puddling, an earthen bund, about 30 cm high, should be made around the field to stop seepage of water from the field. Puddling is a very important operation in transplanted rice. It helps to kill the harmful weeds and to create beneficial physical, biological, and chemical conditions for rice plant growth. Soil surface is left in a more even condition. Puddle the field by three to four times of puddler in standing water. Apply uniformly half of nitrogen and total quantities of phosphorus and potash on drained surface at the time of last puddling and incorporate in the top 10–15 cm deep soil.

#### 8.2.7.6 Transplanting

Transplanting should be started when seedlings are ready to transplant in the field. The seedlings are uprooted from the nursery at the optimum age (four to five weeks or 25-30 days). Transplanting of the healthy seedlings may be done at the four to five leaf stages or when they are about 15-20 cm high. Delayed transplanting leads to poor tillering, early flowering of the main tillers, and reduction in yield. Transplant two to three seedlings per hill at  $20 \times 15$  cm distance under normal conditions. Number of seedlings per hill should be increased if old seedlings are used. For 45-day-old seedlings the number of seedlings per hill should be five or six. In each case seedlings should be transplanted at 2–3 cm depth. Seedlings should not be planted deeper than 2-3 cm as deeper planting delays and inhibits tillering (Singh 2001). Fifty hills per square meter should be maintained to assure adequate population in rice field. Transplanting is advantageous as it enables the cultivator to have optimum plant population at desired spacing in the field; it enables the cultivator to have an opportunity to give a thorough cultivation and puddling operation to the field which keeps down the weeds, and the nurseries occupy only a small area of the field; and the control of diseases and insect, pests and irrigation and manuring of young crop is easier and cheaper than a broadcast or direct sown crop (Fig. 8.2).



Fig. 8.2 The growth process of two black rice varieties during a transplanting period in organic paddy field (Budiman and Wibowo 2012)

#### 8.2.7.7 Fertilizer Application

For best result, follow fertilizer recommendation chart of the particular site. Apply full dose of phosphorus and potash and half dose of nitrogen before last puddling. Remaining half dose of nitrogen should be applied in two equal doses, first at tillering stage (25–30 days after transplanting) and second at panicle initiation stage (55–65 days after transplanting). In the case of transplanting of old age seedlings, two-thirds to three-quarters of total nitrogen should be applied at the time of transplanting and rest 25 days later. Method of application of nitrogen fertilizer is crucial in paddy in view of the fact that hardly 30–40 % of the applied nitrogen is actually used by the rice crop (Singh 2001). The unused portion also does not stay in the soil rather it is lost mainly through leaching and denitrification. Till now there is no practical and economic way to completely check this loss but recently some techniques have been developed which can minimize the losses substantially. Urea-coated fertilizer, neem-coated fertilizer, or using nitrogenous fertilizer in deep soil is the way to reduce the loss of nitrogen.

#### 8.2.7.8 Water Management

Since the water requirement of rice is higher than that of any other crop of a similar duration, assured and timely supply of water has a great influence on the yield of the crop. It is roughly estimated that about 2,500 l of water is needed to produce 1 kg of rice seeds through conventional rice farming. In the life cycle of rice plant, there are certain critical stages when water requirement is high. The water requirement is high during the initial seedling period covering about 10 days. Tillering to flowering is the most critical stage when rice crop should not be subjected to any moisture stress. Ensure enough water from panicle initiation stage to flowering (heading). Application of small quantities of water at short intervals to keep the soil saturated is more effective and economical than flooding at long intervals. Flooding is not necessary if the soil is saturated with water and bio-fertilizers have not been used. However, flooding suppresses the weed growth. It increases the availability of many nutrients, particularly phosphorus, potassium, calcium, iron, and silica. Until the transplanted seedlings are well established, water should be allowed to stand in the field at a depth of two to five centimeters. There after about five centimeter of water level may be maintained up to the dough stage of the crop. Water should be drained out from the field 7-15 days before harvest depending on the soil type to encourage quick and uniform maturity of grain.

#### 8.2.7.9 Weeding

In upland rice cultivation, weeds are a major problem. Hence, effective weed control is very important from the very beginning. Stagnant water in the field reduces weed infestation drastically. Benthiocarb at the rate of 2 kg (a.i., active

ingredient) per hectare should be applied six to seven days after seeding of rice. It should be followed by one manual weeding 40–50 days after sowing.

Weed can cause yield reduction up to 30-40 %. The most commonly found weeds in transplanted black rice are

- I. Echinochloa colonum
- II. Echinochloa crusgalli
- III. Cyperus iria Eclipta alba
- IV. Celosia argentea
- V. Dactyloctenium
- VI. Setaria glauca
- VII. Monocharia spp.
- VIII. Cyperus difformis
  - IX. Scirpus spp.
  - X. Fimbristylis litoralis

Weeds must be removed once at four to five weeks after transplanting (before first top dressing) and second time at 50–60 days after transplanting (before second top dressing). A number of herbicides have been found effective in controlling annual weeds of grassy and broad leaf nature. They are Butachlor 5 % GR, Atrazine 50 % WP, 2, 4-D Sodium Salt 80 % WP, Ammonium Salt of Glyphosate 71 % SG and Basalin, etc.

# 8.3 Diseases

Diseases are important factors that cause drastic yield reduction in rice field when plant is in standing condition. Black rice is not seriously affected by diseases because of its ability of resistance against disease. Black rice contains many chemical compounds like anthocyanins in its leaf and grain which make diseases unsuitable as their host. Diseases cause on an average, about 10 % reduction in yield, which may go up from 50 to 90 % in severe cases. Symptoms and suitable control measures for major diseases are described below.

## 8.3.1 Blast

Blast is caused by *Pyricularia oryzae*. It attacks standing rice twice in its life cycle, i.e., once at the tillering stage (leaf blast) and the other at heading stage (neck blast). In leaf blast, boat- or eye-shaped spots with gray or dark brown margin appear on the leaf and leaf sheath. The central portion of the young spot becomes pale green or dull grayish green and appears watery soaked but in older spots the central portions turn gray or straw colored. In severe condition spots may coalesce to form large blighted spots. Similarly, at the time of panicle emergence, the stem below the





ear head gets infected and turns into brown to black spots which may cover the entire stem and stop the translocation of food. The grains remain empty. This stage of infection is known as neck blast. Application of heavy dose of nitrogen, high atmospheric relative humidity (86–98 %), and night temperature around 20 °C for few hours are conducive for the disease (Fig. 8.3).

#### **Control Measures**

The following measures should be adopted for effective control of the disease:

- 1. Treat the seed with organomercurial compounds such as Agrosan GN, Ceresan, or Thiram @ 2.5 g/kg of seed.
- 2. In endemic areas where the disease appears every year, spray the crop with 0.1 % Hinosan 50 EC or Benlate or Bavistin; four to five spray would be required to protect the crop. One spray should be given in nursery, two during tillering, and one to two during panicle emergence stage.

# 8.3.2 Bacterial Leaf Blight

Bacterial leaf blight is caused by *Xanthomonas oryzae pv oryzae*. The 'Kresek' occurs in early stage of plant growth in which the plant withers and dries up. In the later stage, the blighting starts from the tip of the leaves from one or both the margins or in the center and proceeds downward. The leaves turn straw yellow. Yellowish bacterial ooze appears on the surface which dries into bead-like encrustations, if there is no rain for a few days. In diseased plants, grains are partially filled or become chaffy causing heavy loss in yield (Fig. 8.4).

#### 8.3 Diseases

Fig. 8.4 Rice infected by bacterial blight



#### **Favorable Condition**

The disease is most likely to develop in areas that have weeds and stubbles of infected plants. It can occur in both tropical and temperate environments, particularly in irrigated and rainfed lowland areas. In general, the disease favors temperatures at 25–34 °C, with relative humidity above 70 %.

It is commonly observed when strong winds and continuous heavy rains occur, allowing the disease causing bacteria to easily spread through ooze droplets on lesions of infected plants. Bacterial blight can be severe in susceptible rice varieties under high nitrogen fertilization (IRRI 2015).

#### **Control Measures**

Adoption of the following steps helps to control bacterial blight:

- 1. Use disease-free certified seed.
- 2. Draining the standing water of field from time to time. This would help in checking the spread of the disease.
- 3. Provide balanced dose of nitrogen. Use of heavy dose of nitrogen aggravates the disease incidence.
- 4. To check the blight phase of the disease, give 3–4 sprays of a mixture of 75 g Agrimycin 100 and 500 g copper oxychloride, e.g., Fytolan, Blitox-50, Cupravit, etc., in 500 l of water per hectare. The first spray should be given just before the start of disease symptoms. Subsequent sprays should be given at an interval of 10–12 days.
## 8.3.3 Sheath Blight

Sheath blight is caused by *Rhizoctonia solani*. The symptoms of the disease appear on leaf and leaf sheath as 2–3–cm-long greenish gray lesions, turning to straw color and surrounded by bluish gray narrow bands. The lesions increase in size and girdle the stem. Hemispherical or spherical grayish black sclerotia are formed on the lesions which fall in the field with slight jerk. In severe infection, sclerotia form even on the grain. In diseased ear, grains remain unfilled.

The fungus has a wide host range and may attack rice. Its sclerotia also survive in soil for long and serves as a source of primary inoculum. Once the infection is established, it spreads through contact between diseased and healthy plants.

#### **Favorable Condition**

Sheath blight occurs in areas with high temperature (28–32 °C), high levels of nitrogen fertilizer, and relative humidity of crop canopy from 85 to 100 %.

Plants are more vulnerable to sheath blight during the rainy season. High seeding rate or close plant spacing, dense canopy, disease in the soil, sclerotia or infection bodies floating on the water, and growing of high yielding improved varieties also favor disease development (IRRI 2015).

#### **Control Measures**

- 1. Crop rotation
- 2. Collection and burning of infected plants and
- 3. Use of balanced dose of nitrogen minimize the disease.

## 8.3.4 Brown Spot

Brown spot is caused by *Cochliobolus miyabeanus* (*Helminthosporium oryzae*). The pathogen infects coleoptile of the seedling and causes blighting. On the leaves, circular or oval, dark brown to purplish brown spots is found. In severe conditions, these lesions may coalesce and cover the entire leaf. The disease symptoms also appear on grains where black spot appears on glumes. The kernels of infected spikelets become shriveled and discolored.

#### **Favorable Condition**

The disease can develop in areas with high relative humidity (86-100 %) and temperature between 16 and 36 °C. It is common in unflooded and nutrient-deficient soil, or in soils that accumulate toxic substances.

For infection to occur, the leaves must be wet for 8–24 h. The fungus can survive in the seed for more than four years. It can spread from plant to plant through air. Major sources of brown spot in the field include

#### 8.3 Diseases

- infected seeds which give rise to infected seedlings
- volunteer rice
- infected rice debris and
- weeds

Brown spot can occur at all crop stages, but infection is most critical during maximum tillering up to the ripening stages of the crop (IRRI 2015).

#### **Control Measures**

The following steps may be taken to control the disease:

- 1. Treat the seed with organomercurials such as Ceresan, Agrosan GN, Thiram @ 2.5 g/kg of seed.
- 2. Add muriate of potash to correct the potash deficiency in soil, because the severity of disease increases in deficient soils. Also, apply balanced NPK fertilizers.
- 3. Give three to four sprays with Dithane M-45 @ 0.25 % at an interval of 10–12 days just before the appearance of initial symptoms of the disease.

# 8.3.5 Stem Rot

Stem rot is caused by *Leptosphaeria salvinii*. Primary symptoms of the disease appear after transplanting as small, black irregular lesions at the water line on sheath. Fungus gradually enters the stem and develops large number of black, smooth, shining sclerotia in the stem. Such infected stem rots and falls. The spikelets remain chaffy or partially filled.

The sclerotia of the pathogen remain viable even in adverse conditions for a long time and germinate during raining season causing primary infection. Sclerotia are disseminated through irrigation water from field to field and intact fresh infection to healthy plants.

#### **Favorable Condition**

The infection bodies or sclerotia are found in the upper soil layer. They survive in air-dry soil, buried moist rice soil, and in tap water. They can also survive on straw which is buried in the soil. The sclerotia float on irrigation water and infect newly planted rice during land preparation.

Infection is high on plants with wounds as a result of lodging or insect attack. The panicle moisture content and nitrogen fertilizer also influence disease development. The presence of the white tip nematode is reported to have a synergistic effect with the disease and incidences become higher (IRRI 2015).

#### **Control Measures**

It is advisable to follow the control schedule as given below:

- 1. Destroy the infected stubbles by burning and/or deep plowing.
- 2. Avoid standing water in the field. This would help in reducing the infection.

# 8.3.6 False Smut

False smut is caused by *Claviceps oryzae sativa*. The symptoms of the disease become visible only after flowering when infected kernel is transformed into a large velvety, yellow to orange pulverulent mass changing to olive green in color, more than twice in diameter than the normal grains. Infected grains are covered with powdery spore mass which get disseminated by wind and reach to healthy flowers and cause infection. Disease appears only on few grains in ear. In severe condition, more grains get affected. Severe disease incidence occurs in the year of heavy rainfall.

#### **Favorable Condition**

The disease can occur in areas with high relative humidity (>90 %) and temperature ranging from 25 to 35 °C.

Rain, high humidity, and soils with high nitrogen content also favor disease development. Wind can spread the fungal spores from plant to plant. False smut is visible only after panicle exsertion. It can infect the plant during flowering stage (IRRI 2015).

#### **Control Measures**

The following control measures are recommended:

- 1. Use certified seed from a reliable source.
- 2. Collect the diseased grains and destroy them as soon as possible. This will check the secondary spread of the disease and will also help in reducing the inoculum for next year.

# 8.3.7 Bacterial Leaf Streak

Bacterial leaf streak is caused by *Xanthomonas translucens f. sp. oryzicola*. There are water soaked to translucent inter-veinal narrow streaks which are restricted by the veins and soon turn yellow to orange brown in the initial stage. These streaks may coalesce to form large patches which may cover the entire leaf surface. Minute yellow to amber colored beads of bacterial exudates are observed abundantly on the streaks. Primary infection of the disease occurs through infected seeds, whereas the secondary spread of the pathogen occurs through rain droplets and irrigation water and also through contact between healthy and diseased leaves.

#### **Favorable Condition**

Bacterial leaf streak occurs in areas with high temperature and high humidity. It is transmitted through seeds and infected stubbles to the next planting season. It can occur in fields where *X. oryzae* pv.*oryzicola* bacteria are present on leaves, in the water, or in the debris left after harvest.

Particularly, the disease is common in tropical and subtropical regions of Asia, Africa (including Madagascar), South America, and Australia. It can affect the plant

during early stages, from maximum tillering to panicle initiation. Mature rice plants can easily recover from leaf streak and have minimal grain yield losses (IRRI 2015).

