Anthocyanin and Iron Absorption of Black Rice Bran Aqueous Extract using *In Vitro* Everted Gut Sac Method

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Anthocyanin and Iron Absorption of Black Rice Bran Aqueous Extract using *In Vitro* Everted Gut Sac Method

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Abstract. Black rice bran in Indonesia countries contained high anthocyanin and iron. As rice bran has not been widely used for food, this research investigated other possible functional effect of rice bran extract. The purpose of this study was to evaluate the absorption of anthocyanin and iron of subvar Cibeusi black rice bran extract of by in vitro everted gut sac method. Experiment was conducted on various part of small intestine using black rice bran, aqueous extract, and residue during various periods (10 – 100 minutes). Twenty four male 2 months old Wistar rats were put in 20-24 hours fasting condition and only given ad libitum deionized drinking water. After dissection, small intestine was taken, cut into duodenum, jejunum, ileum, and then reverted. The two ends of each of them were tied, filled with 0.9% NaCl solution, and the put into tube contained mucous solution and samples with constant oxygen saturated. The results showed part that the highest anthocyanin absorption of 0,0160 mg/g was obtained in jejunum. The highest anthocyanin absorption was obtained at 40 minutes of 0.0165 mg/g. Results also showed part of the small intestine can highest absorbed iron in duodenum compared to other parts of 3.65 ppm. Black rice bran extracts can be highest absorbed of 3.78 ppm. The type of material that can be absorbed highest in black rice bran extracts of 4.23ppm. The highest iron absorption at 10 minutes of 3.48 ppm.

Keywords: black rice bran, absorption, anthocyanin, iron, rat everted gut sac

1. Introduction

Rice is the staple food of more than half the world's population and provides 35-39% of the energy consumed by 3 billion people in Asia. Based on the color of the pericarp was white, red and black rice. Red and black rice has not yet become a staple food as well as white rice, although the two colored rice has a high nutritional value. Black rice is local variety that contains the best pigment, the color of rice genetically regulated and can vary due to the difference in genes that regulate the color of aleuron, endosperm, and the composition of the starch in the endosperm. The aleuron and endosperm black rice produce high-intensity anthocyanin [1].

Black rice part consists of rice hull, rice, and bran. Anthocyanin content in rice hull and rice 1.89 ± 0.05 mg/g and 0.17 ± 0.01 mg/g. Bran is a by-product of the processed rice consists of aleuron, pericarp, lemma. The majority of black rice anthocyanin is on the layer. The content of anthocyanin of black rice bran of 10.70 ± 0.03 mg/g [2]. Utilization of bran as food material relationships with resource-constrained barrier components to absorption fitic acid, so that needs to extraction for reduction of the components. Anthocyanin content various in materials and parts of such material, caused by genetic, rays, temperature and cultivation pattern factors [3]. Some of the types of rice also contains some minerals, and some vitamins are known as vitamin B complex. Especially grains whole grains are a source of iron, thiamin, riboflavin and niacin [4].

The composition of black rice according to the Indonesian Nutritionist Association (2009), per 100 g of the edible part (BDD) consists of 12.9 g water; 351 kcal of energy; 8 g protein, 1.3 g fat; 76.9 g carbohydrates; 20.1 g fiber; 0.9 g ash; minerals calcium 6 mg; 198 mg phosphorus; 0.1 mg of iron; sodium 15 mg; potassium 105 mg; copper 0.1 mg; Zinc 1.6 mg; Vitamin thiamine 0.21 mg; 0.06 mg of Riboflavin.

According to Meng et al. (2005), the content of iron in rice is primarily determined by the absorption of iron from the soil and the transport and accumulation of iron in rice. The content of iron in the soil, with 5% of the Earth's crust, a layer of iron is the fourth largest elements in abundance on Earth, only small to oxygen, silicon, and aluminum. The content of iron in the soil varied from 1-20%, with an average of 3.2%, but a normal concentration in plants only 0.005%. The main causes that the iron in the soil that exists mostly in the form of hydrogen oxide, oxide, phosphate, or other deposit components. The concentration of dissolved forms for absorption by plant roots may be smaller than the 10-10 moll l-1, which cannot be found at the plant.

