Total anthocyanin, flavonoid and phenolic content of pigmented rice landraces from South Sulawesi

by Rinaldi Sjahril

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A P Hanifa[1,2], J P Millner[2], C R M McGill[2], R Sjahril[3]

[1] AIAT South Sulawesi, Jl. Perintis Kemerdekaan KM.17.5 Makassar, Indonesia
[2] School of Agriculture and Environment, Massey University, New Zealand
[3] Faculty of Agriculture, Hasanuddin University, Tamalanrea Makassar, Indonesia

Email: rinaldi.sjahril@gmail.com

Abstract. The precious traits in the coloured grains of red, deep purple, or black has attracted more attention due to its health benefits. These health benefits have been attributed in part to phytochemicals in the rice seed, including phenolic, flavonoid and anthocyanins which are known as source of antioxidants. This study was aimed to investigate the content of total phenolic, flavonoid and anthocyanin from seventeen pigmented rice collected from the upland regions of Toraja, Enrekang and from lowland of Jeneponto, South Sulawesi. The grains were dehusked using handheld rice husker (Kett TR130) then were grinded into fine powder before extraction. Flavonoids and anthocyanins were quantified by UV-Vis spectrophometry and total phenolic content was determined using Folin-Ciocalteu method. Results showed that black rice glutinous rice varieties from lowland (*Le'leng*) had the highest content of total anthocyanins, flavonoids and phenolics.

1. Introduction

Pigmented rice is one of the untapped treasures amongst landrace diversity in Indonesia. Their preservation is pivotal for environmental sustainability and to enable valuation of their potential for future utilisation to strengthen food security. Pigmented rice is increasingly popular due to its health benefits. For example, red rice is becoming popular in Japan as a functional food because of the high content of polyphenols [1]. In addition, compared with common white rice, black rice posses higher protein content, vitamins and minerals [2]. Coloured rice also has wide range of biological properties including anticarcinogenic, antiallergic, antiatherosclerosis properties as well as amelioration of iron deficiency [3].

The bioactive content of pigmented rice has attracted the most recent research for use as a functional food. Phenolics are the initial substances in flavonoid's biosynthethic pathway. In addition, phenolic properties and benefits such as reducing the risks from heart disease, diabetes, inflammation have been confirmed [4]. Anthocyanins are naturally occurring color substances from flavonoid group. Positive health effects of the pigments present in the bran layer of rice have been reported [5] including free radical scavenging and antioxidant activity [6].

In food fortification and animal-human clinical research, rice is the most studied cereal [7]. The trend is likely to climb as South America, Europe and Africa are also becoming interested in the antioxidant potentials of their rice varieties [8]. In line with the emerging market of healthy food nowadays, more information regarding bioactive contents in coloured rice grains is important.

Pigmented rice may get higher value both in market and society. Thus, the bioactive contents can be an economic leverage for the farmers.

This research was aimed for initial assessment of bioactive content in pigmented rice husked grain that were collected from Toraja, Enrekang, Jeneponto before multi sites experiment.

2. Methods

2.1 Rice samples

Fifteen pigmented rice varieties were collected in form of paddy from three regencies in South Sulawesi, during May-July 2017. Two modern-national varieties (one white, Ciherang and one red, Inpari 24) were grown also for comparison (Table 1). For simplicity henceforth when landraces are referred to this will include the two national varieties.

Table 1. Source and type of the rice seed landraces assessed for bioactive content

Variety	Altitude (m asl)	Regency
Black rice		
Kobo	900	Toraja
Lotong	780	Toraja
Ambo	903	Toraja
Lallodo	905	Toraja
Lotong Tanduk	908	Toraja
Le'leng	115	Jeneponto
Red rice		
Ra'rang	836	Toraja
Lea	791	Toraja
Maminyak	727	Enrekang
Mandoti	789	Enrekang
Balan	536	Enrekang
Kamida	847	Enrekang
Jambu	607	Enrekang
Bakka Eja	115	Jeneponto
Punu Eja	115	Jeneponto
National/modern variety		
Inpari 24 (red)		
Ciherang (white)		

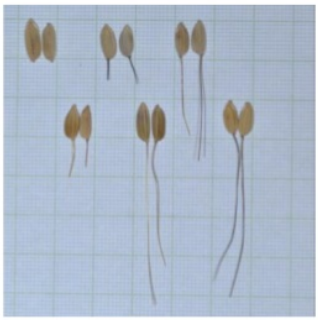

Figure 1. Comparison of husked black rice materials
Left to right-bottom up : Ko'bo, Lotong, Ambo, Lallodo, Lotong Tanduk, Le'leng

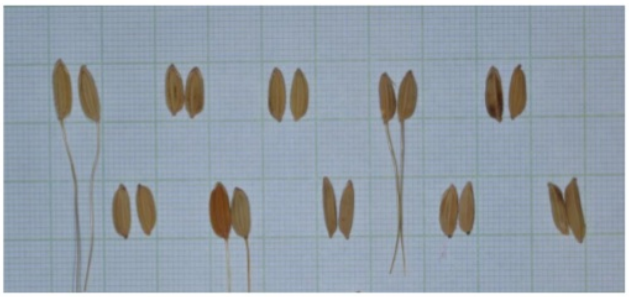

Figure 2. Comparison of husked and dehusked red rice grains.
Left to right- bottom up : Kamida, Jambu, Bakka Eja, Punu Eja, Inpari 24, Mandoti, Barri Ra'rang, Lea, Maminyak, Balan

2.2 Timing

Assessment of seed's bioactive content was undertaken at the School of Agriculture and Environment, Massey University, Palmerston North from March to May 2018.