#### **Control Measure**

The following measures provide effective control:

- 1. Use certified seed from a reliable source.
- 2. Give two sprays of Streptocycline 12 g or Agrimycin-100, 75 g in 500 l of water per hectare. The first spray should be given when the initial symptom of the disease is observed followed by another spray after 10–12 days.

# 8.3.8 Tungro Virus

This disease is caused by a virus. Older leaves turn yellow orange starting from the tip and margin. Plants become stunted and bear poor panicles with empty glumes showing dark brown color on young leaves. Interveinal chlorosis is observed.

The virus is transmitted from diseased plant to healthy plant by the nymph, male and female of rice green leaf hoppers. After 30 min of feeding of diseased plants, hoppers become capable to transmit the virus which continues transmitting till virus remains in stylet. Most of the hoppers lose their transmitting ability after 24 h.

#### **Favorable Condition**

Tungro disease viruses are transmitted from one plant to another by leafhoppers that feed on tungro-infected plants. The most efficient vector is the green leafhopper. Leafhoppers can acquire the viruses from any part of the infected plant by feeding on it, even for a short time. It can, then, immediately transmit the viruses to other plants within 5–7 days. The viruses do not remain in the leafhopper's body unless it feeds again on an infected plant and reacquires the viruses.

Tungro infection can occur during all growth stages of the rice plant. It is most frequently seen during the vegetative phase. Plants are most vulnerable at tillering stage. Tungro incidence depends on the availability of the virus sources and vector population. Primary sources for tungro include

- stubble of previous crops
- new growth from infected stubbles that had not been properly plowed under and harrowed effectively
- volunteer rice
- infected plants in nearby rice fields

Seedlings raised in nurseries or seedbeds can also be infected with Tungro prior to transplanting and can be a primary source of virus. Transplanting seedlings from nurseries in tungro-infected areas has also shown to increase infection rates in the field, particularly, in cases where seedbed is in a tungro-endemic area or when the nursery duration is 5–6 weeks. However, this is not believed to be a very strong

mechanism in initiating epidemics, because the competitiveness of tungro-infected seedlings is low; they can die rapidly after transplanting (IRRI 2015).

#### **Control Measure**

The following steps may provide effective control:

- 1. The green stubbles should be uprooted and burnt after harvest.
- 2. Isolated plants showing disease symptoms in the beginning should be uprooted and destroyed so that the insect does not get inoculum to spread on healthy plants.
- 3. The vector can be controlled effectively by spraying Diazinon @ 1.5 kg (a.i.)/hectare. In all, five sprays should be given including one in nursery after 10 days of sowing followed by 15, 30, 45, and 60 days after transplanting in the main field. Seven Dimecron 100 also control the vector population. Furadan and Carbofuran 75 wettable powder as seed dresser or pre-transplanting dip have also been found to give good control of the insect.

# 8.3.9 Khaira Disease

Khaira disease is caused by zinc deficiency, usually appears in nursery stage but may appear in patches after 10–15 days of transplanting. Growth of the diseased plant is stunted. High-yielding varieties show chlorosis between the veins of new leaves, where brown spots are formed. On lower leaves, a large number of small, brown to bronze spots appear which coalesce to form bigger spots and ultimately the entire leaf turns bronze colored and dries. Root growth also restricted and usually the main roots turn brown. The finer roots are destroyed. In severe cases, plants fail to grow further and produce no ears but sometimes there is natural recovery after 6 weeks of age.

#### **Control Measures**

- 1. Spray a mixture of 5 kg zinc sulfate and 2.5 kg lime in 1000 l of water after 10 days of sowing in nursery.
- 2. Provide second spray as above on 20th day in the nursery.
- 3. Give third spray as above in the field after 15–20 days of transplanting if symptoms appear in the field.

# 8.4 Insect Pests

In the early stages of the rice crop, several common insects such as the leaf folder, whorl maggot, and armyworms can cause highly visible damage symptoms; however, the damage is rarely enough to reduce yield because the crop can compensate for early damage over the rest of the growing season. Generally, it is not recommended to spray in the early stages of crop growth (0–40 DAP) because the plant can recover from much of the damage without any loss to yield (IRRI).

Though rice is attacked by a large number of insect pests right from nursery to the harvest stage of the crop; black rice has some chemical compounds in its leaves and its stem which avoid insects far from the plant. Insects remain far from the black rice which may be due to its distinct scent. A brief description of the major insects, their nature of damage, and appropriate control measures are given below.

## 8.4.1 Stem Borer

Six species of stemborer found to attack rice. These are the yellow stemborer, white stemborer, striped stemborer, gold-fringed stemborer, and the pink stemborer. Damage caused by the larva of stem borer results in 'Dead heart' and 'White head' of rice plant. Both types of damages result from the feeding of larvae within the stem. They bore and feed inside the stem. They cut the growing pores of the plant from the base causing the plant to die. The drying of growing part of the plant is known as 'dead heart.' Dead heart is created in early life of the plant before flowering while 'White head' occurs at flowering resulting in drying of the entire panicle. Moths may be seen flying around or floating on water in the field.

#### **Favorable Condition**

The stem borer larvae bore at the base of the plants during the vegetative stage. On older plants, they bore through the upper nodes and feed toward the base. The yellow stem borer is a pest of deep water rice. It is found in aquatic environments where there is continuous flooding. Second instar larvae enclose themselves in body leaf wrappings to make tubes and detach themselves from the leaf and falls on to the water surface. They attach themselves to the tiller and bore into the stem. Striped stem borer is most abundant in temperate areas during winter. The pink stem borer is found in upland rice which is grown near sugarcane or related grasses. The presence of alternate hosts encourages the pink stem borer to develop, multiply, and survive during winter or dry season. Unlike other species of stem borers, the pink stem borer lays bare eggs between the leaf sheath and the stem.

High nitrogenous field favors population build up of the stem borers. Fields planted later favor more damage by the insect pests that have built up in fields that have been planted earlier. Stubble that remains in the field can harbor stem borer larvae and or pupae (IRRI 2015).

#### **Control Measures**

Survey the field and if 5 % or more dead hearts are seen, apply 3 % Furadan granules @ 30–33 kg per hectare or Thimet 10 % granules @ 10 kg per hectare in 3–4 cm standing water in the field. Apply there granules at 20–25 days and again at 50–60 days after transplanting.

# 8.4.2 Gall Midge

In gall-affected plants regular tillers are transformed into tubular galls, resembling the leaf of onion. These are known as 'onion leaf.' This is a sucking type of insect and sometimes causes serious damage to the growing crop. Tillers that have galls do not produce panicles (Singh 2001).

#### **Favorable Condition**

The Asian rice gall midge is found in irrigated or rainfed wetland environments during the tillering stage of the rice crop. It is also common in upland and deep water rice. The adults are nocturnal and can easily be collected using light traps. During the dry season, the insect remains dormant in the pupal stage. They become active again when the buds start growing after the rains.

The population density of the Asian rice gall midge is favored mainly by cloudy or rainy weather, cultivation of high tillering varieties, intensive management practices, and low parasitization (IRRI 2015).

#### **Control Measures**

Survey the field and if 5 % or more crop damages are seen, apply 3 % Furadan granules @ 30–33 kg per hectare or Thimet 10 % granules @ 10 kg per hectare in 3–4 cm standing water in the field. Apply there granules at 20–25 days and again at 50–60 days after transplanting.

# 8.4.3 Rice Hispa

The adults of this insect scrap on upper surface of leaf blade, leaving only lower epidermis. Damaged areas show white streaks parallel to midrib. Grubs mine between two epidermal layers producing irregular longitudinal white mines.

#### **Favorable Condition**

The presence of grassy weeds in and near rice fields works as alternate hosts harbor and encourage the pest to develop. Heavily fertilized field also encourages the damage. Heavy rains, especially in premonsoon or earliest monsoon periods, followed by abnormally low precipitation, minimum day night temperature differential for a number of days, and high RH are favorable for the insect's abundance. The rice hispa is common in rainfed and irrigated wetland environments and is more abundant during the rainy season (IRRI 2015).

#### **Control Measures**

Spray the crop with Dimecron 100 EC @ 0.5 ml in one liter of water or Fenitrothion 50 EC @ 1 ml one liter of water or dust, 5 % BHC @ 20 kg per hectare. In case of spray, amount of solution should be prepared on the basis of area to be covered (Singh 2001).

# 8.4.4 Leaf Roller

Leaf roller (*Cnaphalocrocis medinalis*) caterpillars cause damage by folding the leaf blades into tubular structures and feeding on the green leaf tissues within these structures. Usually, only one caterpillar is found within a fold. The larvae feed within this portion of the leaf cause typical white streaks by consuming all but the epidermis of the inside of the leaf. Pupation occurs within the rolled portion.

## **Favorable Condition**

Heavy use of fertilizer encourages rapid multiplication of the insect. High humidity and shady areas of the field, as well as the presence of grassy weeds from rice fields and surrounding borders, favor the development of the pest. Expanded rice areas with irrigation systems, multiple rice cropping, and insecticide induced resurgences are important factors in the insect's abundance. Rice leaf folders occur in all rice environments and are more abundant during the rainy seasons. They are commonly found in shady areas and areas where rice is heavily fertilized. In tropical rice areas, they are active year round, whereas in temperate countries they are active from May to October. The adults are nocturnal and during the day, they stay under shade to escape predation. Moths fly short distances when disturbed (IRRI 2015).

## **Control Measures**

If more than one damaged leaf is seen in each hill, protect the crop by spraying Ekalux 25 EC @ 2 ml in one liter of water or Nuvacron 40 EC @ 1 ml in 1 l of water or BHC 5 % dust @ 20 kg per hectare.

# 8.4.5 Armyworm

Armyworms are caterpillars that attack rice. There are at least three armyworm species that attack rice in Asia. These are the rice swarming caterpillar, common cutworm, and the rice ear-cutting caterpillar. The caterpillars are greenish brown in color with a pair of prominent dorso-lateral stripes on the body. Larvae feed voraciously on leaves, resulting in skeletonising of leaf blades. They have a climbing habit and cut panicles from base and do damage at night. The insect migrates from one field to another.

## **Favorable Condition**

Adult armyworms survive better and produce more eggs when the temperature is at 15 °C maximum, and when plants are naturally fertilized. Periods of drought followed by heavy rains and the presence of alternate hosts also sustain the development of armyworms. The larvae usually feed in the upper portion of the rice canopy on cloudy days or at night, while the adult feeds, mates, and migrates at night and rests in daytime at the base of the plant. In dryland fields, armyworm pupa can be found in the soil or at the base of the rice plants. In wetlands, they pupate on the plants or on grassy areas along the field borders (IRRI 2015).

## **Control Measures**

- 1. Use BHC 10 % dust @ 20 kg per hectare.
- 2. Spray crop with Endosulfan 35 EC @ 1.25 l in 1000 l of water per hectare.

# 8.4.6 Mole Cricket

Mole cricket cause problem on raised nursery beds and in upland fields. The adults feed on the young roots and basal portions of the plant below the ground. They may kill the plant by cutting at the base.

## **Favorable Condition**

Mole crickets occur in all rice environments. They are more common in non-flooded upland fields with moist soil. In flooded rice fields, mole crickets are usually seen swimming in the water. They are also found in permanent burrows or foraging galleries in levees or field borders. The entrances to burrows in the soil are marked by heaps of soil. The nymphs feed on roots and damage the crops in patches (IRRI 2015).

#### **Control Measures**

Apply Furadan 3 % G @ 33 kg per hectare or Thimet 10 % G @ 10 kg per hectare for the control of this pest.

# 8.4.7 Green Leaf Hopper

Generally, leaf hopper feeds on leaves and upper part of plants by sucking the cell sap. It also transmits tungro virus diseases. Two species of green leafhoppers (GLH) spread tungro: *Nephotettix malayanus* and *Nephotettix virescens*. The disease is characterized by slight stunting and reduced tillering. Young leaves are often molted with pale green to whitish stripes. Infected plants bear a few light spikelets.

#### **Favorable Condition**

Green leafhoppers are common in rainfed and irrigated wetland environments. They are not prevalent in upland rice. Both the nymphs and adults feed on the dorsal surface of the leaf blades rather than the ventral surface. They prefer to feed on the lateral leaves rather than the leaf sheaths and the middle leaves. They also prefer rice plants that have been fertilized with large amount of nitrogen. Staggered planting encourages population growth of GLH.

## **Control Measures**

Treat the crop with Dimecron 100 EC @ 0.5 ml of insecticide in 1 l of water or Sevin 50 wettable @ 1 g of insecticide in 1 l of water or Furadan 3 % G @ 30 kg per hectare. Granular insecticides should be used in 2–3 cm standing water.

# 8.4.8 Plant Hopper

Two species of planthopper infest rice. These are the brown planthopper (BPH), *Nilaparvata lugens* (Stal); and the white backed planthopper (WBPH), *Sogatella furcifera* (Horvath). White backed plant hopper is common in the early crop season (July–August), whereas brown plant hopper appears during later part of the crop season (October–November). Both the plant hoppers cause severe damage by sucking plant sap. This serious damage is called as 'hopper burn.' A large number of plant hoppers are confined to basal portion of the plant. Due to sucking plant sap by these pests, there is yellowing of plants followed by rapid drying. Brown plant hopper in addition to causing direct damage also transmits 'grassy stunt virus disease.' The disease is characterized by narrow, erect leaves which are usually yellow to yellowish green with numerous dark spots. If the plant produces panicles, they are small and have dark brown unfilled grain.

#### **Favorable Condition**

Planthoppers can be a problem in rainfed and in irrigated wetland environments. It also occurs in areas with continuous submerged conditions in the field, high shade, and humidity. Closed canopy of the rice plants, densely seeded crops, excessive use of nitrogen, and early season insecticide spraying also favors insect development (IRRI 2015).

## **Control Measures**

If plant hopper population reaches five to ten per hill, treat the crop with Furadan 3 % G @ 33 kg per hectare or Thimet 10 % G @ 10 kg per hectare or Sevidol 4.4 % G @ 12 kg per hectare or Dimecron 100 EC @ 0.5 ml of insecticide in one liter of water or Rogor 30 EC @ 1.75 ml of insecticide in 1 l of water.

Care should be taken to direct the sprays toward the base of the plants, particulary for the control of plant hoppers as they remain on basal part of the plants.

# 8.4.9 Swarming Caterpillar

This pest causes severe damage in nursery beds. Newly hatched larvae cut leaves and cause plants to look sickly with withered tips. Older larvae feed voraciously and cause almost complete defoliation. These larvae migrate from fields and do damage at night.

#### **Control Measures**

In case of moderate to severe damage spray Ekalux 25 EC @ 2 ml in 1 l of water or Nuvacron 40 EC @ 1.25 ml in 1 l of water or BHC 5 % dust @20 kg per hectare.