Iron content in rice showed the highest found in rice hull, followed by the husk and bran [5]. While the highest iron content found in the black rice, followed by Brown rice, white rice, glutinous rice, and grains have the lowest iron content [6]. Iron nutrition may be fixed by increasing the supply of black rice. It is also important to study the physiological mechanisms of absorption of iron and usage on black rice and is associated with the gene, for the improvement of iron nutrition by cross-breeding and gene cloning.

Iron content in black rice outer layer according to Xia et al. (2003) showed a higher content of (16.46 mg/100 g) than black rice according to the Indonesian Nutritionist researches. Laokuldilok et al. (2011) detect any type of anthocyanin component of cyanidin-3-glucosides (C_3G) and peonidin-3-glucosides (P_3G) on black rice bran. The results of the analysis of black rice bran match analysis by Kaneda et al. (2005), Zawistowski et al. (2009) as well as Kong and Lee (2010).

Gastrointestinal tract consists of the mouth, stomach, intestine (duodenum, jejunum, ileum, and colon) and rectum. The consumed of the iron and anthocyanin will be entered and absorption in the GI tract. The iron and anthocyanin that are not absorbed are forwarded to the colon and excreted through the feces. The degradation of the iron and anthocyanin by microbial occurs in the colon, thus enabling absorption. According to the research of Matuschek et al. (2006) have demonstrated that it does not occur in the colon absorption. The absorption of the iron and anthocyanin occur through two stages in the gastrointestinal, passive diffusion and active transport.

The methods of absorption process used in *in vitro, in situ*, and *in vivo*. *In vitro* methods have some advantages, that is simple, fast, cheap and has good repeatable. Also, this method has a lot of variables that can be controlled. Animal are commonly used for experiments in *vitro* is a rat, rabbit, hamster. *In vitro* method that is widely used because it is simple, yet "reproducible" enough is the everted gut sac technique [11].

The purpose of this research was to evaluate the absorption of iron and anthocyanin black rice bran of Cibeusi varieties with *in vitro* everted gut sac methods in the different of intestine, the type of material and a different of time.

2. Materials and Methods

2.1. Material

Black rice bran from the Cibeusi varieties. Extraction treatment will be obtained extracts and extract residue black rice bran, which was used for this research

2.2. Chemicals and Research Equipment

Chemicals used consist of deionisation water, methanol, acetic acid, HCl, NaCl are specification pro analysis (p.a.). Oxygen is obtained from PGN (Perusahaan Gas Negara). Research equipment used in this research consist of shaker water bath, rotary evaporator, freeze dryer, UV-Vis, the other glasses. The everted gut sac methods according to the Crane and Wilson are modified by Yuwono (1987).

2.3.Everted gut sacs

We prepared the everted gut sac according to the technique of Wilson and Wiseman (1954) that are filled with a Ringer solution (pH 7.4). The sacs were hanged in an incubatory medium containing 200_mol/l of Fe SO4 (Smith et al., 2002) (pH 3 for stomach). The everted gut sacs were maintained at 37°C and bubbled with gas (O_2/CO_2, 95/5).

2.4.Black rice bran extract sample preparation

Black rice bran extracted by adding 3% acetic acid solution in deionization water with a ratio of 1:10. The next step is done shaking with shaker water bath for 1 hour, speed for 125 rpm. Maceration is carried out by storage in a dark room for one night at a temperature of 4 ° C. then centrifuged at 5000 rpm, 4ºC for 10 minutes. The residue obtained by extraction was done 2 times more, whereas the filtrate is filtered. The filtrate is obtained is dried with a freeze dryer that was previously removed solute using a rotary evaporator. Extract of acetic acid-water deionisasi obtained, packaged and stored in the dark at a temperature of 20 ° C until use. The extract and residue is used for *in vitro* research.

2.5.Animals

The animals used in this study i.e. Wistar rats (2 months), obtained from LPPT 4^{th} unit in Gadjah Mada University.

