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Fatty Acid Composition and Physicochemical Properties in Germinated Black Rice

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Abstract

The objective of the research was to determine the effect of the germination of black rice. Black rice was soaked for 12 h and further germinated for 12, 24, 36, and 48 h. Non-germinated black rice was used as a control. Black rice was germinated for 19-20 h. Fat and moisture content, total sugar, cooking quality, sensory analysis, and fatty acid composition of germinated black rice were further evaluated. Results show that fat content decreased during germination, but moisture content and total sugar increased. Physicochemical properties, such as length expansion ratio, volumetric expansion, water absorption, gel consistency, and alkali values were in the ranges of 0.77-0.91 mm, 2.42-3.38, 1.64-2.04, 66-82.5 mm, and 2-3.5, respectively. From sensory analysis, most of panelists were favorable for the germinated black rice at 24 h germination. SFA and MUFA tended to decrease during germination but PUFA tended to increase. Thus the germination is effective to improve nutritional values of black rice.

Keywords: germination, black rice, total sugar, fat and moisture content, fatty acid composition

Introduction

Rice (*Oryza sativa*) is the main product from rice grain milling where all husk layers are peeled off, and the whole or part of bran layer has been separated (SNI Beras 6128:2008). In general people consume white rice in Indonesia. But actually there are many other types of rice in Indonesia, such as red rice, black rice, and brown rice. The difference of the types of rice husk is its color. Black rice is not yet a staple food as white rice. It is suggested that the colored rice has a high nutritional value. Black rice contains anthocyanin (a type of antioxidant), iron, vitamin B1, and minerals more than in brown rice and white rice. Anthocyanin in black rice

is bioactive compounds that may be useful for the inhibiting the initiation stage of chemical reactions that cause cancer, binding free radicals, and cardio protective capacity (Smith et al., 2000).

Americans describe black rice as "Indonesian Black Rice" or black jasmine rice of Thailand as "Thai Jasmine Black Rice". In the times of ancient kingdoms, black rice is food that is consumed only among kings, sultans, the nobles, or as tribute offerings across the kingdom. In Keraton Surakarta, black rice is known as the "Wulung Rice". It refers as "Cempo Ireng" or "Jlitheng Rice", "Melik Rice", and "Gadog Rice" in Sleman, Bantul, and Subang, West Java, respectively.

Meanwhile, black rice also has been consumed in China since thousand years ago. It was limited to be consumed by the emperor and his family, and is referred as "forbidden rice". Rabble dares who consumed black rice would be beheaded.

Germination is one of the appropriate methods to improve the nutritional and functional components in rice, where the germination process may improve the content of vitamins, minerals, fiber and other components. Several studies on the germination of cereals showed that germination increased the concentrations of oligosaccharides and amino acids, such as in rice (Manna et al., 1995), wheat (Yang et al., 2001), and oats (Mikola et al., 2001). In Japan, a new product of rice was found through the process of germination. This type of rice was known as Pre-germinated Brown Rice (PGBR). PGBR is believed to be healthier than white rice, because it contains vitamins, minerals, and fiber those are quite high (Hsu et al., 2008).

The purposes of this research were to determine the changes in nutritional quality of black rice, especially the total sugar content, fat content and fatty acid profiles those occur during the process of germination, physicochemical properties and preference test of the germinated black rice. Germination process, which was easy to do and effective for the improvement of rice nutritional value, was expected to be well accepted and can contribute to the improvement of the food nutritional value of the community.

Materials and Methods

Black rice variety Cempo Ireng was obtained from producers in Condong Catur, Sleman, Yogyakarta. Glucose standard, HCl, NaOH, Nelson reagent, arsenomolibdat, lead acetate, KOH, petroleum ether, hexane, and

methanol were obtained from Merck KGaA (Darmstadt, Germany)

Germination

Germination was done at room temperature in a dark room. The ratio of black rice to water was 1:10 (w/v). The soaking time was 12 h. Germination times were 0 (control), 12, 24, 36, and 48 h. Water was added into black rice every 12 h during germination. Moisture content and length of the germinated rice was analyzed.

Analysis of Germinated Black Rice

Rice was dried at 50°C for 4 h after germination. It was then milled into rice flour and stored for further analysis. Moisture and fat content were analyzed using AOAC method (1990). Total sugars were analyzed using the Nelson-Somogyi method.