# 8.4.10 Gundhi Bug

Both nymphs and adults cause damage by sucking the plant sap and particularly the milky juice of the developing grains in early morning or at dusk. The grains are then either empty or partly filled and shriveled. This insect is popularly known as Gundhi bug because it produces unpleasant smell when touched or disturbed.

#### **Control Measures**

In case there are five or more insects per meter row length, as a control measure applies BHC 5 % dust @ 20–25 kg per hectare.

## 8.4.11 Rats

Rats do not like wide, open spaces because they are more vulnerable to attack from predators. They like to hide and burrow in weedy areas near major irrigation canals, in village gardens, and in other non-crop areas which provide good cover. Before planting is the best time to organize community rat control campaigns. These can be done until three weeks after planting (IRRI 2015).

Figures 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 8.10, 8.11, 8.12, 8.13, 8.14, 8.15, 8.16, 8.17, 8.18, and 8.19 credit IRRI (2015).

#### **Effective Community Control**

- 1. Flooding, digging, or fumagating rat burrows.
- 2. Scare rats out of areas with high vegetation cover or around villages (using netting, dogs, clubs, and others to catch rats).
- 3. Use dogs to locate active rat burrows, then do Step 1.
- 4. Hunt rats at night using flashlights, clubs, bow and arrows, and nettings.
- 5. Set local kill traps along runways of rats.
- 6. Use registered rat poisons that are placed in covered bait stations (but not where children, pets, or livestock have easy access).

## 8.4 Insect Pests

Fig. 8.5 Rice leaf infected with sheath blight



Fig. 8.6 Rice leaf infected with *brown* spot





Fig. 8.7 Rice plant infected with stem rot



Fig. 8.8 Rice grain infected with false smut

## **Management Action**

- 1. Keep rice bunds (banks) in the crops less than 30 cm wide to prevent rats from burrowing.
- 2. Keep the edges of the field, the bunds, and surrounding areas clean and free of tall weeds and hiding areas for rats.
- 3. Plant at the same time as your neighbors within 2 weeks of each other.
- 4. Strategic use of trap barrier system (TBS) during the rice season with the most rodent damage.

#### 8.4 Insect Pests

Fig. 8.9 Rice leaf infected with bacterial leaf streak



Fig. 8.10 Rice plant infected by tungro virus





Fig. 8.11 Rice stem borer



Fig. 8.12 Rice gall midge

- 5. Keep area around fields, homes, and villages clean, no piles of wood or brush, no garbage heaps, and no weedy areas.
- 6. Keep grain stores and surrounding area clean.



Fig. 8.13 Rice hispa



Fig. 8.14 Rice leaf roller



Fig. 8.15 Rice army worm



Fig. 8.16 Mole cricket



Fig. 8.17 Rice green leaf hopper



Fig. 8.18 Rice planthoppers



Fig. 8.19 Rat

# 8.5 Harvesting and Threshing

Nowdays, harvesting and threshing are done simultaneously at the same time by machine, the combined harvester. Thus now it is easy to harvest and thresh crop in commercial level. But before harvesting one should ensure the crop as soon as it matures. Timely harvesting ensures good grain quality, consumer acceptance, since the grain is less likely to break when milled. Grain may be lost due to damage by rats, birds, insects, shattering, and lodging if it is delayed. The right stage for harvesting is when about 80 % panicles have ripened spikelets. At the time of harvest the upper portion of the spikelets should be straw colored. Tropical rice is usually harvested at 20 % or more moisture about 30 days after 50 % flowering, when grains will provide optimum total and head rice yields. Moisture content at

harvest is lower during the dry season than in the wet season because of sun drying while the grains are in the intact plant.

For small-scale farming, the crop is generally cut with serrate by hand. The plants should be cut quite close to the ground and left in the field for a few days to dry. Later on they should be collected in bundles and stacked for threshing.

Threshing of rice on small farms is still a major problem in developing countries. The most common methods of threshing are trampling by bullocks, rubbing with bare human feet (in hills) or lifting the bundles, and striking them on the raised wooden platform. On big farms pedal threshers or power-driven stationary threshers are also in use.

Sun drying to 14 % moisture is a common practice but is unreliable during the wet season. Many mechanical dryers have been designed but have not been popular with farmers and processors. After drying, the rough rice is winnowed to remove the chaff using either a hand winnower or a manually operated wooden winnower.

## 8.6 Post-harvest Loss

Rice losses occur at all stages of the post-harvest chain. Though quantitative losses are usually simple to assess, qualitative ones are more difficult to define and rely more on subjective judgements and cultural perceptions. Accepted figures for quantitative post-harvest losses in rice range from 10 to almost 40 % with the following breakdown:

harvesting: 1–3 %, handling: 2–7 %, threshing: 2–6 %, drying: 1–5 %, storage: 2–6 %, milling: 2–10 %.

These figures were collected in Southeast Asia (de Padua 1988), and were later confirmed for other parts of Asia and Africa by field activities of FAO's Prevention of Food Losses (PFL) program, among others. They have become the standard values for rice losses.

The timing of the rice harvest influences the level of losses. Depending on the variety, delay in harvesting of a mature rice crop leads to lower yields because of lodging and shattering and the exposure of the ripe grain in the field to insects, birds, and rodents. It also leads to post-harvest losses by lowering milling yields and recovery of head grains. Traditional threshing techniques are a frequent cause of loss. Often a considerable amount of grain is scattered around and gets eaten by poultry and household pets. However, while this quantity can be considered lost for human consumption, it becomes productive within the total household economy. Threshed rough rice is commonly stored either in sacks or in bulk. The sacks or bags provide a means of separating varieties for specific requirements but do not

provide protection against insects and rodents. Good store management, proper dunnage, and adequate hygienic conditions significantly limit the losses. On the large scale, bulk storage and controlled atmosphere storage, if properly organized, are efficient and relatively inexpensive. However, efficient operation requires considerable capital investment and trained labor which often go beyond the single farmer's capability.

Storing rice as rough rice has advantages over storing milled rice, since the hull protects the kernel against insects and fungal attacks. This possibility depends to some extent on the local economic situation and on supply and demand for rough rice and milled rice at different times in the season (FAO).

## 8.7 Yield

A well-managed crop yields about 4–6 metric tons per hectare. Freshly threshed rice spoils rapidly because of high moisture content in the grain. Thus they should be dried well in sun for few days before storage. The moisture content may range from 10 to 12 % for well-dried rice crop. The crop should be stored in well-dried sacks or granary and dry the rice between 2 and 3 months alternatively.

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# Chapter 9 Health Benefits of Black Rice

It is a common sense that a healthy diet results positive health and a bad diet results health negatively. A university-led study is not necessary to arrive at that conclusion. In the human body, free radicals damage cells. Free radicals are unstable and highly reactive molecules. It is normal for our bodies to produce these free radicals in small amounts but many factors, such as metabolic stress and UV radiation, can increase the formation of these free radicals. Free radicals cause oxidative damage within the body which may eventually result in DNA and protein damage and even cell death. Antioxidants are able to neutralize these free radicals and help to prevent oxidative damage. Foods that are rich in antioxidants seem to neutralize these free radicals in order to better preserve cells. In addition, foods that are rich in antioxidants also result in blood clot reduction and lower blood pressure. The arrays of foods that are rich in antioxidants include blueberries, artichokes, Russet potatoes, and dark beans. All black foods are typically rich in antioxidants simply because of their pigment levels. Black beans and black rice are great examples of abundant sources of antioxidants. Studies have shown that antioxidant supplementation can exert a preventative effect against the development of serious conditions like cancer and may improve overall health.

- 1. It is a proven fact that antioxidant supplementation lowers inflammation in the body. Inflammation is a subject of significant research interest in recent times because it is heavily involved in the pathology of serious conditions including diabetes and cardiovascular disease.
- 2. Black rice is rich in both anthocyanin and tocopherols (another type of powerful antioxidant) which are also known as vitamin E. Recent research has shown that antioxidants may work synergistically, meaning that foods containing two or more types of antioxidant may deliver greater health benefits than the sum of each antioxidant alone.
- 3. Black rice is a great source of iron, potassium, and B vitamins and it is relatively high in protein. Aside from being an extremely rich source of nutrients, black rice is cheaper and lower in sugar than other super foods like berries.

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Black rice contains B-complex vitamins, riboflavin, thiamin, B5 and B6, minerals, iron, calcium, magnesium, etc. and Vitamin E. Phenolics have been reported as the major hydrophilic antioxidants in rice, while carotenoids, tocopherol, and gamma-oryzanols as the principle lipophilic antioxidative constituents (Min et al. 2011). These substances are associated with the prevention of cardiovascular diseases, certain type of cancer, and other diseases related to aging (Kim et al. 2006a, b; Shen et al. 2009). Lots of studies have demonstrated antioxidant activity and radical scavenging ability of the pigmented rice and its extract in both in vitro and in vivo models (Hu et al. 2003: Ichikawa et al. 2001: Nam et al. 2006: Oki et al. 2002; Toyokuni et al. 2002) as well as other biological effects of the extracts including antimutagenic and anticarcinogenic activities (Hyun and Chung 2004; Nam et al. 2006), reduction of the atherosclerotic plague formation (Xia et al. 2003), aldose reductase inhibitory activity (Yawadio et al. 2007), and attenuation of some metabolic abnormalities associated with high fructose diets including glucose intolerance and hyperlipidemia (Guo et al. 2007). These researches assure the health benefits of the pigmented black rice. It has been shown that consumption of colored rice causes decrease of oxidative stress and simultaneous increase of antioxidant capacity in the tested models (Hu et al. 2003). Black rice might provide health benefits to reducing risk of chronic diseases such as cardiovascular problems, cancers (Xia et al. 2006), diabetes and its complications (Walter and Marchesan 2011), as well as iron-deficiency anemia (Wang and Guo 2007), because of the existences of phytochemicals such as phenolic compounds (Shen et al. 2009). Phenolics are defined as molecules with at least one aromatic ring bearing one or more hydroxyl groups, and are one of the major bioactive substances in black rice (Shen et al. 2009).

A series of research reports have proven that black rice is linked with health benefits and its successes and recent research suggests that there may be many more that we are currently not aware of. High blood pressure, circulation, and overall blood health also seems to be maintained well by those who add this rice to their diet. There is evidence that correlate black rice consumption with improvement of cardiovascular health status (Sangkitikomol et al. 2010a, b) and inhibition of cancer invasion (Chen et al. 2006). Cyanidin-3-O-glycoside has been shown to have antioxidant activity 3.5 times more potent than Trolox, a vitamin E analog (Zuo et al. 2012). Black rice is recently known as a 'super food.' 'Super foods' are certain types of foods that offer many consequential health benefits aside from their inherent caloric and nutritional value. Regular consumption of this rice may cut down or completely eliminate many future health problems. It may be useful in avoiding long-term problems like Alzheimer's and diabetes. It is possible that it will discover even more health-related benefits as future research is going on. Thus black rice varieties have been the subject of exploratory research for its potential biomedical applications. Previous work have demonstrated that black rice extract

- 1. Induces superoxide dismutase (SOD), catalase (Chiang et al. 2006), and glutathione peroxidase activities (Auger et al. 2002)
- 2. Suppresses reactive oxygen species (ROS) and nitric oxide (NO) radicals (Hu et al. 2003) and
- 3 Inhibits xanthine oxidase, one of the generators of superoxide anions (Zuo et al. 2012).

Zuo et al. (2012) demonstrated that black rice extract prolong the mean life span of fruit flies by 14 %. Furthermore, this effect is accompanied by an upregulation of CuZnSOD (SOD1), MnSOD (SOD2), and catalase (Zuo et al. 2012). It is reported that anthocyanins and tocols in black rice bran significantly prevent lipid oxidation. The capability of anthocyanins is greater than the tocols in retarding cholesterol oxidation. The prevention of lipid oxidation could reduce the inflammation associated with chronic diseases. Both of the hydrophilic and lipophilic antioxidants contribute to the health-promoting function (Zhang et al. 2013). Similarly, dietary intake of AEBR reduces platelet hyperactivity, hypertriglyceridemia, and body weight gain, and facilitates in the maintenance of optimal platelet function in dyslipidemic rats induced by high-fat diets (Yang et al. 2011). It is possible to live healthy life not only by including black rice extract in their foods but also with continuous physical exercise (Watanabe et al. 2013).

## 9.1 Enhances Health and Increases Longevity

Legend tells that this ancient grain was once eaten exclusively by the Emperors of China. This most popular rice is fabled to enrich health and ensure longevity. This medium-size heirloom rice is treasured for its delicious roasted nutty taste, soft texture, and beautiful deep purple color. It is extremely high in flavonoid and antioxidants. According to Chinese herbal medicine, black rice is considered as a blood tonifier. Everybody knows rice bran is byproducts of the hulling of rice, but it is an important food resource in Korea. Various studies have been reported immune-enhancing effects of rice bran cultured with Lentinus edodes. Black rice bran contains anthocyanin, and the effects of antioxidant have been reported yet. The capacity of bran (crude fermentation polysaccharide) CFP and (crude fermentation polysaccharide-S. cerevisiae) CFP-S seem to act as a potent immune modulator causing augmentation of immune cell activity, and enhance the immune function through regulating cytokine production capacity by activated macrophage and splenocyte in mice (Kim et al. 2011). Anthocyanin extracted from black rice may be utilized as a possible antioxidant agent against ROS (Park et al. 2008). Phetpornpaisan et al. (2014) reported that Kamklaing rice bran (KRB) extracts had a high content of cyanidin-3-glucoside, caffeic acid, and ferulic acid and possessed antioxidation and wound healing effect. This was associated with increasing migration and collagen synthesis in normal human dermal fibroblast cells when compared to the controls, MMP-2 and MMP-9 inhibitory effects in gelatin zymography, and immunomodulation with concentration-dependent bimodal effects in human monocytes. These results suggest that Kamklaing rice bran is a promising byproduct that contains functional ingredients which may be useful in the control of skin aging and chronic disease development. Similarly, anthocyanins are reported to have health-promoting properties. Oxidative stress plays a major role in the pathogenesis of many degenerative diseases induced by free radicals, such as cardiovascular disease, stroke, and cancer. ARE at high doses ( $\geq$ 800 mg/L) induces cytotoxicity. However, at 600–1000 mg/L it reduced intracellular oxidative stress (P < 0.05) in a dose-dependent manner, and at 200 mg/L it significantly enhanced the expression of the LDLR gene in HepG2 cells. Thus it was concluded that ARE can be beneficial for health promotion by reducing oxidative stress and enhancing LDL clearance, regulating LDLR production on the cell surface membrane, thereby maintaining lipid homeostasis (Sangkitikomol et al. 2010a, b).