2.6.Determination of Iron

Iron concentration was measured from inside the sacs using atomic absorption spectro-photometry (Perkin-Elmer serie 23 80). Sample absorbance results are used for the determination of iron concentration according to a standard curve. Iron concentration is determined in the absence and the presence of aqueous extract of black rice bran used with different concentrations (5, 10, 20 and 30 mg/ml) through the studied parts of the digestive tube. Incubation period were 1, 5 and 15 min. Iron absorption is expressed in mol/l. Number of experiences used for the determination of iron concentration is at least six times for several conditions used in our experimental protocol.

2.7.Determination of Anthocyanin content

Determination of anthocyanin content according to the methods of Cheng and Breen (1991) in Prior et al. 2005, i.e. measuring the absorbance of samples at pH 1 and pH 4.5 which is measured at a wavelength of 510 and 700 nm and is calculated by the formula: = Abs [(A_{510}-A_{700}) pH1-(A_{510}-A_{700}) pH4.5].

2.8.Statistical Methods

Method of calculating statistically performed with SPSS 12.00 with the analysis of the general linear model univariate and one-way ANOVA to know correlation treatment. If there is a real difference manifested with $P \leq 0.05$.

3. Results and Discussion

In vitro analysis by everted gut sac was done to examine part of the gut that can greatest absorpted, get the that can be absorpted by the intestine, followed by how long it takes to absorpted the largest and last of the relationships with the types of materials that can be absorbted by the intestine.

3.1.The Absorption of anthocyanin-based on parts of the Intestine and on Difference of Concentration on Jejunum by Everted gut sac Method

Intestine consists of three parts consist of the Duodenum (D), Jejunum (J) and Ileum (I). The third part of the intestine can be known to be part of the intestine that can the greatest absorpted by using with 20 mg anthocyanin in 80 ml of NaCl 0.9% solution, the method of in vitro everted gut sac is presented in Figure 1. In this study used 3 rats which were divided into 3 groups.

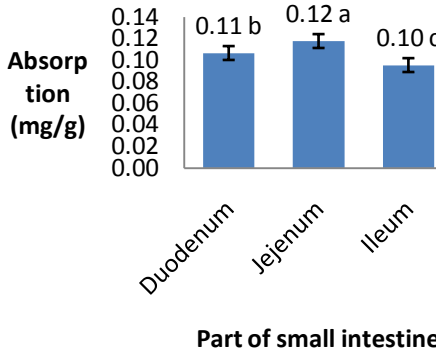

Figure 1. The absorption of anthocyanin based on difference in the part of the intestine with in vitro everted gut sac method (levels of anthocyanin in mucosal solution 20 mg/80ml)

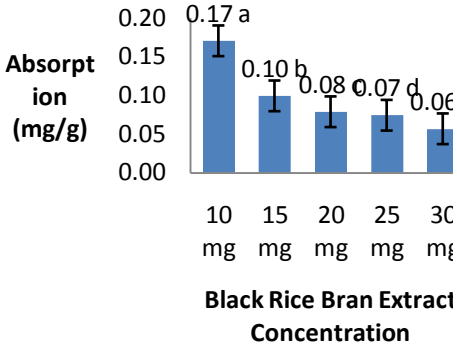

Figure 2. The absorption of anthocyanin based on difference of concentration on jejunum with in vitro everted gut sac methods

Figure 1 shows that the third part of the intestine between the treatments section of intestine were significance ($p > 0.05$). Parts of the intestine can the greatest absorpted is the jejunum i.e. of 0.0160 mg/g, followed by duodenum of 0.0144 mg/g and the last of the ileum 0.0130 mg/g. This is possible because on the surface of the intestinal absorption of fine especially in the jejunum, there are many creases form of intestinal mucosa valvula conniventes which is very important in the absorption. In the area of intestinal villi villi-covered by epithelium consisting of microvilli and has a strong activity. The presence of blood capillaries and lymph nodes on each flap allows the strong absorption occurs [15]. Active transport may also play a role in the intestine, as happened in the same competition against the carrier or the transport system saturation occurs can limit crossing the membrane.