Analysis of Physicochemical Properties

Analysis of length expansion was performed as follow: five black rice grain lengths was measured, they were heated in the boiling water for 10 min. Their lengths were further measured after cooking. Length expansion was calculated as follow:

$$\text{Length expansion} = \frac{X_p}{Y_p}$$

where X_p is the cumulative length of the cooked rice grains and Y_p is the amount of rice grains.

Analysis of volumetric expansion was performed as follow: 8 g of black rice volume were measured. They were then boiled for 30 min and further cooled for 10 min. The volume the boiled grains were measured. The volumetric expansion was calculated as follow:

$$\text{Volumetric expansion} = \frac{V_a}{V_b}$$

where V_a is the volume of cooked rice and V_b is the volume of raw rice.

Analysis of water absorption was performed as follow: 8 g of black rice were weighed and further boiled for 30 min. They were cooled for 10 min and then weighed. The water absorption was calculated as follow:

$$\text{Water absorption} = \frac{b - a}{a}$$

where a is the weight of raw rice and b is weight of cooked rice.

Analysis of gel consistency was performed according to Cagampang et al., (1973) based on the length of the gel that was formed by 100 mg of rice flour in 10 mL solution of KOH 0.2 N solution in test tube 13x100 mm at room temperature.

Analysis of alkali value was performed as follow: six rice grains were soaked in 10 mL of 1.7% KOH solution for 24 h at 30°C and then they were observed for their damage (Little et al., 1958).

Analysis of Fatty Acid Profiles

About 100 mL of hexane was added into 20 g of black rice flour. Mixture was incubated at 40°C in the water bath shaker for 1 h. Supernatant was separated by filtration and solvent was evaporated using a rotary vacuum evaporator at 40°C and a pressure of 335 kPa. Then 0.1 g fat extract was transferred into a test tube and dissolved by adding 10 ml of hexane. About 0.1 mL of KOH 2 N in methanol was further added into the solution and mixed using vortex for 30 s. Subsequently, it was centrifuged for 15 min at 3000 rpm. About 2 mL of the supernatant was transferred into a GC tube. This analysis was performed using Shimadzu GC GC-9AM, the type of capillary column with a length of 50 m and a diameter 0.22 mm, N₂ carrier gas and the pressure of 2.5 kg/cm². The temperature of column was 180-230°C, injector temperature was 250°C, temperature detector was 250°C, the detector types was

FID (Flame Ionization Detector), and the injection sample was 1-5µL.

Sensory Analysis

Samples were tested duplicate for each analysis and there were five variables. Data were analyzed using the one-way ANOVA and Bonferroni test with a significance level of p<0.05.

Results and Discussion

Germination

Germination was started by the absorption of water (imbibition), further activation of enzymes and hormones, the changes of food reserves (carbohydrates, fats, and proteins), the initial growth of the embryo, the outbreak of the skin and the appearance of root and shoot growth. The influx of water in grain caused the activation of gibereline hormone. This hormone encouraged the formation of hydrolytic enzymes, such as α-amylase and protease, ribonuclease, β-glukonase and phosphatase. These enzymes diffused into the endosperm and hydrolyzed the reserve food to become sugars, amino acids and nucleosides, which supported the growth of the embryo during germination and seeding growth (Pranoto et al., 1990).

It was found that there were no sprouts at initial germination until 12 h because there was still insufficient the required energy to germinate (**Table 1**). Black rice was starting to sprout after 19-20 h. The sprout length was 0.45 cm at 24 h and increased 5.2 times after 48 h of germination. It is suggested that biochemical processes, which occur during germination, also have an important role for utilization of food as a source of energy for growth that occurs in the endosperm.

Table 1. Length of sprout and water content during germination

Germination (h)	Length of sprout (cm)	Moisture content (%)
0	0 ^a	33.50±0.26 ^a
12	0 ^a	33.90±0.29 ^{ab}
24	0.45±0.07 ^a	37.76±2.01 ^{ac}
36	1.10±0.42 ^a	39.29±1.21 ^{bc}
48	2.35±0.49 ^b	41.20±1.28 ^c

The number followed by the same letter in the same column indicate no significant differences

Macromolecular compounds, such as protein, starch and lipids, are used as the building blocks of growth and energy for growth after they were breakdown by the enzymes.