## 9.2 **Protects Heart Health**

It is reported that black rice reduces dangerous atherosclerotic plaque formation within the arteries, which is essential for keeping arteries clear as well as avoiding heart attacks and stroke. The particular anthocyanins phytochemicals present in black rice assist to maintain healthy cholesterol levels by reduction of total cholesterol, LDL "bad" cholesterol, and total triacylglycerol concentrations (www. healthbenefitstimes.com). Studies show that black rice contains a large amount of anthocyanins, an ingredient that is capable of lowering the risk of heart attack. It does this by preventing the buildup of plaques in the arteries which is the most common reason why heart attacks occur. Black rice intake is associated with reduced levels of plasma cholesterol, triglycerides, and low-density lipoprotein in rats. Lee et al. (2008) reported that anthocyanin pigments are highly effective in reducing cholesterol levels in the human body. Anthocyanin extract of black rice is reported to reduce the blood plasma levels of cholesterol, triglycerides, LDL (low-density lipoprotein) and raise HDL (high-density. lipoprotein), and potent for cardiovascular disease therapies (Kim et al. 2006a, b; Xia et al. 2006; Wang et al. 2007; Zawistowski et al. 2009). Zawistowski et al. (2009) also reported supplementation of atherogenic experimental diets with black rice extract (BRE) effectively decreased lipid levels in hypercholesterolemic rats. Similarly, previous studies have demonstrated that anthocyanin extracted from black rice inhibits atherosclerosis. Thus it is clear that anthocyanins are more capable of controlling cholesterol levels than any other food supplement available today (Fig. 9.1).

Jung et al. (2006) conducted an experiment which demonstrated that brown and black rices have cardioprotective effects. Abdel and Aly (2011) research suggested a mechanism of the cardioprotective effect of black rice. On the other hand, Xia et al. (2003) showed that adding black rice outer layer fraction to a hypercholesterole mice diet significantly affected the plasma LDL levels of Apo E-deficient



**Fig. 9.1** HDL/TC—cholesterol ratio in experimental rats. NC—negative control, CI—positive control with 0.5 % cholesterol, CII—positive control with 0.5 % cholesterol and 0.05 % cholic acid, CI + BRF and CII + BRF—positive controls (I and II) with BRF (black rice fraction). \*Significant difference at p < 0.05; \*\*significant difference at p < 0.01 as compared with CI group and CII group respectively (Zawistowski et al. 2009)

mice. Similarly, oryzanol decreases levels of total LDL and VLDL cholesterol in hypercholesterolemic rats (Suh et al. 2005) and in mildly hypercholesterolemic men (Berger et al. 2005). Finne Nielsen et al. (2005) have shown that anthocyanins extracted from black currants significantly increase total cholesterol, LDL, VLDL cholesterol, and triacylglycerols in the plasma of Watanabe heritable hyperlipidemic rabbits. The livers of rats fed with experimental diets containing 3 % BRF, cholesterol, and cholic acid weighed significantly less than counterparts not fed with the BRF. Similarly, the findings of Yang et al. (2011) suggest that dietary intake of anthocyanin extract from black rice (AEBR) reduces platelet hyperactivity, hyper triglyceridemia, and body weight gain, and facilitates in the maintenance of optimal platelet function in dyslipidemic rats induced by high-fat diets. Ling et al. (2001) reported that diet supplemented with black rice improved the lipid profile and increased glutathione peroxidase activity in rabbits. Ling et al. (2001) laboratory has also identified the antiatherogenic effects of black rice and its pigment fraction in a series of studies. In the same way, both Ling et al. (2002) and Xia et al. (2003) reported that supplementation of black rice pigment fraction to the diet significantly inhibited atherosclerotic plaque formation in rabbits and in apolipoprotein (apo) E-deficient mice. These studies also revealed that the anti-atherosclerotic properties of black rice do not arise from the dietary fiber or vitamin E components and the anthocyanin components of black rice are responsible for the anti-atherosclerotic effect. Early studies have already shown that polyphenols such as plant anthocyanins are beneficial to cardiovascular health. Hu et al. (2003) also reported that two major anthocyanins cyanidin-3-glucoside and peonidin-3-glucoside show antioxidative and anti-inflammatory activities in vitro.

An experiment was conducted to observe the anti-atherosclerotic effects of black and red rices and to find out their mechanism, 24 New Zealand male white rabbits (average body weight 1.91 kg) were divided randomly into three groups (white rice, black rice, and red rice groups). The rabbits were fed a cholesterol-enriched diet (cholesterol 5 g/kg, lard 35 g/kg) containing 300 g/kg white, black, and red rice powders, respectively. The rabbits were sacrificed 10 weeks later. The aorta was dissected and stained in 0.5 % oil red-isopropanol solution. The plaque area and total area of aorta and serum lipids were measured by an automatic image analyzer. The results showed that the plaque area (% of total surface) in the black and red rice groups was significantly lower than that in the white rice group (P < 0.05). Serum TG, TC, LDL-C, ApoB, and ApoAI/ApoB were not significantly different among the three groups (P > 0.05). However, the concentrations of HDL-C and ApoAI were significantly higher in the black and red rice groups than those in the white groups (P < 0.05). No significant difference was found between the black and red rice might be effective in reducing atherosclerotic plaques on the aorta of rabbits fed a cholesterol-enriched diet. The effectiveness of black and red rices against atherosclerosis might be related to the high level of serum HDL-C and ApoAI (Chen et al. 2000).

Red or black rice consumption reduces or retards the progression of atherosclerotic plaque development induced by dietary cholesterol. Yao et al. (2013) findings provide an important evidence that anthocyanins may partly contribute to the inhibitory effects of black rice on cholesterol absorption, and thus may be applied for the prevention and treatment of hypercholesterolaemia. Especially, pigs fed C3G show significantly lower total cholesterol concentration compared to pigs fed brown rice bran (Kil et al. 2006). The study on rats also demonstrates that brown and black rices have cardioprotective effects (Kim et al. 2006a, b). Similarly, Xia et al. (2003) concluded that the inhibition of atherosclerotic lesions of the black rice pigment fraction is attributed to the improvement in cholesterol accumulation and reduction in oxidative stress and inflammation. A study showed that black and red rices can lower serum triglyceride levels and prevent hepatomegaly and thereby, possibly reducing the risk of CVD (cardiovascular disease) (Gadang et al. 2010).

The effect of white and black rices consumption on lipid profile, hydroperoxides, thiobarbituric reactive substances, and oxidized low-density lipoprotein (LDL) induced by hypercholesterolemia was investigated in 24 male rabbits; a purified normal diet (NC, n = 6), a high-fat/cholesterol (1.0 g/100 g) diet (PC group, n = 6), a high-fat/cholesterol diet with 25 g/100 g white ground rice (PCWR group, n = 6), and 25 g/100 g black ground rice (PCBR group, n = 6) for 10 weeks. Results indicated that serum high-density lipoprotein cholesterol was higher (P < 0.05) in the PCBR compared with the PC and PCWR groups. Hydroperoxides and thiobarbituric reactive substances were significantly lower (P < 0.05) in the PCBR compared with PCWR and PC groups. Cyanidin-3-glucoside (Cy-3-Glu) and peonidin-3-glucoside have been tested in vitro against copper-mediated low-density lipoprotein. Cy-3-Glu was excelled peonidin-3-glucoside by increasing the lag time of NC from 80 to 500 min in the presence of 2.0 µM of Cy-3-Glu. Hierarchically, black rice rabbits group gave the best results compared with other groups. This result indicates a suggested mechanism (anthocyanins protection; Cy-3-Glu) of the cardioprotective effect of black rice (Abdel and Aly 2011). This enhance in serum HDL cholesterol and apo A-I concentrations, and the increase of antioxidant and decrease on oxidative status may be mechanisms of the antiatherogenic effect of red or black rice (Ling et al. 2001). Similarly, the findings of Yao et al. (2014) also provide an important evidence that anthocyanins may partly contribute to the inhibitory effects of black rice on cholesterol absorption, and thus may be applied for the prevention and treatment of hypercholesterolaemia. The capability of the anthocyanin extract is relatively greater in stablizing cholesterol but lower in inhibiting fatty acids oxidation (Zhang et al. 2013).

## 9.3 Reduce Atherosclerosis

Black rice features a powerful impact on the heart's health. The nutrition retained within this variety assists in the protection against high blood pressure levels and plaque formation within the heart. Atherosclerosis, represented by hardening of the artery walls, is really a cardiovascular disease. Eating black rice is considered to cut deaths significantly from such causes. The rice also enhances heart outcomes by reduction of LDL cholesterol as well as total cholesterol. The inhibition of atherosclerotic lesions by the black rice pigment fraction is attributed to the improvement in cholesterol accumulation and reduction in oxidative stress and inflammation (Xia et al. 2003).

The influence of the supplementation of black and white rice outer layer fractions on atherosclerotic plaque formation induced by hypercholesterolemia was investigated in rabbits. Male rabbits (n = 32) were randomly divided into four groups. They were fed non-purified diet (normal group), a lard (3.5 g/100 g) with high cholesterol (0.5 g/100 g) diet (HC group); the HC diet with 5 g/100 g white rice outer layer fraction (WRF group); or the HC diet with 5 g/100 g black rice outer layer fraction (BRF) for 2 months. The atherosclerotic plaque area in rabbits fed the BRF diet was 66 % lower than that of the HC or WRF rabbits (P < 0.001). Supplementation of the black rice outer layer significantly (P < 0.05) lowered aortic 8-hydroxy-2'-deoxyguanosine (8-OHdG) (-52 %, -44 %) compared with the WRF or HC diets (P < 0.05). There were no differences in aortic 8-OHdG levels between rabbits that fed the BRF and normal diets. The BRF diet significantly (P < 0.05) decreased the malondialdehyde (MDA) level of serum (-37 %) and aortic artery (-50 %) compared with the WRF diet. There were no differences in the concentrations of serum total cholesterol (TC), LDL cholesterol (LDL-C), HDL-C or the ratio of apoprotein (apo) I/apoB among the HC, WRF, and BRF groups. Similarly, there were no differences in the serum vitamin E concentration, erythrocyte, and aorta superoxide dismutase (SOD) activities among rabbits fed these diets. The serum concentration of most fatty acids except 18:1 did not differ between the WRF and the BRF groups. It was concluded that the inhibition of atherosclerotic plaque formation derived from the black rice outer layer fraction in rabbits might be mediated by antioxidative or anti-inflammatory effects (Ling et al. 2002).

An illustration is presented here where two experiments were conducted to investigate the effect of feeding cyanidin 3-glucoside (C3G) black rice bran on nutrient digestibility, blood measurements, growth performance, and pork quality of pigs. In Exp. I, a total of fifteen pigs (19.91??.80 kg, average initial body weight) were used in assay of nutrient digestibility and blood measurements. All pigs were allotted to five treatments with three replicates according to a completely randomized design (CRD) in an individual metabolic crate. Treatments included

- (1) CON: basal diet,
- (2) BRB-2: basal + brown rice bran 2 %,
- (3) BRB-4: basal + brown rice bran 4 %,
- (4) CRB-2: basal + C3G high black rice bran 2 %, and
- (5) CRB-4: basal + C3G high black rice bran 4 %.

The digestibility of dry matter (DM), crude protein (CP), crude fat (CF), crude ash (CA), and crude fiber (CF) was not affected by dietary treatments. Serum triglyceride (TG) and high density lipoprotein (HDL) cholesterol concentrations were not affected by addition of C3G high black rice bran. However, at the end of experiment, pigs fed rice bran showed decreased tendency in total cholesterol concentration. Especially, pigs fed C3G high black rice bran showed significantly lower total cholesterol concentration compared to pigs fed brown rice bran (p < 0.03). There was numerically lower total cholesterol concentration with increasing levels of black rice bran in the diet. In terms of serum glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT), there were no significant differences among treatments, even though pigs fed CRB-4 showed the lowest GOT concentration compared to other pigs. In Exp. II, sixteen finishing pigs (average initial body weight 89.96? 35 kg) were divided into four treatments to investigate the effect of feeding C3G high black rice bran on growth performance and pork quality. There were no significant differences in average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) among the treatments. Pigs fed C3G high black rice bran showed numerical decrease in ADG and increase in FCR while not effecting feed intake. There was no significant difference in live weight, carcass weight, carcass rate, backfat thickness, and carcass grade. However, pigs fed C3G high black rice bran tended to show lower back fat thickness than pigs fed basal diet. Pigs fed C3G high black rice bran showed a tendency of decreased TBA value than pigs fed basal diet, although there was no overall significant difference among treatments. In conclusion, nutrient digestibility, blood measurements, growth performance, and pork quality were not significantly affected by feeding C3G high black rice bran to pigs. However, C3G high black rice bran might have an effect on lowering serum total cholesterol and decrease the TBA value in pork compared to control group and these effects might be due to high concentration of antioxidative compounds in C3G high black rice bran (Kil et al. 2006).

# 9.4 Control Hypertension

A research paper published in the "American Journal of Clinical Nutrition" investigating the consumption of major flavonoid groups (flavonoids, flavones, flavones, flavan-3-ols, proanthocyanidins, and anthocyanins) determined that anthocyanins are the group, most significantly associated with prevention of hypertension (American Journal of Clinical Nutrition, 93:338–347). The antioxidant capabilities of anthocyanin and tocol extracts from black rice bran have been evaluated and it was found that the capability of the anthocyanin extract is relatively greater in stabilizing cholesterol but lower in inhibiting fatty acids oxidation (Zhang et al. 2013).

## 9.5 Reduces Stroke Risk Levels in Women

An expansive Swedish survey was coordinated among women at Karolinska Institute led to a conclusion that diets which are comprised with foods rich in antioxidants result in lower risk levels of stroke. Women who have been diagnosed with heart disease and are known to have higher risks associated with stroke were also included in these findings. American Heart Association, medical journal "Stroke" published findings that are concentrated on elderly and middle aged women's diets. Those women who did not suffer from heart disease experienced a 17 % risk reduction, while those with some form of heart disease reduced their risk of stroke by 57 % when they adopted a diet loaded with antioxidants like black rice. The HOPE study (Heart Outcomes Prevention Evaluation) is another study that supported the findings of the Swedish study. The HOPE study specifically followed 10,000 patients at risk for stroke in excess of four years and these patients also experienced lower risks through increased intakes of vitamin E.