According to research by Matuschek et al. (2006) on the part of the intestine the duodenum, jejunum, ileum towards anthocyanin, suggesting that is where absorption jejunum the most optimal when compared to other parts of the intestine because it has the most extensive absorption area with the villi-villi. Anthocyanin on intestinal absorption, by the active transport of sugars that is affected by sodium (Intestinal Sodium-Dependent Glucose Transport/SGLT-1). Glucose is the carrier who brought in through SGLT-1 pierce the intestinal brush border membrane [12]. The process of sodium-potassium pump is active will simplify the entry of sugar into cells. When three sodium ions enter past the alpha and beta receptors, such as chloride compounds, amino acids, glucose, and water as well as organic compounds that others will follow the movement sodium so that nutrients can be utilized by cells and partly transported towards the next cells. Other types of sugar or glucose binding relationships also will follow the movement of the sodium so it will be absorbed into the cells. These assumptions are supported by some of the results of research that finds the existence of natural anthocyanin (anthocyanidin-sugar) in plasma, tissues of the body and in the urine [13]. Plural form of anthocyanin in segments GI tract also influenced its structure. The stomach is where absorption of anthocyanin [14], especially those that bind to the glycosides i.e. delphinidin glycosides. Then the relationships that are absorbed, in bile is transformed into a form of metilated [13]. Absorption in the intestine most quickly in the form of glycosides, cyanidin after it was directly forwarded to the bile in the form of metilated and glucoronide [13]. Absorption of anthocyanin based on the difference of concentration on jejunum with everted gut sac Method. The results obtained from the analysis of the determination of the greatest percentage anthocyanin that can Intestine absorption with levels various of the tested i.e. 10 (A), 15 (B), 20 (C), 25 (D), 30 (E) mg of anthocyanin in 80 ml of NaCl 0.9% solution, can be seen in Figure 2.

Figure 2 suggesting that the treatments being tested turned out treatment using 25 mg anthocyanin in 80 ml of 0.9% NaCl solution which has the highest absorption in the intestine. Although treatment 25 mg when compared to all the treatment in the calculation of statistical significance with other treatment. For the treatment of 30 mg turns decline in absorption. In other

words be seen that with the increasing concentration of anthocyanin added in 0.9% NaCl solution than the anthocyanin absorption in the intestine, except on the treatment of 30 mg of anthocyanin. This shows that turns with the method of in vitro everted gut sac above with the addition of 25 mg anthocyanin is not beneficial in increasing the absorption of anthocyanin in the intestine and possibly there is a saturation factor in the absorption anthocyanin in the intestine. Decrease the absorption of anthocyanin on the treatment of 30 mg, may be caused by the formation of ties (chelate) of the relationships with the iron so it's hard to be absorpted, led by the intestine.

3.2. The Absorption of Anthocyanin based on Difference of Times and Fraction on Jejunum With Everted gut sac Method

Determination of the time needed for the greatest intestine absorpted to use the method of in vitro everted gut sac with time variations 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 minutes. Use the relationships with 25 mg in 80 ml of 0.9% NaCl solution and rats as much as 3 tail, its analysis results can be seen in Figure 3.

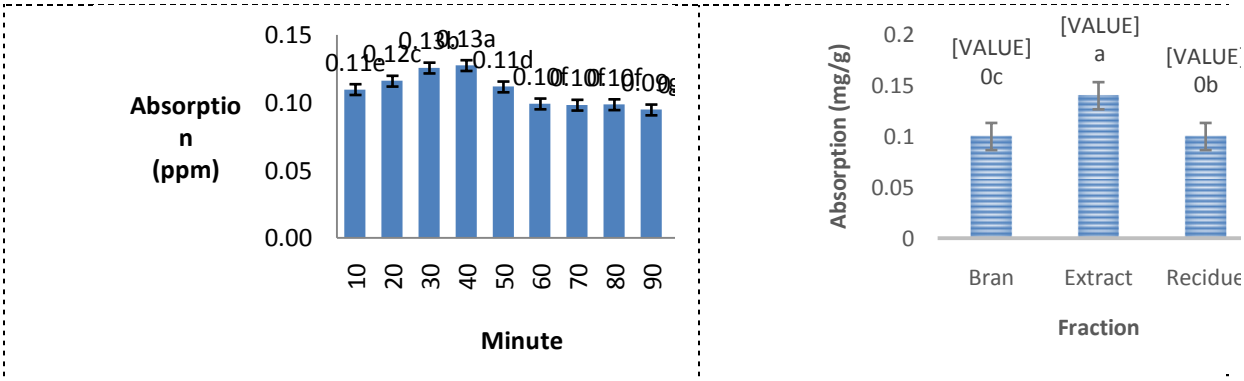

Figure 3. The absorption of anthocyanin-based on difference of times on jejunum with in vitro everted gut sac methods (levels of anthocyanin in aqueous solution mucous 25 mg/80ml)