The water content is an important parameter in the food stuff for determining the physicochemical properties, organoleptic, and biological activity. From **Table 1** it was found that the moisture content of the germinated black rice increased with an increase in germination time. Germinated black rice absorbed water from its surrounding. An increase in water content during germination was caused by an increase in the number of cells in the black rice that needed water for their metabolism (Nonogaki et al., 2010).

Chemical Composition

It was found that fat content decreased with an increase in the germination time (**Table 2**). Fat content was almost equal to the black rice in Japan (Yoshida et al., 2010), which was about 2.2% to 3.7%. During

germination, fat was hydrolyzed into fatty acids and glycerol by lipase. Free fatty acids and glycerol were then transferred to the embryo. It is suggested that a decrease in fat content can provide benefits for peoples who have cardiovascular disease and hypercholesterolemia.

Total sugar on black rice increased with an increase in germination time (**Table 2**). The total sugar was a mixture of reducing sugar and non-reducing sugar, which were the result of starch hydrolysis. Total sugar increased due to an increase in α -amylase activity, which hydrolyzed starch. Before germination the seed was in dormancy and α -amylase activity was still low so that the total sugar content was still low. An increase in the total sugar content may also be due to hydrolysis of simple sugars and sugar reduction due to invertase (Traore et al., 2004).

Table 2. Chemical composition

Germination (h)	Fat (%)	Total sugar (%)
0	3.63±0.12 ^a	0.46±0.09 ^a
12	3.20±0.26 ^a	0.58±0.09 ^{ac}
24	2.84±0.50 ^a	0.67±0.04 ^{ad}
36	2.55±0.27 ^a	0.89±0.00 ^{cd}
48	2.44±0.23 ^a	2.06±0.13 ^b

The number followed by the same letter in the same column indicate no significant differences

Physicochemical Properties

Length expansion of the black rice during germination was between 0.76 and 0.91 mm (**Table 3**). There was no correlation between the length of the black rice and germination time. Volumetric expansion decreased with an increase in time of germination (**Table 3**). It is suggested that a decrease in volumetric expansion was due to a hydrolysis of the black rice starch. According to Greenwood and Munro (1979), starch is an important component because the structure is less compact and less able to resist for the development of mass and will push granules gaps so that the granules will expand. Starch that was hydrolyzed due to germination reduced or even eliminated the possibility of the volumetric product expansion.

Volume length ratio was expressed as the ratio between the volume of the cooked rice and raw rice. There will be the expansion of the starch granule during the cooking of rice. The average volume of the cooked rice in Indonesia was 3.5 times compared to the volume of raw rice (Suismono et al., 2003).

According to Winarno (1997), the expansion of the granules occurs when the kinetic energy of the water molecules become stronger than the intermolecular attractive forces in the starch granules so that the water can get into the grains of starch. Because the number of hydroxyl groups in the starch

molecules is very large, then the ability to absorb water is also very large. An increase in time of the germination caused a decrease in starch content, which caused a decrease in water absorption as can be seen in **Table 3**. Water absorption is expressed as the ratio between the amount of the absorbed water and the weight of raw rice. The average of water absorption of Indonesian rice was 2.5 times.

The consistency of the gel is determined from the cold paste viscosity of starch. From **Table 3** it was found that an increase in germination time resulted in an increase in gel consistency. It was due to differences in the composition of fats, proteins, and amylose (Lai., 2001). Tang et al., (1991) suggested that gelation occurred due to the intermolecular interactions involving molecules of amylose and amylopectin during starch cooling. Rice that has a gel consistency values above 50 mm means that the rice yield soft textured.

Alkali value associated with how much the damage of black rice as a result of a strong base. This is associated with gelatinization temperature. In the range of 1-3, the gelatinization temperature was higher than 74°C. In the germinated black rice, all rice had gelatinization temperature higher than 74°C and they belong to the high gelatinization temperature.