#### 9.6 Improves Digestive Health

Black rice and other whole grain rice varieties like wild, red, or brown rices have similar amount of fiber with about 2–3 g per half cup serving. Fiber binds to waste and toxins within the digestive tract, helping to pull them out and to contribute to regular bowel function. The fiber in black rice helps to prevent constipation, bloating, and other unwanted digestive symptoms. The dietary fiber found in black rice can also help to feel full after eating and to stay satisfied for a longer period of time between meals, potentially aiding in weight loss. Studies show that a diet high in dietary fiber from whole grain rice varieties is protective against obesity, heart disease, diabetes, and digestive disorders like irritable bowel syndrome (IBS). This is because fiber has the important job of clearing the body toxins, helping to reduce

inflammation and to clear the arteries as it removes waste and plague from the body. Black rice can also help to prevent or cure cases of diarrhea since fiber adds bulk to your stool. Like other colored rice types, black rice is partially milled so that some of the fibrous parts remain. Fiber acts as the broom which sweeps our colon and clean toxins, sludge, cholesterol, etc. It also helps feel us full, faster, and longer. Like brown rice, black rice provides higher dietary fibers and is also great for our gut. It facilitates bowel motions and helps prevent constipation. Higher fiber also helps to prevent assimilation of toxic materials in intestine and results in their elimination from body.

# 9.7 Anti-inflammatory Action

Inflammation is the human body's response to the presence of harmful bacteria, pathogens, viruses, or substances; and it is one of the reasons that the body is able to heal itself. Chronic inflammation is now cause of many diseases such arthritis, diabetes, heart disease, and even cancer. Drugs which control chronic inflammation are often expensive and come with numerous side effects especially when they are usually to be taken for long life. This may be the reason why anti-inflammatory foods and herbs are the health craze today. Spices and herbs such as turmeric and ginger and fish oils like omega-3S are just some of the safer and natural anti-inflammatory supplements currently being studied. Recently conducted research suggests that black rice can protect against (chemically induced and possibly other types of) inflammation. The full breadth of uses and possible applications for black rice are still being studied (www.cnn.com).

Researchers at Ajou University in Suwon, South Korea tested brown rice and black rice for their effectiveness in protecting against skin inflammations and found that the black rice bran suppress dermatitis but the brown rice bran not. This scientific study suggests that black rice may be a useful therapeutic agent for the treatment and prevention of diseases associated with chronic inflammation. In a study published in the "American Chemicals Journal of Agricultural and Food Chemistry," researchers found that mice fed with a diet supplemented with 10 % black rice bran significantly reduced inflammation and they also found that black rice bran inhibits the release of histamine, a chemical that triggers inflammation. Black rice consumption can alleviate allergic dermatitis symptoms such as swelling and allergy. Similarly, Korean scientists injected a group of mice with black rice bran extract directly into their skin. A second group was given a placebo to compare the results. In mice that received the bran, there was a 32 % reduction in skin inflammation that did not occur in the control group. The scientists also fed mice a diet made of 10 % black rice bran and discovered that its consumption also seemed to be related to an improvement in allergic dermatitis symptoms such as swelling. Such findings reinforce black rice bran's anti-inflammatory benefits and hold much promise for it to prevent future allergic reactions, asthma symptoms, and other inflammatory conditions. Asthma sufferers may also find relief by adding black rice to their diet.

Feeding mice a standard diet with added 10 % black rice bran significantly suppressed DNFB-induced allergic contact dermatitis on the skin of the mice. By contrast, a non-pigmented brown rice bran extract did not inhibit the TPA-induced edema and failed significantly to suppress production of pro-inflammatory biomarkers (mediators). These in vivo findings further demonstrate the potential value of black rice bran as an anti-inflammatory and antiallergic food ingredient and possibly also as a therapeutic agent for the treatment and prevention of diseases associated with chronic inflammation (Choi et al. 2010). If BR is orally administered, its main constituent C3G may be metabolized to cyanidin and PA which express potent anti-inflammatory effects by regulating NF- $\kappa$ B and MAPK activation (Min et al. 2010). Similarly, chronic diet intake of anthocyanin-rich extract from black rice may enhance plaque stabilization in old apoE-deficient mice. The underlying mechanism is related mainly to inhibiting pro-inflammatory factors and improving the serum lipid profile (Xia et al. 2006). In vivo findings also demonstrate the potential value of black rice bran as an anti-inflammatory and antiallergic food ingredient and possibly also as a therapeutic agent for the treatment and prevention of diseases associated with chronic inflammation (Choi et al. 2012).

## 9.8 Reduces Allergy

Scientists now believe that adding black rice bran into our diet may be a potential allergy treatment. Inhibitory effects of extracts of pigmented rice bran on in vitro allergic reactions were determined by Choi et al. (2007). Kamklaing (Thai black rice) bran is a promising byproduct that contains functional ingredients which may be useful in the control of skin aging and chronic disease development (Phetpornpaisan et al. 2014).

Main constituent of black-colored rice (BCR) is cyanidin-3-O- $\beta$ -D-glucoside (C3G) which exhibits an antiallergic effect, and orally administered C3G is mainly metabolized to protocatechuic acid in rats. To understand the relationship between the metabolism of C3G and its pharmacological effect, Han and his colleague isolated C3G from BCR; anaerobically incubated it with fecal microflora, investigated its metabolite(s) by LC-MS/MS, and measured the antiscratching behavioral effects of C3G and its metabolites. C3G was metabolized to protocatechuic acid via cyanidin. Protocatechuic acid and cyanidin were identified as the metabolites. The activities transforming C3G to protocatechuic acid and cyanidin were 28.2 ± 11.7 and 21.8 ± 5.2 nmol/h/mg fecal microfloras, respectively. C3G and its metabolites showed inhibitory effects against histamine or compound 48/80-induced scratching behaviors in mice. C3G more potently inhibited scratching behaviors following oral

administration than following intraperitoneal administration. However, protocatechuic acid and cyanidin showed more potent inhibition when administered intraperitoneally than when administered orally. These metabolites also inhibited the expression of allergic cytokines, IL-4, and TNF- $\alpha$  and the activation of their transcription factor, NF- $\kappa$ B, in RBL-2H3 cells stimulated with IgE-antigen. These findings suggest that C3G-rich BCR may be a beneficial food for diseases involving scratching behaviors such as chronic dermatitis, rhinitis, and psoriasis (Han et al. 2009a, b).

Antioxidant nature of black rice is effective in safeguarding skin. Black rice also helps to prevent skin from damage by pollutants or sun. It helps to prevent aging of skin as well as decreases signs of aging such as wrinkles, dark spots, and fine lines. Black rice provides antioxidants and other proteins to skin which help in restoring skin elasticity and maintain firmness.

## 9.9 Detoxifies the Body

Lots of researches have demonstrated that black rice consumption can help to detoxify the body and cleanse the liver of harmful toxic build up. It has been reported that black rice bran (Oryza sativa L.) is enriched with abundant antioxidative and hepatoprotective components, including cyanidin 3-O-β-d-glucoside and peonidine 3-O-\beta-dglucoside (Ling et al. 2001). The phytonutrients found in black rice help the body to reduce inflammation and to cleanse the body of harmful substances that can contribute to a wide range of conditions. It acts as cleaning agent which helps our colon to free from toxins, cholesterol, and other unhealthy stuff that we digest. In addition, fiber is slow digesting which means it will help feeling full and will prevent from snacking, and thus reduces the chance of weight gain. Jang et al. (2012) reported that dietary BRE supplementation improved serum lipid profiles and significantly enhanced mRNA expression levels of fatty acid metabolism-related genes, primarily via β-oxidation and ω-oxidation in the liver which show that a BRE-supplemented diet could be useful in reducing the risks of hepatic steatosis and related disorders including hyperlipidemia and hyperglycemia. In a recent animal study, the antioxidant status of blood, the liver, and the aorta were evaluated in rabbits that were either fed refined white rice or black rice. Results showed that rabbits fed black rice experienced less oxidative stress (free radical damage), contained more antioxidants in their blood, experienced detoxification in the liver, and also had reduced plaque buildup in the arteries.

Black rice outer layer fraction is a good source of dietary soluble fiber, oil, and plant sterols (Cara et al. 1992; Vissers et al. 2000; Xia et al. 2003) which accounts for the lower lipid accumulation in livers of rats fed atherogenic diets without the BRF. ARBE is significantly beneficial to liver health and that Cy-3-G is the

predominant anthocyanin in ARBE exerting this effect. The antioxidant activity of anthocyanins is an important mechanism by which ARBE exerts hepatic health benefit (Hou et al. 2013). Anthocyanin-rich extract from black rice also protected liver against CCl4 injury in mice and the results showed that anthocyanin is significantly beneficial to liver health (Hou et al. 2013). Yoon et al. (2014a, b) reported that pretreatment of HepG2 cells with BRE strongly protects the cells against TBHP-induced oxidative stress by reducing cell death, caspase-3 activity, and ROS generation and also by preventing ERK1/2 deactivation and the prolonged JNK activation. Mycelia fermentation is also utilized as a feasible strategy for enhancing the hepatotherapeutic effect of black rice and herbs (Chung et al. 2015).

Black rice (*Oryza sativa* L.) is often associated with blood lipid control. It is reported that C3G significantly decreases serum levels of total cholesterol, free cholesterol, triglycerides, and free fatty acids in rats fed a high-fat/cholesterol diet (HFCD). Similarly, hepatic cholesterol, triglyceride levels, and the activities of hepatic lipogenic enzymes (malic enzyme and glucose-6-phosphate dehydrogenase) were significantly reduced by C3G supplementation which suggest that C3G can ameliorate HFCD-induced hyperlipidaemia in part by modulating the activities of hepatic lipogenic enzymes (Um et al. 2013).

A study evaluated the protective effect of anthocyanin-rich extract from black rice (AEBR) on chronic ethanol-induced biochemical changes in male Wistar rats. Administration of ethanol (3.7 g/kg/day) to Wistar rats for 45 days induced liver damage with a significant increase (P < 0.05) of aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyl transferase (GGT) in the serum, and the hepatic malondialdehyde (MDA) level. In contrast, administration of AEBR (500 mg/kg) along with alcohol significantly (P < 0.01) decreased the activities of liver enzymes (AST, ALT, and GGT) in serum, the MDA levels and the concentrations of serum and hepatic triglyceride (TG) and total cholesterol (TCH). Rats treated with AEBR showed a better profile of the antioxidant system with normal glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and glutathione *S*transferase (GST) activities. The results demonstrated that AEBR has a beneficial effect in reducing the adverse effect of alcohol (Hou et al. 2010).

Similarly, Jiang et al. (2015) conducted a research to investigate whether cyanidin-3-O- $\beta$ -glucoside (Cy-3-G) could exert protective role on liver injury and its further mechanisms of the antifibrosis actions in mice. The results demonstrated that the treatment of Cy-3-G (800 mg/kg diet) for 8 weeks significantly attenuated hepatotoxicity and fibrosis in carbon tetrachloride (CCl4) administrated mice. Cy-3-G strongly downregulated the expression of  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), desmin, and matrix metalloproteinase (MMPs) which showed its suppression effect on the activation of hepatic stellate cells (HSCs). In addition, Cy-3-G favorably regulated oxidative stress and apoptosis in liver. Furthermore, Cy-3-G ameliorated the infiltration of inflammatory cells such as neutrophils and leukocytes, meanwhile suppressed the production of proinflammatory cytokines and growth factors. In conclusion, daily intake of Cy-3-G could prevent liver fibrosis progression in mice induced by CCl4 through inhibiting HSCs activation which provide basis for clinical practice of liver fibrosis prevention.

## 9.10 Lipid Profile Improvement

It is observed that diet containing black rice reduce the level of plasma cholesterol, triglycerides, and low-density lipoprotein. For high-density lipoprotein values, the diet that provides an increase in the levels is the black rice. The diet containing black rice is more effective in controlling the lipidemia in rats compared with the whole rice diet (Salgado et al. 2010). Supplementing BRF diets decreases lipid levels in the plasma, heart, and liver as well as plasma LDL, total cholesterol and triglycerides in Wistar Kyoto rats. Supplementation of anthocyanin-rich extract improves the lipid profile by decreasing serum triglyceride, total cholesterol, and non-HDL cholesterol. Thus intake of anthocyanin-rich extract from black rice may enhance plaque stabilization in old apo E-deficient mice. The underlying mechanism is related mainly to inhibiting pro-inflammatory factors and improving the serum lipid profile (Xia et al. 2006). Oryzanol which is a component of the BRF can also affect lipid metabolism especially by decreasing cholesterol absorption (Berger et al. 2005; Cicero and Gaddi 2001; Lichtenstein et al. 1994; Suh et al. 2005), although there are some reports that cyanidin-3-O-glucoside does not affect the lipid profile of hypercholesterolemic rats (Frank et al. 2002).

# 9.11 Reduces Risk of Diabetes

Harvard School of Public Health scientists have estimated that replacing about two servings a week of white rice with the same amount of black rice would lower diabetes risk by 16 % (Archives of Internal Medicine, June 14, 2010: 170 (11); 96-99). Insulin resistance is strongly associated with non-alcoholic fatty liver disease. Numerous studies have suggested that natural anthocyanins are potent antioxidants associated with prevention of diabetes. Jang et al. (2015) hypothesized that black rice containing C3G may reduce the risk of hepatic fat accumulation and improve insulin resistance. A clinical evaluation published in "American Journal of Clinical Nutrition" investigated the effects of consumption of major flavonoid groups concluded that anthocyanins are the only group significantly associated with a lower risk of Type II Diabetes (Wedick 2012). Some scientific studies were done using rats with dosage adjusted to human equivalency of 120 mg C3G; it was demonstrated that C3G inhibited lipid peroxidation (cell membrane damage leading to cell death), improved activity of superoxide dismutase (antioxidant defense mechanism of the body), and demonstrated a hypoglycaemic effect (lowering of blood sugar levels) in eight weeks (Nasri 2011). C3G decreases blood sugar levels and is therefore considered to exhibit strong anti-diabetic effects (alleviation of diabetic progression) associated with metabolic syndrome (antioxidant and anti-inflammation). C3G anti-diabetic activities include the following:

#### 9.11.1 Vision and Eye Health

C3G defends the retina from the potentially harmful effects of light and UV rays. Jia et al. (2013) result suggests that BRACs improves retinal damage produced by photochemical stress in rats via AP-1/NF-B/Caspase-1 apoptotic mechanisms.

# 9.11.2 Cardiovascular Health

C3G may support to maintain healthy cholesterol levels already within the normal range and it also support healthy blood flow and vasodilation (widening of the blood vessels).

## 9.11.3 Glucose Metabolism

C3G supports the maintenance of healthy blood sugar levels through vascular mechanisms and the promotion of superoxide dismutase (SOD) activity.

# 9.11.4 Weight Loss

In vitro research suggests that anthocyanins may influence gene expression for fat metabolism signaling the body to burn rather than store fat.