Figure 4. The absorption of anthocyanin-based on difference of fraction on jejunum with in vitro everted gut sac methods (levels of anthocyanin in mucosal solution 70 mg/80ml)

The absorption is observed every 10 minutes, from minutes to 0 to 100 minutes to find out how many minutes the absorption occurs the highest anthocyanin. From Figure 3 it can be seen that statistically different treatment between real ($p > 0.05$), except in the 70 and 80 is no different. Time needed to be able to absorb the relationships with the longer time (minutes to 10 up to 40) the more the relationships with the absorbed, but it turns out after 40 minutes experience decreased absorption that is going on 50 minutes. When viewed from the treatment from time to time, then the percentage increase in the absorption of the relationships with the longer lower up to 100 minutes. The results showed that the relationships begin to be absorbed from the early observations have occurred either absorption. Absorption of anthocyanin occurs optimally until minute 40, then decrease the absorption of up to 100 minutes. Research on steady-state phase occurred at 30 [15] factors associated with the saturation of the relationships. This phenomenon is in line with the opinion Aiache certain molecules (iron) with ions-valence 2 or 3 will form bonds (chelate) which are not absorbed. Gastrointestinal tract (GI tract) tract includes the mouth, stomach, intestine (duodenum, jejunum, ileum, and colon) and rectum. The relationships with the consumed will be entered in the GI tract absorption and will occur. The relationships that are not absorbed are forwarded to the colon and excreted through the feces. Degradation anthocyanin by microbial occurs in the colon, thus enabling absorption occurs. According to the research of Matuschek et al. (2006) have demonstrated that in the colonic absorption does not occur

The capabilities of the greatest anthocyanin absorption intestine based on the fraction used, namely bran, bran extracts, and bran extract residues black rice, in 80 ml of 0.9% NaCl solution and rats as much as 3 tail, its analysis results can be seen in Figure 4.

Figure 4 that statistically between the treatment using black rice bran significance with rice bran extract real with black but not significance with black rice bran extract residues ($p > 0.05$), while the black rice bran extract significance with black rice bran extract residues ($p > 0.05$). The results showed that black rice bran extracts can be highest absorption than other types of material. According to research conducted by Meng et al. (2005) showed that the bioavailability iron rather low in plant foods, about 10%, on the rice about 1%, likewise anthocyanin on the black rice bran.

Anthocyanin includes flavonoids, absorbed the body in very small amounts compared to the other types of flavonoids [16]. The main components of anthocyanin of the black rice is cyanidin-3-glucosides and peonidin-3-glucosides. Naturally, the anthocyanin are in the form of glycosides (tied with sugar molecules) and some of the results of the study mentioned that the consumption of the relationships will be expelled through the urine in the form of still bound with sugars [17]. This proves that absorption in the body is low. Plural form of anthocyanin can be through, among others, the direct absorption in mucosal epithelial cells in the form of glycosides or through the process of hydrolysis to break down sugar molecules into a form of aglicon. The anthocyanin that are not absorbed in the body, will be brought directly to the colon and will be fermented by the microbiota.

3.3. The Absorption of iron based on parts of the Intestine and on Difference of Concentration on Jejunum by Everted gut sac Method.

Absorption of iron based on parts of the intestine by everted gut sac method intestine is composed of 3 parts are the duodenum, jejunum, and ileum. The third part of the intestine can be known to be part of the intestine which can absorbed the greatest Fe by the use of black rice bran extract 70 mg in 80 ml of 0.9% NaCl solution by using the method of in vitro everted gut sac the presented in Figure 5 below. In this study used 3 rats which were divided into 3 groups.