Table 3. Physicochemical properties

Germination (h)	Length expansion (mm)	Volumetric expansion	Water absorption	Gel consistency (mm)	Alkali value
0	0.80±0.14 ^a	3.38±0.01 ^a	2.04±0.08 ^a	66.00±5.66 ^a	3.5±0.71 ^a
12	0.91±0.13 ^a	3.28±0.11 ^{ab}	1.88±0.03 ^{ab}	74.50±2.12 ^{ab}	3±0.00 ^{ab}
24	0.76±0.15 ^a	3.14±0.05 ^{ac}	1.76±0.04 ^{bc}	75.00±2.83 ^{ac}	3±0.00 ^{ab}
36	0.90±0.23 ^a	2.80±0.15 ^{bcd}	1.70±0.00 ^{bd}	78.00±2.83 ^{ad}	2±0.00 ^{ab}
48	0.77±0.09 ^a	2.42±0.11 ^d	1.64±0.05 ^{cd}	82.50±2.12 ^{bcd}	3±0.00 ^{ab}

The number followed by the same letter in the same column indicate no significant differences

According to Damardjati (1995) the rice, which had high gelatinization temperature, required more water and a longer time of cooking compared to that of the lower gelatinization temperature. In addition the rice that has high gelatinization temperature has less expansion compared to the rice that has a low gelatinization temperature.

Fatty Acid Composition

The composition of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) during germination of black rice is presented in **Table 4**. MUFA was dominant at the germination period between 0 and 24 h, followed by PUFA and SFA. Further increase in germination time resulted in changing fatty acid composition. PUFA became dominant in period between 36 and 48 h, followed by MUFA and SFA. Germination tended to lower the value of the SFA. It is the same as reported by Hahm et al., (2008) that SFA decreased on a sesame seed during germination. Capric acid ($C_{10:0}$) and palmitic acid ($C_{16:0}$) tended to decrease during germination, while myristic acid ($C_{14:0}$) and lauric acid ($C_{12:0}$) acid tended to increase.

MUFA tended to decrease during the germination, except at 12 h. Oleic acid was the predominant fatty acid contained in the sample. It is suggested that a number of unsaturated fatty acids (essential fatty acid)

can inhibit the emergence of blockage of blood vessels (atherosclerosis).

PUFA increased with an increase in time of germination. Unsaturated fatty acids, which were dominant in the PUFA's sample, were linoleic ($C_{18:2}$) followed by linolenic acid ($C_{18:3}$). Linoleic acid increased during the germination but linolenic acid tended to decrease. Substitution of high saturated fatty acid diet by consuming linoleic acid food has been recommended in preventing coronary heart disease (Galli et al., 1994).

Sensory analysis

Organoleptic properties are the parameter level of consumer acceptance. From 8 panelists, the germinated black rice, which was germinated for 24 h, was the most acceptable rice to the panelists in terms of flavor, texture, appearance, and preference. Germination for 0 and 12 h were less favorable because they had a bit bitter taste and hard texture. Germination for 36 and 48 h was less favorable because they had already long sprout appearance and they had bad taste. The 24 h germination had a slightly sweet taste, the texture was a bit hard, and the appearance was still acceptable as long the sprout has not been long yet.

Table 4. Fatty acid composition

Germination (h)	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	SFA	MUFA	PUFA
0			0.43	20.39	39.94	35.57	1.75	20.73	39.94	37.32
12			0.43	19.47	40.46	35.79	1.54	19.90	40.46	37.33
24	1.58	0.22	0.52	20.99	39.93	36.44	1.45	21.90	39.93	37.89
36	0.30	0.30	0.51	16.50	36.79	37.11	1.45	17.62	36.79	38.56
48	0.41	0.42	0.70	17.06	36.34	38.97	1.71	18.59	36.34	40.68

Conclusion

This study shows that black rice germination increased water content and total sugar content. Fat content decreased because it was used for metabolism. The physicochemical properties of the black rice including NPV and NPA decreased during germination, but consistency gel increased. Gelatinization temperature was higher than 74°C. SFA and MUFA tended to decrease with an increase in germination time, while PUFA increased. Sensory test in black rice showed that germination for 24 h was the most preferred because it had a slightly sweet taste, the texture was little bit harder, and the appearance was still acceptable. Panelists suggested that they concerned about the presence of sprouts on black rice and it need to be processed into other products.

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