Increased levels of glycated low-density lipoprotein (glyLDL) are commonly elevated in diabetic patients. Previous studies have demonstrated that glyLDLs increase the production of reactive oxygen species (ROS), activate NADPH oxidase (NOX), and suppress mitochondrial electron transport chain (mETC) enzyme activities in vascular endothelial cells (EC). Cyanidin-3-glucoside (C3G) reduces oxidative stress and insulin resistance in diabetic animals (Xie et al. 2012).

When researchers analyzed white and brown rice consumption in relation to type 2 diabetes risk, they discovered that white rice was more prone to contribute to insulin resistance as well as diabetes risk. The substitution of whole grains which includes brown or black rice, instead of white rice, white bread, pasta, and sweetened cereals may lower the chance of type 2 diabetes along with other problems, with keeping the weight under control as well as your energy levels balanced. Black rice is a whole bran grain where the fiber is kept, and fiber has the capacity to help glucose (sugar) from the grain to be absorbed through the body over an extended period of time.
#### 9.12 Improves Eye Vision

Anthocyanins present in black rice safeguard eyes from degenerative problems of eye. A diet full of black rice may decrease chances of development of cataract or diabetic complications associated with vision. Latest research points to preventative as well as curative effects of anthocyanins on impaired vision. Animal research indicates that anthocyanins may be used in management of ophthalmological diseases just like myopia and glaucoma (www.healthbenefitstimes.com).

## 9.13 Help in Satiation and Weight Management

Satiation is a complex neurobiological phenomenon mediated by hormones and enzymatic reactions that send signals to the brain associated with the recognition of the feeling of fullness (satiety). Black rice has a super-satiation effect that can greatly assist all those for whom weight management is a concern. There are eleven distinct anthocyanin species many of which are localized in the dark purple flavonoid pigments in rice husk (pericarp). Due to different localization patterns, these are not released and digested in the same place at the same time in our digestive tract. The effects are felt over time. They are in a sense "time released." The recognition process and digestion of the multiple species of anthocyanins slows the pace at which food moves through the digestive tract. In addition, the anthocyanins carried in the fiber slow down the oxidation of the food causing it to move slower and take longer to be processed (Yoshimura et al. 2012). Black rice has antioxidants woven through a carrier that is nutritionally well balanced, high in fiber (soft milled), and very low in sugar. Other foods with high levels of antioxidants (blueberries) may also be high in sugar. Sugar oxidizes quickly and may neutralize any satiation benefit.

An antioxidant found in the dark pigments of black rice effects the gene expression of fat metabolism. It turns off the gene expression for fat storage and turns on the gene expression for fat metabolism. Fat is burnt instead of storing it. This makes black rice an effective and safe vehicle for weight loss (Tsuda et al. 2006). Scientific studies using rats (with dosage adjusted to the human equivalency of 250 mg C3G) indicated that in 30 days, C3G reversed the weight gain of a high-fat diet to the level of a normal diet. Weight gain was significantly lower in the high-fat diet plus anthocyanins as compared to the high-fat diet only (Kwon 2007). Hence, C3G acts as a catalyst that promotes lipolysis to occur at a velocity that exceeds the capacity of the body to store the free fatty acids. The fatty acids are converted into energy rather than being stored. The overall effect of C3G results in prevention of obesity from diet. Similarly, Lee et al. (2015) found that higher ratio of black rice to white rice is associated with lower risk of abdominal obesity in Korean man. The inclusion of 3 % BRF in the diet does not affect gains in body weight which agrees with the findings of Xia et al. (2003) and Ling et al. (2002) in

mice and rabbits, respectively. These are the points which are safety level of BRF when fed at this level. Researchers suggest that intake of  $\geq 10$  % black rice to white rice in a normal diet could lower the risk of abdominal obesity in men. This dietary modification could serve to improve public health. However, further longitudinal studies on the association between black rice intake and abdominal obesity are warranted (Lee et al. 2015).

It is crucial for those along with pre-diabetes, diabetes, or any other forms of metabolic syndrome as well as insulin resistance to consume 100 % whole grains, instead of processed "white" types which lack fiber. Exactly the same can probably be said for those who have a problem with slimming down, since the fiber as well as nutrients in whole grain rice helps to shut off hunger signals as well as helps prevent over eating. Research has revealed that whole grain rice types might help to avoid insulin resistance which is associated with an elevated risk for obesity. In China, black rice is becoming common as weight reduction food. However, this can be related to high fibers contained in black rice which provides sense of full stomach as well as decreases craving for food. High fiber as well as anthocyanin also assists in reducing absorption of cholesterol. Animal study has additionally shown that black rice extract reduce serum triglyceride levels, decreases platelet hyper activity, and helps prevent weight gain.

Researchers investigated the mechanisms underlying the anti-obesogenic properties of black-colored rice (BCR; *Oryza sativa* L. cv. Heugkwangbyeo) with high levels of anthocyanins. Stimulation of cultured adipocytes with BCR extract caused a significant decrease in cellular lipid reduction, and C57BL/6 J mice fed BCR extract for 8 weeks exhibited significant reductions in body weight and body fat. Similarly, the plasma metabolites in mice rendered obese by intake of a high-fat diet were analyzed after being fed different diets for 8 weeks: control, *Garcinia cambogia*, control rice (*O. sativa* L. cv. Ilpumbyeo) and BCR. The major components related to this distinction were mannose, isoleucine, threonine, and phenylalanine. Also, body weight, glucose, and total cholesterol were positively correlated with the levels of propionic acid, butyric acid, cholesterol, alanine, ribitol, glucose, and galactose whereas the high-density lipoprotein (HDL)/non-HDL ratio were negatively correlated with those of stearic acid, cholesterol, valine, leucine, glucose, and galactose (Kim et al. 2013).

## 9.14 Reduces Cancer Growth

Tumor metastasis is the most important cause of cancer death and various treatment strategies have targeted on preventing the occurrence of metastasis. Traditionally, cancer treatment strategy primarily focused on preventing the occurrence of metastasis. Results show that anthocyanins from black rice could suppress CAL 27 cell metastasis by reduction of MMP-2, MMP-9, and NF-B p65 expression through the suppression of PI3 K/Akt pathway and inhibition of NF-B levels (Fan et al. 2015). Oral administration of AEBR (100 mg/kg/day) to BALB/c nude mice

bearing MDA-MB-453 cell xenografts significantly suppressed tumor growth and angiogenesis by suppressing the expression of angiogenesis factors MMP-9, MMP-2, and uPA in tumor tissue which suggests the anticancer effects of AEBR against human breast cancer cells in vitro and in vivo by inducing apoptosis and suppressing angiogenesis (Hui et al. 2010). Choi et al. (2013) reported that feeding a diet supplemented with 10 % (w/w) black and brown rice brans inhibited growth of transplanted tumors in mice. Chang et al. (2010) also reported that in BALB/c nude mice bearing MDA-MB-453 cell xenografts, oral administration of BRACs (100 mg/kg/day) suppressed tumor growth and angiogenesis and BRACs-induced apoptosis in MDA-MB-453 cells via an intrinsic pathway. In the same way, Suttiarporn et al. (2015) pointed out structures of some bioactive phytochemicals in the bran extract of the black rice and riceberry that demonstrated anticancer activity in leukemic cell. It is also identified that black rice pigments have a higher content of bioactive substances such as antimutagenic substance than the nonpigmented rice grain (Sig et al. 2012).

A molecular evidence was provided associated with the antimetastatic effects of peonidin 3-glucoside and cyanidin 3-glucoside, a major anthocyanins extracted from black rice (*Oryza sativa* L. indica), by showing a marked inhibition on the invasion and motility of SKHep-1 cells. This effect was associated with a reduced expression of matrix metalloproteinase (MMP)-9 and urokinase type plasminogen activator (u-PA). Peonidin 3-glucoside and cyanidin 3-glucoside also exerted an inhibitory effect on the DNA binding activity and the nuclear translocation of AP-1. Furthermore, these compounds also exerted an inhibitory effect of cell invasion on various cancer cells (SCC-4, Huh-7, and HeLa). Finally, anthocyanins from *O. sativa* L. indica (OAs) were evidenced by its inhibition on the growth of SKHep-1 cells in vivo (Chen et al. 2006) (Fig. 9.2).



**Fig. 9.2** Cell survival inhibitory effect of BRACs on breast cancer cells. Breast cancer cells MCF-10A, MCF-7, and MDA-MB-453 were treated for 24 h with 25, 50, 100, 200, and 400 µg/mL BRACs or not (control). Cell survival rate assay was performed by flow cytometry. ap < 0.05; bp < 0.01 compared with control (Luo et al. 2014)

It is proven that anthocyanins can inhibit the growth of breast cancer by inducing cell apoptosis and scavenging reactive oxygen species (ROS) (Xu et al. 2010). However, limited references are available about the potential in antimetastasis by anthocyanins (Adams et al. 2010; Tsuda 2012). Studies show that bioactive anthocyanins isolated from black rice may act as potential candidate for cancer metastasis inhibition (Neve et al. 2006) (Fig. 9.3).

Luo et al. (2014) demonstrated that nutritional ingredients such as BRACs have anticancer potential. Long-term BRACs (150 mg/kg/day) supplemented diet can slightly reduce tumor growth, but significantly inhibit cancer cells pulmonary metastasis from subcutaneous ErbB2 positive breast tumor xenograft. Further, BRACs treatment decrease cancer cell metastatic abilities, including migration, adhesion, motility, and invasion. These cancer metastasis inhibitory effects may be partly due to the enzymatic activity suppression of transfer promoting factor u-PA by BRACs. Effects of major anthocyanins (peonidin, peonidin 3-glucoside and cyanidin 3-glucoside) extracted from black rice exerts an inhibitory effect of cell invasion on various cancer cells (Chen et al. 2006). Chen et al. (2006) reported that anthocyanin extract of black rice is able to inhibit the growth of liver cancer cells. In the same way, mice fed with black rice pigment fraction diet also had lower CD4(+)



**Fig. 9.3** BRACs reduced primary tumor growth in MDA-MB-453 xenograft. The MDA-MB-453 xenografts were established on BALB/c nude mice. **a** The anesthetic mice of control (*left panel*) and BRACs (*right panel*) treatment groups and the isolated primary tumors by day 42. **b** Body weights of mice were measured every five days from the day BRACs treatment started. **c** Tumor volumes were measured and calculated every three days. **d** All mice were euthanized at the end point of the experiment and tumors were removed and weighed. *ap* < 0.05; *cp* < 0.001 compared with control (Luo et al. 2014)



**Fig. 9.4** Effects of BRACs on Pulmonary Metastasis and Ki-67 Expression in vivo. **a** The pulmonary metastases on the surface of lungs (×70), *arrowhead* indicates metastases. **b** The expression of Ki-67. *Brown* or *gray* staining cells were counted as Ki-67 positive (proliferating) cells (×400). The percentage of Ki-67 positive nuclei was calculated with the aid of an image computer analyzer. p < 0.05 compared with control (Luo et al. 2014)

T lymphocyte expression (P < 0.05) and weaker inducible nitric oxide synthase expression (P < 0.05) compared with mice fed AIN-93G diet and the white rice outer layer fraction diet respectively (Xia et al. 2003) (Fig. 9.4).

Kim et al. (2013) also demonstrated that antioxidant activity of black rice is better than that of red rice, and the lung cancer cell (A549) viability of red rice is better than that of black rice. Anthocyanin, a phenolic compound, has been reported to have an anti-inflammatory effect against lipopolysaccharide (LPS)-induced changes in immune cells. However, little is known about the molecular mechanisms underlying its anti-inflammatory effects. Results showed that BR-WG-P significantly inhibit LPS-induced proinflammatory mediators, including production of NO and expression of iNOS and COX-2. In addition, secretion of proinflammatory cytokines including TNF- $\alpha$  and IL-6 is also significantly inhibited. Moreover, BR-WG-P and anthocyanin inhibited NF-kB and AP-1 translocation into the nucleus. BR-WG-P also decreased the phosphorylation of ERK, p38, and JNK in a dose-dependent manner. It suggested that BR-WG-P might suppress LPS-induced inflammation via the inhibition of the MAPK signaling pathway leading to decrease of NF-kB and AP-1 translocation. All of these results indicate that BR-WG-P exhibits therapeutic potential associated with the anthocyanin content in the extract for treating inflammatory diseases associated with cancer (Limtrakul et al. 2015).

Black rice pericarp extract could inhibit proliferation, change the cell cycle distributions and induce apoptosis in human prostatic cancer cell PC-3. Its inhibitory effect may be through promoting activation of the JNK, p38 signaling pathway, and all the results suggest that black rice pericarp extract may have an inhibitory effect on prostatic cancer (Jiang et al. 2013). Fermentation might be a good process to increase the antimutagenicity. The protective effects of rice extracts might be due to the presence of antimutagenic components that are supposed to be flavonoids which might scavenge of the toxic compounds or/and inhibit bacterial enzyme. Thus, the results make such rice potentially useful in dietary antioxidant and chemoprevention (Sadabpod et al. 2014). Increasing evidence from animal, epidemiological, and clinical investigations also suggest that dietary anthocyanins have the potential to prevent chronic diseases including cancers.

#### 9.15 Increases Life Span

Antioxidants in black rice have prolonged the life of fruit flies by 14 %, say scientists from China. Antioxidants inhibit the effects of reactive oxygen species in the body which attack cells and are responsible for aging. Black rice, rich in antioxidants and widely consumed in China, is claimed to possess anti-aging activity. Zhen-Yu Chen and colleagues from The Chinese University of Hong Kong fed black rice extract to fruit flies and observed that the flies lived longer than they normally would. Their mean life span increased from 44 to 50 days. The extract contained two anthocyanins cyanidin-3-O-glucoside and petunidin-3-O-glucoside. The team fed different amounts of the extract to fruit flies. They found that 30 mg/ml of the extract increased their life span. The anthocyanins increased the expression of antioxidant enzymes and a gene that suppresses progressive neurodegeneration. The compounds also decreased expression of the gene Methuselah, an action that has previously been shown to increase lifespan. The team also tested the rice extract on flies that had been exposed to a weed killer that causes neurodegeneration to a group of normal flies and a group of flies with the Alzheimer's gene. The weed killer produces reactive oxygen species and is normally used to study the pathogenesis of neurodegenerative diseases such as Parkinson's disease. The team found that the extract extended the lives of one group of normal flies and one group of the Alzheimer's flies, but the team said that it is premature to conclude that black rice extract could attenuate age-related neurodegeneration such as Alzheimer's disease. However, Xu add that

if the number of flies used in the experiment could be increased to 2000 for example (from groups of 200), it would be expected that the researchers might get more significant difference in the survival rate and lifespan.

Anthocyanin, an established antioxidant, can potentially be detrimental during stressful conditions, at least in *D. melanogaster* which suggests that all anthocyanins are not beneficial and extend longevity.