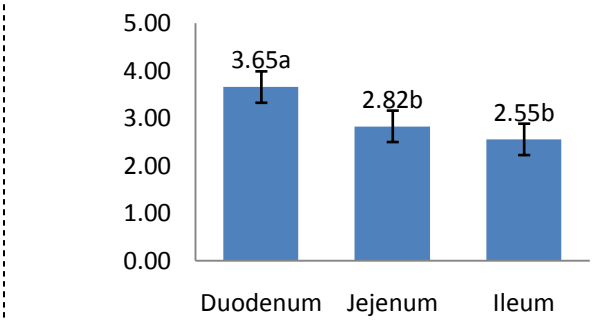

Figure 5. The absorption of iron based on the difference in the part of the intestine within vitro everted gut sac method

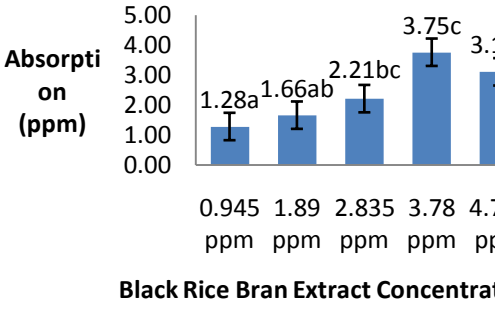

Figure 6. The absorption of iron based on the difference of concentration on jejunum with in vitro everted gut sac methods

Figure 5, showed that based on the results of the ANOVA statistical analysis a randomized complete design (RAL) ($p \leq 0.05$), the third part of the intestine between the treatments section of intestine significance ($p > 0.05$) between the duodenum, with jejunum and ileum, whereas between jejunum and ileum is not significance. Parts of the intestine can the largest Fe absorbed is the duodenum, namely of 3.65%, followed by 2.82% of jejunum and ileum the last of 2.55%. This is possible because, on the surface of the intestine absorption especially in the duodenum and jejunum, there are many creases form of intestinal mucosa valvula conniventes very important in absorption. In the area of intestinal villi villi-covered by epithelium consisting of microvilli and has a strong activity. The presence of blood capillaries and lymph nodes on each flap allows the strong absorption of Fe occur [15]. Active transport may also play a role in the intestine, as happened in the same competition

against the carrier or the transport system saturation occurs can limit crossing the membrane. The phenomenon of absorption of Fe in rice bran extract from black, the same as that stated by Aiache et al. (1982); Galesloot and Tinbergen (1985) in which the largest iron can be absorbed in the intestine in the Duodenum or Jejunum on top,

The results obtained from the analysis of determination of the absorption of Fe based on largest absorption jejunum with various levels of the tested from 0.945; 1.89; 2,835; 3.78 and 4,729 ppm black rice bran extracts in 80 ml of NaCl 0.9% solution, can be seen in Figure 6.

Figure 6 showed that all the treatments tested turned out treatment using 3.78 ppm black rice bran extract in 80 ml of 0.9% NaCl solution which has the largest average absorption in the intestine of 3.75%. However, based on the results of the ANOVA statistical analysis a complete randomized design (RAL) ($p \leq 0.05$), 3.78 ppm treatment when compared with the treatment of 2.835 and 4.729 ppm and did not significance, but significance with more concentration treatments small. For treatment 4.729 ppm turns decline in absorption. In other words be seen that with the increasing concentration of Fe is added in aqueous NaCl 0.9% Fe absorption in the intestine so smooth part of the jejunum, except at the treatment 4.729 ppm Fe. This shows that turns with the method of in vitro everted gut sac black rice bran extract addition on top of 3.78 ppm is not beneficial in increased absorption of iron in rice bran extract, black in the jejunum and the possibility there is saturation factor in black rice bran extract absorption containing Fe in the jejunum.

Decreased absorption of Fe on the ppm 4.279 treatment, may be caused by the formation of ties (chelat) of the anthocyanin with the iron so it's hard to absorption, led by jejunum.