2.3 Sample preparation and extraction

A rice husker (Kett TR-130) was used to remove the husk and prevent the pigment loss from the pericarp layer's outer. The rice seed was then ground in a grinder and sieved using 30 mesh sieve to get fine powder. Samples of rice flour were then stored in sealed polyethylene containers in triplicates. All samples are stored immediately at 5 °C until analysis.

One gram of rice powder was added to 30 ml of a mixture of ethanol and citric acid (1.0 mol L^{-1}) in the ratio 10:20. The extraction was performed in a water bath for 80 minutes at 35°C under constant stirring. After processing, the extract was filtered (qualitative Whatman #1 paper), the residue and filter paper rinsed using the extraction solvent into a polyethylene bottle until a volume of 50 mL is reached. The extract is stored at -20 °C in the polyethylene bottles, protected from light.

2.4 Analyses of total anthocyanins, flavonoids, and total phenolics

Flavonoids and anthocyanins from the rice grains were quantified using UV-Vis spectrophotometry at λ = 374 nm and λ = 535 nm, respectively. The content of flavonoids and anthocyanins were determined using equation 1 and 2, respectively [9]. The content of total flavonoids is expressed as mg quercetin (a plant pigment (flavonoid)) equivalents (CE) per 100 g of rice dry weight and total anthocyanins expressed as mg cyanidin-3-glucoside equivalents (CGE) per 100 g dry weight sample (mg CGE 100 g−1 DW).

Equation 1 $$\text{Total flavonoids (TF)} = \frac{A_{374nm} \times \text{dilution factor}}{76.6}$$

Equation 2 $$\text{Total anthocyanins (TA)} = \frac{A_{535nm} \times \text{dilution factor}}{98.2}$$

Total phenolic content was determined using the Folin–Ciocalteu method [10];[11] with slight modifications [12]. To a 5.0 mL flask, 3.0 mL of ultrapure water, 250 µL of Folin–Ciocalteu reagent 0.2 N and 250 µL of sample extract are added. The solution is stirred for 5 min and 250 µL of a 10% Na_2CO_3 solution (w/v) and 4.25 ml ultrapure water added. The mixture is incubated at 25 °C in a water bath for 60 min. The absorbance is recorded at 761 nm using a spectrophotometer (Biochrom Libra S60PC, United Kingdom). The content of phenolic compounds was determined using a standard curve of gallic acid in the range 100–700 µmol L^{-1}.The results are expressed in mg of gallic acid equivalents per 100 g fresh weight sample (mg GAE 100 g^{-1} DW).

2.5 Data analysis

Data were analysed by analysis of variance, when significant treatment effect is identified then followed by Tukey's posthoc test by using SPSS 15.0.

3. Results and Discussion

3.1 Total Anthocyanin Content

The total anthocyanins content in seventeen different varieties of pigmented rice are presented in Table 2. The total anthocyanins content differed among the tested varieties, ranged from 2.89 to 187.23 mg CGE/100 g DW. Black glutinous rice varieties namely Le'leng, contained highest tot al anthocyanins, followed by Lallodo.

Table 2. Total Anthocyanin Content of Pigmented Rice from South Sulawesi

Variety	Total Anthocyanin (mg CGE/100g dry weight)
Black rice	
Kobo	78.90d
Lotong	92.04c
Ambo	11.33f
Lallodo	169.04b
Lotong Tanduk	35.35e
Le'leng	187.23a
Red rice	
Ra'rang	3.07g
Lea	2.91g
Maminyak	4.42fg
Mandoti	4.00fg
Balan	2.89g
Kamida	7.41fg
Jambu	3.25fg
Bakka Eja	3.55fg
Punu Eja	4.26fg
National variety	
Inpari 24 (red)	3.45fg
Ciherang (white)	3.04g

Values followed the same letter are not different (P>0.05)

The results showed the anthocyanin were more pronounced in black rice group which had grain with a dark pericarp. However, despite it being classified as a black rice, Ambo had partly less dark kernel based on visual appearance, in hence the bioactive content was much lower than other black rice varieties (Fig. 3).Other study also confirmed that rice anthocyanin content is correlated with grain color [13]. Among all of studied pigmented rice, it was verified that black rice has the highest content of total anthocyanins [14].