A study was focused on the anti-aging effect of black rice under stress-induced premature senescence (SIPS) of WI-38 human diploid fibroblasts cells caused by hydrogen peroxide ( $H_2O_2$ ), a well-established experimental model of cellular aging. The protective effect of the MeOH extract from black rice against  $H_2O_2$ -induced premature senescence was investigated using WI-38 cells by evaluating the cell viability, lipid peroxidation, and cell life span.  $H_2O_2$ -treated WI-38 cells exhibited the phenomena of SIPS, the loss of cell viability, the increase of lipid peroxidation, and shortening of the cell lifespan. However, the treatment with black rice attenuated cellular oxidative stress through increase in the cell viability and inhibition in lipid peroxidation. In addition, the life span of WI-38 cells showed extension of the population doubling level suggesting that it would delay aging process which suggest that the MeOH extract from black rice may delay the aging process by attenuation of oxidative stress under stress-induced premature senescence (SIPS) cellular model (Choi et al. 2012).

Zuo et al. (2012) performed an experiment in which the OR wild type fly was maintained on a control diet and two experimental diets containing 10 mg ml<sup>-1</sup> BRE (BRE10) and 30 mg ml<sup>-1</sup> BRE (BRE30). Results demonstrated that BRE30 could prolong the mean lifespan of fruit flies by 14 %, accompanied with the upregulation of mRNA SOD1, SOD2, CAT, and Rpn11, and with downregulation of Mth. It was also found that BRE30 could attenuate the paraquat-induced neurodegeneration in OR wild type flies accompanied by upregulation of SOD1 (CuZnSOD), SOD2 (MnSOD), CAT (catalase), and Rpn11. In addition, BRE30 supplementation increased the survival time of OR wild type flies and Alzheimer transgenic flies A $\beta$ 42 33,769 with chronic exposure to paraquat. Hence it was concluded that BREs could extend the lifespan of fruit flies, most likely by regulating the genes of SOD1, SOD2, CAT, *methuselah* (Mth), and Rpn11 at the transcriptional level.

Phetpornpaisan et al. (2014) also reported the immunomodulation effect in hPBMC model and its usefulness in prevention of chronic disease and anti-aging of skin. The brown and black rice bran extracts exhibited significant inhibitory effects on the production of interleukin-1 alpha (IL-1alpha) and tumor necrosis factor alpha (TNF-alpha) in the same macrophage culture experiment. A possible mechanism of the immunomodulating activities of the rice bran extracts and the immunopharmacological significance of these findings are discussed.

A study investigated the antibacterial effect of a bioprocessed polysaccharide (BPP) isolated from *Lentinus edodes* liquid mycelial culture supplemented with black rice bran against murine salmonellosis. BPP was not bactericidal in vitro; however, it stimulates uptake of the bacteria into RAW 264.7 murine macrophage cells as indicated by increased colony forming unit (CFU) counts of the contents of the lysed macrophages incubated with *Salmonella typhimurium* for 30 and 60 min. After two hours of postinfection, the bacterial counts drastically increased in the macrophages but after 4 and 8 h of postinfection, BPP extract-treated cells showed

lower bacterial counts than the vehicle (saline phosphate pH 7.4 buffer, PBS) treated control. BPP elicited altered morphology and markedly elevated inducible nitric oxide (NO) synthase (iNOS) mRNA and protein expression in the infected macrophage cells. BPP also activated leukocytes in S. Typhimurium infected mice as determined by spleen lymphocyte proliferation and IFN- $\gamma$  levels in mice serum. ELISA analysis on cytokine production by Th1 and Th2 immune cells from splenocytes of infected mice showed significant increases in the levels of the following Th1 cytokines: IL-1B, IL-2, IL-6, and IL-12. Histology assays of the livers of mice infected with a sublethal dose  $(1 \times 10^4 \text{ CFU})$  of S. Typhimurium showed that BPP administered daily through an intraperitoneal (ip) or oral route, protected against necrosis of the liver, a biomarker of in vivo salmonellosis. The life span of mice similarly infected with a lethal dose of S. Typhimurium (1  $\times$  10<sup>5</sup> CFU) was significantly extended by ip injection or oral administration of the BPP without side effects. These results suggest that the activity of BPP against bacterial infection in mice occurs mainly through the activation of macrophage-mediated immune response resulting from augmented Th1 immunity. The significance of the results for microbial food safety and human health and further research needs are discussed (Kim et al. 2014).

BRE pretreatment protects the cells against oxidative stress by reducing cell death, caspase-3 activity, ROS generation, and also by preventing ERKs deactivation and the prolonged JNKs activation. Pretreatment of BRE increases the activation of ERKs and Akt which are prosurvival signal proteins. However, this effect is blunted in the presence of ERKs and Akt inhibitors. These results suggest that activation of ERKs and Akt pathway might be involved in the cytoprotective effect of BRE against oxidative stress and these findings provide new insights into the cytoprotective effects and its possible mechanism of black rice against oxidative stress (Yoon et al. 2014a, b).

## 9.16 Protects from Osteoporosis

Osteoporosis is an age-associated skeletal disease that exhibits increased adipogenesis at the expense of osteogenesis from common osteoporotic bone marrow cells. Research shows that black rice extract (BRE) consumption may protect from osteoporosis. An experiment was conducted where BRE stimulated the alkaline phosphatase (ALP) activity in both C3H10T1/2 and primary bone marrow cells. Similarly, BRE increased mRNA expression of ALP and osterix. Oral administration of BRE in OVX rats prevented decreases in bone density and strength. By contrast, BRE inhibited adipocyte differentiation of mesenchymal C3H10T1/2 cells and prevented increases in body weight and fat mass in high-fat diet fed obese mice, further suggesting the dual effects of BRE on anti-adipogenesis and pro-osteogenesis. UPLC analysis identified cyanidin-3-O-glucoside and peonidin-3-O-glucoside as main anti-adipogenic effectors but not for pro-osteogenic induction. In mechanism studies, BRE selectively stimulated Wnt-driven luciferase activities. BRE treatment also

induced Wnt-specific target genes such as Axin2, WISP2, and Cyclin D1. These data suggest that BRE is a potentially useful ingredient to protect against age-related osteoporosis and diet-induced obesity (Jang et al. 2012).

## 9.17 Enhances Hair Growth

In China and some other Asian countries, homemade fermented black rice water is utilized to wash hair. Black rice water provides antioxidants and nutrition for protection of scalp and hair. Black rice provides biotin, Vitamin B along with other proteins necessary for hair growth. Thus black rice helps to make hair smooth and glossy. It safeguards hair damage from wind and sun. Today, numerous hair formulations can be found in market with black rice extract. Homemade black rice shampoo made out of black rice water and aloe vera gel for safeguarding hair damage. This particular evident uses black rice nourishing wine made out of black rice, mulberry, Chinese angelica, dried rehmnnia root as well as yellow rice wine particularly ratio for hair nourishment as well as growth (www.healthbenefitstimes. com).

## 9.18 Reduces Risk of Asthma

The anthocyanins in black rice have shown to fight free radicals within the body and particularly the ones that induce inflammation within the airways and liable for mucous secretion related to asthma. This tends to work as an adjunct to something to assist manages asthma exacerbations (www.healthbenefitstimes.com).

## 9.19 Antioxidative Effects

Black rice is a very good source of antioxidants; a substance well known for helping individuals flush out body wastes on a regular basis. Antioxidants are also found in coffee and tea but are definitely more prevalent in black rice. The bran/hull of the black rice, i.e., the outermost layer of the rice grain contains highest levels of the antioxidant. Black rice has a deep black or purple color which is an indication of its high antioxidant properties. Research show that the anthocyanin content of black rice is higher than any other grain including brown rice, red rice, red quinoa, or other colored whole grain varieties. Anthocyanin has the ability to help, prevent a myriad of common but serious illnesses. Anthocyanins have been correlated to prevent cardiovascular disease, protecting against cancer that can be caused by free radical damage, improving brain function, reducing inflammation, and more. Whole grain like brown and red rice also contain beneficial antioxidants, but only black rice contains anthocyanin. Additionally, black rice also contains an important antioxidant Vitamin E which is useful in maintaining eye, skin, and immune health.

Black rice anthocyanin molecules are known to have potent antioxidant properties which help to protect our bodies from cardiovascular diseases and cancers (www.blackrice.com). Cornell University researchers found that antioxidant content is approximately six times higher in black rice than in common brown/white rice. The main anthocyanin compounds in black rice include cyanidin-3-O-glycoside and peonidin-3-Oglycoside (Zhang et al. 2006). The black rice bran has higher content of phenolics, flavonoids and anthocyanins (Journal of Agricultural and Food Chemistry, 2010, 58(13): 7580–7587). Antioxidants have been linked to prevention and even treatment of a wide range of medical conditions. In addition to its almost legendary antioxidant properties, black rice is also a well-known anti-inflammatory food which helps cut down on general infections and illnesses. Recent studies show that antioxidants are found to slow down the aging process. Research into the uses and applications for antioxidants has revealed that they are linked to the prevention/treatment of the following areas/conditions:

- Anti-aging
- Heart disease
- Diabetes
- Alzheimer's disease
- Cancer
- Inflammation

Antioxidants are also known to actively break down the nasty types of arterial plaque and chemically fight high cholesterol levels. The magical thing about black rice is that it can do all of this without any harmful side effects. Black rice has long been consumed in Japan and China and is considered to be a healthy food because of its antioxidant content that able to prevent oxidative stress. Oxidative modification of low-density lipoprotein (LDL) may play an important role in the development of atherosclerosis. Methanol-HCl 1 % black rice extract has more potential antioxidant activity in compared with rutin. It is known that the antioxidant capacity results mainly from the seed capsule, not the endosperm. The anthocyanin pigments contribute little to the total antioxidant capacity of red (0.03-0.1 %) and black (0.5-2.5 %) rice cultivars. Hence, the antioxidant capacity is derived mainly from other phenolic compounds (Xq et al. 2012). Extracts from ancient rice brans, especially from black rice bran possess strong scavenging activities for reactive oxygen species (ROS). Anthocyanins compounds are found to possess both strong ROS scavenging activities to suppress cell damaging effects of UVB, indicating that both Cy-3-glu and cyanidin are the active components involved in the antioxidative of black rice brans (Kaneda et al. 2006).

## 9.20 Acts as GABA Rice

The digestion of grain proteins results in many nutrients including *y*-aminobutyric acid (GABA). GABA is a neurotransmitter in the brain and spinal chord and induces tranquilizing effects. It is used as a medication to increase blood flow to the brain, to inhibit cancer cell proliferation and provide other beneficial health effects. A Thai purple (black) rice variety exhibited the highest GABA content of all 21 rice varieties tested which indicates the importance of purple rice cultivars for adding nutritional value to functional food products (Karladee and Suriyong 2012).

## 9.21 Works as Nature's Great Super Foods

Black rice grain is low in sugar but packed with high amount of healthy fiber and plant compounds that combat heart disease and cancer. Research suggests that the dark plant antioxidants which mop up harmful molecules can help to protect arteries and to prevent the DNA damage that leads to cancer. A spoonful of black rice bran contains more health-promoting anthocyanin antioxidants than are found in a spoonful of blueberries but with less sugar and more fiber and vitamin E antioxidants. Especially, black rice bran would be a unique and economical material to increase consumption of health-promoting antioxidants. Black rice could also be used to provide healthier, natural colorants. Scientists at the 240th National Meeting of the American Chemical Society in Boston; Victoria Taylor, senior dietician at the British Heart Foundation, said:

In reality, it is unlikely there is a single food that will have a great impact on lowering risk of heart disease. Healthy eating is about a balanced diet overall. It is great if you can eat more of some groups of healthy foods, like having five portions of fruit and vegetable a day, but there is still no conclusive evidence that 'super foods' alone make a real difference to your heart health (www.dailymail.co.uk).

one spoonful of black rice bran equivalent to 10 spoonfuls of cooked black rice contains the same amount of antioxidants as a spoonful of fresh blueberries

Said Associate Professor Zhimin Xu from Louisiana State University Agricultural Centre when reporting on the research at the 240th National Meeting of the American Chemical Society (ACS).

Black rice is the new super food making waves now. Moreover blueberries; black rice is being hailed as the new superfood by US scientists and it is cheaper too. Researchers at Louisiana State University analyzed bran samples from black rice grown in the southern United States and found them with low sugar, high fiber plant compounds that have been proven to prevent heart disease and fight cancer. In ancient China, black rice was reserved especially for eating by Emperors and was believed to have great health properties. Recently, a group of Chinese scientists have found some truth behind these health claims. Just a spoonful of black rice bran contains more health-promoting anthocyanin antioxidants than are found in a spoonful of blueberries, but with less sugar and more fiber and vitamin E antioxidants.

## 9.22 Black Rice is a Panacea

Chinese black rice has medicinal value. It is a fatigue fighter. Consuming Chinese black rice frequently means keeping one's hair dark, black, and shiny. Chinese Traditional Medicine (TCM) doctors recommend Chinese black rice for their elderly patients. They say it increases their appetite and is a good treatment for digestive disorders. In addition, they recommend using it as a diabetic preventative. For anorexics, they suggest stir frying black rice because it helps to reduce the condition. For general people, they suggest black rice to consume to tonify and keep blood dark red and increase *ai*. For this, they suggest cooking it with loguats and sweet potatoes. However, they also say to avoid this kind of rice when suffering from a ving, kidney deficiency disease. For the elderly, TCM practitioners recommend cooking one cup of glutinous white rice for half an hour and in another pot, cooking one cup of Chinese black rice for the same amount of time. Western medical research says that black-pigmented rice husk has antioxidant of anti-inflammatory value. They find it scavenges free radicals and also suppresses low-density lipoprotein molecules, the so-called bad fat cells. Many ongoing studies are seeking new uses of black rice and its seed coats in neutraceutical and functional food formulations. It is high in iron, fiber, and antioxidants, particularly Vitamin E and lower in sugar than its fruit counterparts, and therefore considered an even better preventative measure against (or alleviator of) chronic inflammation, allergies, high cholesterol, high blood pressure, diabetes, Alzheimer's, heart disease, certain cancers, and even aging (www.blackrice.com).