3.4. The Absorption of iron based on Difference of Times and Fraction on Jejunum With Everted gut sac Method

The results obtained from the analysis of determination of Fe absorption based on the fraction on the largest jejunum absorption in black rice bran extracts (4.23%) compared to bran and rice bran extract black residue. The levels of the tested i.e. 3.78 ppm from black rice bran extract in 80 ml of NaCl 0.9% solution, can be seen in Figure 7.

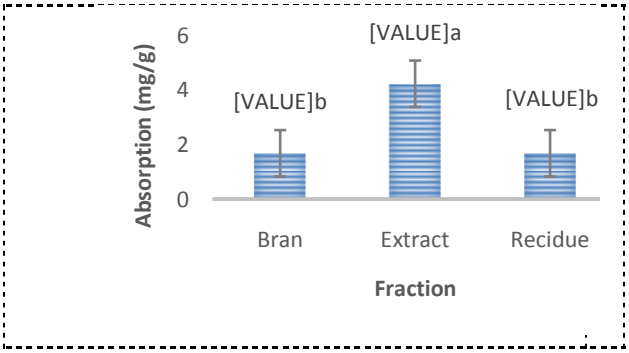

Figure 7. The absorption of iron based on difference of fraction on jejunum with in vitro everted gut sac methods

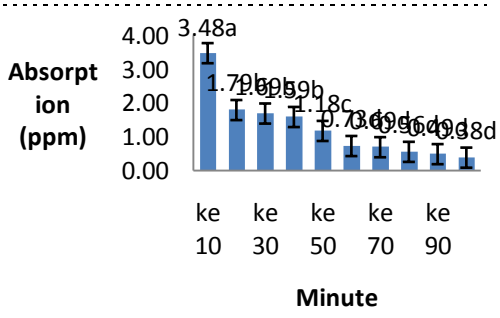

Figure 8. The absorption of iron based on difference of times on jejunum with in vitro everted gut sac methods

Figure 7, showed that based on the results of the Anova statistical analysis a randomized complete design (RAL) ($p \leq 0.05$), it is known that black rice bran extract fraction significance with the fraction of bran and rice bran extract black residue. This is caused by the black rice bran extract is already extracted the bran ingredients causing more easily absorbed by the jejunum.

To be able to figure out how long it will take for the jejunum can absorption black rice bran extract containing Fe used the method of in vitro everted gut sac with variations the time 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 minutes. Use extract 25 mg in 80 ml of 0.9% NaCL solution and rats as much as 3 tail, its analysis results can be seen in Figure 8.

Figure 8, showed the results of the Anova statistical analysis a randomized complete design (RAL) ($p \leq 0.05$), it can be seen that the significance ($p > 0.05$). On 10 minute the biggest absorption in jejunum (3.48%) compared to the next minute. The time required to be black rice bran extract absorbed in longer time slowest the absorption of Fe, but it turns out after 10 minutes has decreased absorption that is happening at the minute to 20 and so on. When viewed from the treatment from time to time, then the percentage increase in absorption of Fe the longer lower up to 100 minutes. This may be caused due to the formation of ties (chelat) of anthocyanin with iron which resulted in absorption in jejunum by more difficult. The formation of the bonding (chelat) factors associated with Burnout. This phenomenon is in line with the opinion Aiache [15] certain molecules (iron) with ions-Valent 2 or 3 will form bonds (chelat) which are not absorbed.

4. **Conclusion**

The results showed parts that the highest anthocyanin absorption of 0,0160 mg/g was obtained in jejunum. The highest anthocyanin absorption was obtained at 40 minutes of 0.0165 mg/g. Results also showed part of the small intestine can highest absorbed iron in duodenum compared to other parts of 3.65 ppm. Black rice bran extracts can be highest absorbed of 3.78 ppm. The type of material that can be absorbed highest in black rice bran extracts of 4.23 ppm. The highest iron absorption at 10 minutes of 3.48 ppm.

5. **Reference**

[1] Kaneda I, Yasui H, Adachi Y, Takada J and Sakurai H. Determination of Trace Element Concentrations in Ancient Rices (Red and Black Rices) and a Present-day Rice (Koshihikari) : Relationship Among the Trace Element Concentrations, Species, Harvest Site and Rice Parts. *Biomed Res Trace Elements*. 2005; 16 (3) : 241-249.