Anthocyanins accumulated on the outer kernel or pericarp. By polishing the rice will remove the outer layer means reduce the anthocyanin contents, and further affect the total polyphenol and antioxidant activity [15]. Thus, to get optimum health benefits of black rice from their high

anthocyanins contents, over-polishing of the grains should be avoided. As a natural pigment, anthocyanins are able to be further developed for food colorant, dye, or cosmetic.

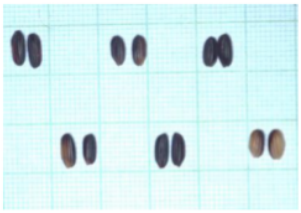

Figure 3. Color comparison of dehusked black rice grains *Left to right-bottom up* : Ko'bo, Lotong, Ambo, Lallodo, Lotong Tanduk, Le'leng

3.2 Total Flavonoid

Total flavonoid contents of the dehusked grain of seventeen varieties of pigmented rice from South Sulawesi are shown in Table 3. The values ranged from 5.45 (Ciherang) to 62.45 mg CE/100g dry weight (Le'leng). The highest content of flavonoids were found in Le'leng and Lallodo. Red rice group have lower flavonoid content in their grains than the grains with black/ dark pericarps.

Table 3. Total Flavonoid Content of Pigmented Rice from South Sulawesi

Variety	Total Flavonoid (mg CE/100g dry weight)
Black rice	
Kobo	43.67b
Lotong	47.18b
Ambo	10.17d
Lallodo	59.56a
Lotong Tanduk	23.60c
Le'leng	62.45a
Red rice	
Ra'rang	9.33d
Lea	7.25d
Maminyak	13.30cd
Mandoti	14.34cd
Balan	8.93d
Kamida	14.33cd
Jambu	15.27cd
Bakka Eja	8.09d
Punu Eja	11.54cd
National/modern variety	
Inpari 24 (red)	8.89d
Ciherang (white)	5.45d

Values followed the same letter are not different (P>0.05)

Flavonoids are included in a group of phenolic compounds. Flavonoids have some subgroups such as flavonone, flavonol and anthocyanin groups etc. Similar to phenolics. They also have health

benefits, such as in cancer prevention, heart disease, diabetes. Determining total flavonoid contents in various pigmented rice varieties enables researchers or breeder to do plant selection or analyse the best method for their extraction, while farmers can select plants that gives better income for the higher value.

3.3 Total Phenolic Contents

According to the experimental results, among tested varieties of black rice grains, red rice grains and one white grains, the accumulation of total phenolic compounds were higher in pigmented rice than the white rice. The range of total phenolics was 45.29 mg GAE /100 g dry weight (white rice Ciherang)-201.49 mg GAE/100g dry weight (black rice Le'leng). The range of phenolic content in black rice group is 49.99-201.49 mg GAE/100g dry weight. The lowest phenolic content in black rice group is found in Ambo, which is almost similar amount of content with white rice Ciherang. Noteworthy amount of phenolics found in most tested red rice varieties, ranging from 77.36-169.14 mg GAE/100g dry weight.

Table 4. Total Phenolic Content of Pigmented Rice from South Sulawesi

Variety	Total Phenolic (mg GAE/100g dry weight)
Black rice	
Kobo	134.59abcde
Lotong	154.35abcd
Ambo	49.99f
Lallodo	189.60ab
Lotong Tanduk	88.42def
Le'leng	201.49a
Red rice	
Ra'rang	141.50abcde
Lea	126.84bcde
Maminyak	137.63abcde
Mandoti	169.14abc
Balan	81.64ef
Kamida	118.00cde
Jambu	109.01cdef
Bakka Eja	77.36ef
Punu Eja	132.24bcde
National/modern variety	
Inpari 24 (red)	107.08cdef
Ciherang (white)	45.29f

Values followed the same letter are not different (P>0.05)

In various plants, phenolics are such a crucial phytochemical. Their important roles in plant development especially in lignin and pigment biosynthesis, structural integrity provider and defences [16]. In rice, phenolic have been reported as the major hydrophilic antioxidants [17].

Finding out the phenolic contents in various rice varieties are beneficial as basic of plant selection. So that, selected groups may be developed not only for the higher yield but also better quality in terms of increased level of bioactive compounds. This bioactive contents of the seeds may beneficial for increasing the value of the landraces in the market. In the meantime, growing interest of healthy diet

and functional food are potential market for farmers to boost their income when effective value chain especially marketing is met.

4. Conclusion

The results indicated the higher bioactive compounds (anthocyanins, flavonoids, phenolics) in pigmented rice that may increase their value for farmers benefit. There is wider opportunity for the bioactive compounds application in various industries for future improvement. Black rice groups with solid dark pericarps are higher in anthocyanin and flavonoids compared with red and white rice. Red rice groups in this experiment results did not show significant amount of anthocyanins and flavonoids, however, noteworthy amount of phenolics was recorded. The highest content of anthocyanins, flavonoids, and phenolics was found in black glutinous rice named Le'leng.

Acknowledgements

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