## 9.23 Other Possible Health Benefits

Black rice is also being looked as a possible prevention from serious illnesses. Some diseases it can prevent include Alzheimer's, diabetes and even cancer. Black rice is lower in sugar, higher in Vitamin E, and higher in iron. It is also considered as aphrodiastic diet. According to the American Chemical Society, it is possibly healthier with more nutritional benefits than brown rice. Glutinous rice, also known as sticky rice, does not contain amylose which makes this rice sticky and helps slow down digestion and lowers insulin levels. However, black glutinous rice is unprocessed, it retains more of its other nutrients making it a healthy food overall. A standardized extract of black rice pigmented fraction (BRE) containing known proportions of cyanidin 3-glucoside and peonidin 3-glucoside exhibit marked antioxidant activities and free radical scavenging capacities in a battery of in vitro model systems. Significant (p < 0.05) prevention of supercoiled DNA strand scission induces reactive oxygen species (specifically, peroxyl radical and hydroxyl radicals) and suppression of the oxidative modification of human low-density lipoprotein is obtained with BRE. In addition, BRE reduces (p < 0.05) the formation of nitric oxide by suppressing inducible nitric oxide synthase expression in murine macrophage RAW264.7 cells without introducing cell toxicity. Black rice contains anthocyanin pigments with notable antioxidant and anti-inflammatory properties for potential use in nutraceutical or functional food formulations (Hu et al. 2003). Similarly, germinated rice contains numerous nutrients including  $\gamma$ -aminobutyric acid (GABA), dietary fiber, inositols, ferulic acid, phytic acid, tocotrienols, magnesium, potassium, zinc,  $\gamma$ -oryzanol, and prolylendopeptidase inhibitor (Shoichi 2004). Beneficial biological activities of these compounds also have been well documented. One of the most interesting findings was a twofold increase in hyaluronan synthase 2 (HAS2) gene expression bv BRP. Semiguantitative RT-PCR showed that BRP increased HAS2 mRNA in dose-dependent manners. ELISA showed that BRP effectively increased hyaluronan (HA) production in HaCaT keratinocytes (Sim et al. 2007).

It is a proven facts that regular intake of germinated brown rice has been found good for health, e.g., it can help to prevent headache, colon cancer, heart disease, and Alzheimer's disease, as well as lower blood pressure and regulate blood sugar level (Kayahara and Tukahara 2000).

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# Chapter 10 Black Rice Recipes

Black rice has been used in traditional Chinese desserts and snacks to make a dessert porridge, black rice cakes, or breads, noodles and many more. Another interesting application for black rice is to use it as a natural food coloring agent. Currently some small manufacturers add black rice either in ground or in extract form to beverages and foods in order to avoid artificial dyes and ingredients which can be harmful in numerous ways. Some popular recipes of black rice are as follows:

- Japanese sautéed firm tofu and black rice
- Chinese spicy sesame beef stir-fry on black rice
- Asian black rice salad
- Black rice salad
- Eggs over black rice
- Black rice risotto
- Black rice with sweet potato scallions
- Brazilian style calamari with black rice
- Sweet black rice porridge
- Sesame black rice

## 10.1 Rice Cooking Methodology

Different varieties of rice are best when cooked using a particular method. Different methodologies are applied to cook rice. One must be sure to follow recipe instructions to get the best flavor and texture from his rice.

## 10.1.1 Absorption Method

This is the most popular method. A set amount of rice is used with a set amount of water for a set amount of time. By the time the water is absorbed and the rice looks tender.

## 10.1.2 Steaming Method

This is usually the preferred method for cooking sticky and clinging rices. Rice is soaked, drained and put in a steaming basket set over a pot and cooked by steam alone without the rice ever touching the boiling liquid.

## 10.1.3 Boiling Method

In this method, the rice is cooked much like pasta. Though this may sound appealingly easy and foolproof, it actually requires almost as much attention as does the absorption method. The rice is sprinkled into a large pot of boiling salted water and then stirred often to prevent sticking. When rice becomes tender, it is thoroughly drained, then rinsed quickly to halt cooking. Sticky and clinging rices do not do well with this method but many other varieties do fine.

## **10.2** Basic Directions for Absorption

## 10.2.1 Rinsing

This step removes surface starch and should only be done when the grains remain quite separate as in Indian basmati rice. For most rice preparations, do not rinse.

## 10.2.2 Measuring

The general ratio is 1 cup rice to 1.5 or 2 cups water, plus 1/8-1/2 teaspoon sea salt. Place rice, salt, and water in a heavy pot with a tight fitting lid.

## 10.2.3 Simmering

Bring water and salt to boil in a heavy pan with a tight fitting lid. Add rice, bring back to a boil, stir once, cover and simmer over low heat until the grains are tender.

#### **10.3** Cooking Black Rice

Black rice comes from exactly the same plant family as other colored rices and includes several varieties like Indonesian black rice and Thai jasmine black rice. The different types of black rice include virtually identical health advantages and all have got a mild, nutty taste which is similar to the taste of much familiar brown rice. Since it is unrefined as well as denser compared to white rice, black rice will take longer to cook. The best results may be accomplished by first soaking black rice for around 1 h just before cooking it, yet ideally for many hours. If you soak rice, you will reduce the amount of cooking time and in addition make the nutrients within the rice more absorbable (www.healthbenefitstimes.com).

Like other unmilled rice, black rice takes longer time to cook than traditional husked rice. It should also ideally be stored under refrigeration and used within 3 months to prevent spoilage. Rinsing and soaking the rice before cooking will help to bring the cooking time down. As a general rule, black rice should be cooked with two cups of water to every one cup of rice, and it will need to cook for 20-30 min after soaking, or 60 min if cooked unsoaked. When black rice is used in desserts, it is sometimes cooked with a mixture of coconut milk, sugar and water to form a sweet, sticky, pudding like rice. Because it is unrefined and denser than white rice, black rice takes longer to cook. The best results can be achieved by first soaking black rice for at least 1 hbefore cooking it, but preferably for several hours. If it is planned ahead of time and rice is soaked, the amount of cooking time required is cut down and also makes the nutrients in the rice more absorbable. After soaking the rice, rinse the rice, clean and then place it on the stove top with two cups of water for every one cup of rice. Boil the rice for at least half an hour if the rice is soaked and for 1 h if the rice is not soaked. Check the texture of the rice after this time to see if it is chewy and cooked through; if not, keep boiling until it reaches the texture one is looking for (www.wholefoodsmarket.com).

	Characteristics	Grain to liquid	Basic cooking method
Arborio rice (white)	Soft and creamy. Best used in risotto recipes	1 cup to 3/4 cups	After an initial toasting of the grains in butter or oil, liquid (usually broth) is added gradually as rice is stirred to create a rich almost saucelike result
Basmati (white imported and brown)	Long-grain, highly aromatic, hulled rice from India. Usually aged for a year to develop its full flavor	White: 1 cup to 1.5 cups Brown: 1 cup to 2 cups	Soak and rinse rice for 30 min. Simmer white basmati 15 min. Simmer brown 45 min

## **10.4** Rice Cooking Tips and Its Serving

	Characteristics	Grain to liquid	Basic cooking method
Brown rice (long grain)	Tends to remain separate and fluffy when cooked. Great for pilafs, rice salads and paella	1 cup to 2 cups	Simmer 45 min
Brown rice (medium grain)	Similar to long grain, but stickier. Great with stir-fries and curries	1 cup to 2 cups	Simmer 45 min
Brown rice (short grain)	Sticky, chewy rice; very good in sushi and puddings	1 cup to 2.25 cups	Simmer 45 min
Brown rice (sweet)	Very sticky. It is what mochi and amasake are made from	1 cup to 2 cups	Simmer 50 min
Forbidden rice	Nutty-tasting black rice, imported from China. Soft textured; purple when cooked	1 cup to 2 cups	Simmer 30 min
Jasmine rice (white or brown)	Aromatic, long-grain rice similar to basmati. The perfect accompaniment to Thai curries	White: 1 cup to 1.75 cups Brown: 1 cup to 2 cups	Simmer white rice for 15 min. Simmer brown rice for 45 min
Kalijira rice (white)	Long-grain rice but on a miniature scale. Sometimes called baby basmati, these tiny grains are nutty and aromatic and cook up quickly	1 cup to 1.5 cups	Rinse well. Simmer 10-15 min
Lundberg countrywild	Long-grain brown rice, blended with Wehani and Black Japonica rices; delicious as a side dish	1 cup to 2 cups	Rinse rice and simmer 45 min
Purple sticky rice	Used as a sweet dessert rice	1 cup to 2 cups Rinse well	Bring to a boil (no salt), cover and simmer for 40 min
Red rice	Imported from Bhutan; has a nutty taste and pink color when cooked	1 cup to 1/2 cups	Bring to a rapid boil over high heat. Cover, reduce heat and simmer for 30– 40 min
Sushi rice (white)	Medium grain, chewy and sticky	1 cup to 1 cup	Rinse and drain several times until water runs clear. Bring to a boil. Cook 1 min. Reduce heat and simmer for 15 min. Rest for 10 min

(continued)

(continued)

#### (continued)

	Characteristics	Grain to liquid	Basic cooking method
Texmati rice (brown)	A cross between basmati and long grain American rice. Delightfully nutty, fragrant rice	Great plain, with curried vegetables or seafood, or use in stuffings	1 cup to 2 cups Simmer 15–20 min
Texmati rice (white)	A cross between basmati and long-grain American rice. Fluffier and milder in flavor and aroma than imported basmati	1 cup to 1.75 cups	Simmer 15–18 min
Wehani rice (red rice)	Long-grain rice, but on a miniature scale. Sometimes called baby basmati, these tiny grains are nutty and aromatic and cook up quickly	1 cup to 2 cups	Simmer 45 min
Wild rice	Technically an aquatic grass seed, but cooked and enjoyed as rice. Delightfully chewy and full-flavored, it can be a little too intense on its own, so it's popular in grain mixes, soups and salads	1 cup to 3 cups	Rinse well. Bring to a boil, cover and simmer strongly for 45 min
Wild and brown rice	20 % lake-harvested wild rice and 80 % long-grain brown rice. A milder alternative to wild rice and a great side dish	1 cup to 3 cups	Simmer 45 min
Wild rice blend	Made from long-grain brown rice, sweet brown rice, Wehani, Japonica, and wild rice. A beautiful blend for sides or soup	_	-

## 10.5 Black Rice Pudding

Black rice pudding is a great dessert and commonly served for breakfast in parts of Southeast Asia (www.epicurious.com).

#### Ingredients

- 1 cup black rice
- 1/2 cup sugar
- 1 (13-1/2-15-oz) can unsweetened coconut milk, stirred well

## Preparation

- (1) Take rice, 3 cups water and 1/4 teaspoon salt to a boil in a 3–4 quart heavy sauce pan, reduce heat to low and simmer, covered with a tight fitting lid for 45 min (rice will be cooked but still wet). Stir in sugar, a scant 1/4 teaspoon salt and 1.5 cups coconut milk and bring to a boil over high heat, then reduce heat to low and simmer, uncovered, stirring occasionally, until mixture is thick and rice is tender but still slightly chewy about 30 min.
- (2) Remove from heat and cool to warm at room temperature, stirring occasionally at least 30 min. Just before serving, stir pudding and divide among 8 bowls. Stir remaining coconut milk and drizzle over pudding.

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# Index

#### A

Absorption method in cooking, 186 Allergy reduction, 161–162 Anthocyanin content, cooking methods and, 71–74 Anthocyanin profile of black rice, 61–67 Anti-inflammatory action, 160–161 Antioxidative effects, 174–175 Applications of black rice, 105–112 food coloring dye, 106–107 Armyworm, 137–138 Aromatic chemistry of black rice, 75–79 Aromatic rice, 13–14 Asthma prevention, 174

#### B

Bacterial leaf blight, 128–129
Bacterial leaf streak, 132–133
Black rice, 21–42, 94–96
black conversion process, 28–29
blueberries, 38
Blast, 127–128
Blueberries versus black rice, 38
Boiling method in cooking, 186
Broadcasting method, 119–122
Brown basmati rice, 10–11
Brown rice, 16, 91–93
Brown spot, 130–131

#### С

Cancer growth reduction, 167–171 Characteristics of black rice, 118–119 Chemicals present in black rice, 59–69 anthocyanin, 61–67 cyanidin-3-glucoside (C3G), 67–69 Chinese black rice, 13, 24–25 Colored rice, 86–91 Colusari red rice, 12 Cooking methodology, 185–186 Cropping seasons for rice, 5–6 Cultivating black rice, 115–149 Cyanidin-3-glucoside (C3G), 67–69

#### D

Degree of milling, 74–75 Detoxification, 162–163 Diabetes risk reduction, 164–165 Digestive health, 159–160 Diseases, 127–134 Domestication of, black rice, 4–5, 26–29 Drilling method, 122 Dry bed method, 124

#### Е

Economic importance, 101–103 Emperor's rice, 26 Eye vision improvement, 166

#### F

False smut, 132 Fermentation, 71 Fertilizer application, 126 Food coloring dye, 106–107 Forbidden rice, 25–26, 52–53

#### G

GABA rice, 176 Gall midge, 136 Genetics of black rice, 29–31 Genetics of rice, 2–4 Grain structure, of rice, 6

© Springer International Publishing Switzerland 2016 U.K.S. Kushwaha, *Black Rice*, DOI 10.1007/978-3-319-30153-2 Green leaf hopper (GLH), 138–139 Growth conditions for rice, 5 Gundhi bug, 140–147

#### H

Hair growth increase, 174 Harvesting, 147–148 Health benefits of black rice, 151–178 hair growth increase, 174 heart health, 154–157 hypertension control, 159 Himalayan red rice, 11 History of black rice, 49–53 Hypertension control, 159

#### I

Insect pests, 134–140 Iron content of black rice, 37

J Japanese method, 122

K

Khaira disease, 134

#### L

Leaf roller, 137 Life span increase, 171–173 Lipid profile improvement, 164 Long grain rice, 8 Long-grain white rice, 86 Lowland cultivation, 123

#### М

Medium grain rice, 8 Mole cricket, 138

#### Ν

Nursery raising, 123–124 Nutrition profiles of black rice, 55–79

#### 0

Osteoporosis prevention, 173-174

#### Р

Paraboiled rice, 17–18 Parboiling, 70–71 Plant hopper, 139 Post-harvest loss, 148–149 Precooking rice, 18 Protein content of black rice, 37 Pudding, 189–190 Purple Thai rice, 12

#### R

Rats, 140–147 Recipes, of black rice, 185–190 Red rice, 93–94 Rice hispa, 136 Rice, 1–18 rough rice, 16 Rough rice, 16

## S

Satiation management, 166–167 Seedlings raising, 124 Sheath blight, 130 Short grain rice, 8–9 Spread process, 29 Steaming method in cooking, 186 Stem borer, 135 Stem rot, 131 Swarming caterpillar, 139–140 Sweet brown rice, 9–10 Sweet rice, 13

## Т

Taxonomy of rice, 2–4 Threshing, 147–148 Transplanting methods of black rice, 122–127 Tungro virus, 133–134

#### U

Upland cultivation, 123 US arborio Rice, 14–15

#### V

Varieties of black rice, 116-119

#### W

Water management, 126 Waxy rice, 13 Weeding, 126–127 Wehani rice, 10 Weight management, 166–167 Wet bed method, 124 Wet milling, 69 White rice, 17

#### Y

Yield, 149