[2] Kong S and Lee J. Short communication: Antioxidants in milling fractions of black rice cultivars. *Food Chemistry*. 2010; 120: 278–281.

[3] Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RI. Concentration of Anthocyanins in Common Foods in the United States and Estimation of Normal Consumption. *J. Agric. Food. Chem*. 2006; 54: 4069-4075.

[4] Meng F, Wei Y and Yang X. Iron Content and Bioavailability in Rice. *Journal of Trace Element in Medicine and Biology*. 2005; 18: 333-338.

[5] Juliano BO. 1985. Some factors Affecting the Properties of Rice Noodles and Extrusion Cooked Rice Flours. In Proceeding of the Thirty-Fifth Australian Cereal Chemistry Conference. The Cereal Chemistry Division of Royal Australian Chemical Institute. Parkville.

[6] Ying M. Content Measurement and Study of Zn, Fe, Ca, Mn, Cu in Rice. *Microelement Health*. 2000; 17 (4): 46-47.

[7] Xia M, Ling WH, Ma J, Kitts DD and Zawistowski J. Supplementation of Diets with the Black Rice Pigment Fraction Attenuates Atherosclerotic Plaque Formation in Apolipoprotein E Deficient Mice. *The Journal of Nutrition*. 2003; 133: 744-751.

[8] Laokuldilok T, Shoemaker CF, Jongkaewwattana S and Tulyathan V. Antioxidant and Antioxidant Activity of Several Pigmented Rice Brans. *Journal of Agriculture and Food Chemistry*. 2011; 59: 193-199.

[9] Zawistowski J, Kopec A and Kitts DD. Effects of a Black Rice Extract (*Oryza sativa* L. indica) on Cholesterol Levels and Plasma Lipid Parameters in Wistar Kyoto Rats. *Journal of Functional Foods*. 2009; 1: 50-56.

[10] Matuschek MC, Hendriks WH, McGhie TK, Reynolds GW. The Jejenum is the Main Site of Absorption for Anthocyanin in Mice. *J. Nut. Biochem*. 2006; 17: 31-36.

[11] Bates TR and Gibaldi M. 1970. Gastrointestinal Absorption of Drugs *in* Swarbrick,J. (ed.). Current Concepts in the Pharmaceutical Sciences: Biopharmaceutics. Lea & Febiger. Philadelphia.

[12] Wollfram S, Block M and Ader P. Quercetin-3-glucoside is Transported by the Glucose Carrier SGLTI Across the Brush Border Membrane of Rat Small Intestine. 2002.

[13] Talavera S, Felgines C, Texier O, Besson C, Lamaison JL and Remesy C. Anthocyanins are Efficiently Absorbed from the Stomach in Anasthetized Rats. *J. Nutr*. 2003; 133: 4178-4182.

[14] Passamonti S, Vrhovsek U, Vanso A, Mattivi F. The Stomach as a Site for Anthocyanin Absoption from Food. *FEBS Letters.* 2003; 544: 210-213.

[15] Aiache, J.M., Devissaguet, J.Ph., Guyot-Hermann, A.M. 1982. Farmasetika Biofarmasi (ed. 2) Technique et Documentation. Paris.

[16] Manach C, Williamson G, Morand C, Scalbert A and Remesy C. Bioavailability and Bioefficacy of polyphenols in humans. Review of bioavailability Studies. *Am. J. Clin. Nutr*. 2005; 81: 230S-242S.

[17] Matsumoto H, Inaba H, Kishi M, Tominaga S, Hirayama M and Tsuda T. Orally Administered Delphinidin-3-rutinoside and Cyanidin-rutinoside are Directly Absorbed in Rats and Humans and Appear in the Blood as the Intact Forms. *J. Agric. Food. Chem*. 2001; 49 (3): 1546-1551.

[18] Galesloot TE and Tinbergen BJ. 1985. Milk Protein 1984. Proceedings of the International Congress on Milk Proteins. Luxemburg 7-11 May 1984. Pudoc Wageningen.