

# Antidiabetic Potency and Characteristics of Corn Flour and Cassava Flour-Based Rice Analog Added with *R. mucronata* Mangrove Fruit Flour and *E. cottonii* Seaweed Flour

Hardoko<sup>1,\*</sup>, Yahya Abdul Hafidz<sup>1</sup>, Bambang Budi Sasmito<sup>1</sup>, Yuniwaty Halim<sup>2</sup>

<sup>1</sup>Department of Fish Processing Technology, Faculty of Fisheries, Brawijaya University, Malang, Indonesia

<sup>2</sup>Department of Food Technology, Universitas Pelita Harapan, Lippo Karawaci, Tangerang, Indonesia

\*Corresponding author. Email: hardoko@ub.ac.id

## ABSTRACT

Rice analog can be made from various sources of carbohydrate other than rice itself and can be designed to become functional rice by adding ingredients that have functions on health. The aim of this research was to determine the antidiabetic potency and characteristics of corn flour and cassava flour-based rice analog that has been added with mangrove fruit flour and seaweed flour. The research was done using experimental method by adding mangrove fruit *R. mucronata* flour (5%, 10% and 15% w/w) and seaweed *E. cottonii* flour (0%, 3%, 5% and 7% w/w). The method used to prepare rice analog was extrusion method. Results showed that addition of mangrove fruit flour and seaweed flour could increase inhibition activity towards  $\alpha$ -glucosidase enzyme but decrease the panelists' acceptance. The selected rice analog that is potential to be antidiabetic food product was formulation of rice analog with addition of 5% *R. mucronata* fruit flour and 5% *E. cottonii* seaweed flour. This rice analog has  $IC_{50}$  of 185.59 ppm, overall hedonic score of 3.65 (slightly dislike to neutral), cooking time of  $7.09 \pm 0.05$  minutes, texture score of 3.44 (slightly sticky), protein content of 3.24% and total dietary fiber of 9.71%.

**Keywords:** analog rice,  $IC_{50}$ , mangrove, seaweeds

## I. INTRODUCTION

Rice is main staple food that is consumed frequently by most of Indonesian people. It can be seen from rice consumption level in Indonesia that reaches 95%. The average rice consumption in Indonesia from 2002-2013 is about 103.18 kg/person/year with decreasing rate of about 0.88% per year. Rice production in Indonesia nowadays is quite high, but in the future, there is a concern that the availability of rice cannot fulfil rice consumption needs. Therefore, there needs to be an effort to support national food security, namely by diversifying food in the form of analog rice [1]

Rice analog is also known as artificial rice. Rice analog is rice made from non-rice sources with carbohydrate content similar to or higher than rice, with a form resembling rice and can come from a combination of local flour [2]. There are two methods to produce rice analog, i.e. granulation method and extrusion method. The difference between these two methods is in dough gelatinization and moulding stages. The moulding result from the granulation method is granular, whereas the results of the extrusion method are oval round and resemble the shape of rice [3].

Other than fulfilling food needs, rice analog can also be utilized as a functional food that is beneficial for health by adding active or bioactive compounds from plants or animals that have positive effects on health. One of bioactive sources is mangrove plant that live on the coast and have many benefits ranging from ecological benefits to benefits as a food source and medicine. There are several bioactive compounds in mangrove, such as saponin, flavonoid and tannins, that can be used as raw materials for medicine [4;5], also steroid and triterpenoid [6]. A type of mangrove that has been used widely as medicine and food source is *R. mucronata* mangrove fruit [7]. Tannin in *R. mucronata* mangrove fruit is reported to be able to reduce hypermotility of intestine during diarrhea [8], whereas alkaloid and triterpenoid can inhibit sedangkan  $\alpha$ -glucosidase [9], therefore it is potential to become antidiabetic agent. Potency of *R. mucronata* as antidiabetic agent has been reported to be quite good [10; 11; 12]. Thus, it is expected that addition of *R. mucronata* fruit flour to rice analog can produce functional rice analog particularly for diabetics.

Another ingredient that has been reported to have antidiabetic properties is *E. cottonii* seaweed [13], which also has hypocholesterolemia properties [14]. It is

suspected because of dietary fiber and bioactive compound in *E. cottonii*. *E. cottonii* seaweed flour contains total dietary fiber of 91.3% (dry basis) and iodine of 19,4 $\mu$ g/g (dry basis). The use of *E. cottonii* seaweed in rice analog development can produce a product that is rich in dietary fiber [15]. Other than source of fiber and bioactive compounds, *E. cottonii* can also act as a thickener in food products.

The main ingredients that are frequently used in rice analog are corn flour and cassava flour as main sources of carbohydrate. Corn flour also contains protein, fat, crude fiber, vitamin and mineral. The nutritional content of 100-gram corn are calorie of 129 kcal, protein of 4.1 g, carbohydrate of 30.3 g and fat of 1.3 g [16]. Meanwhile, the nutritional content of 100-gram cassava flour are calorie of 363 kcal, the water content of 9.1 g, protein of 1.1 g, carbohydrate of 88.2 g and fat of 0.5 g [17]. The addition of ingredients with hypoglycemic properties to rice analog can produce antidiabetic rice analog. The problem is how much ingredient can be added to produce rice analog that resembles natural rice.

The development of rice analog with antidiabetic properties is important for Indonesian society because there are many diabetics that are difficult to change the rice consumption habit. Moreover, diabetics in Indonesia also occupy the top position. Diabetes is characterized by hyperglycemia and metabolic disorder on carbohydrate, fat and protein, related to absolute or relative lack of insulin work or secretion [18; 19]. Control of hyperglycemia after eating is believed as an important thing in diabetes treatment. Control can be done by consuming food with hypoglycemic properties or able to inhibit starch hydrolyzing enzyme, such as  $\alpha$ -glucosidase [20].

Considering the Indonesian society that cannot be separated from rice as staple food, and the benefits of *R. mucronata* mangrove fruits that are potential to cure diabetes, a research to develop corn flour and cassava flour-based rice analog added with *R. mucronata* mangrove flour combined with *E. cottonii* seaweed flour, should be done. This is also to determine its  $\alpha$ -glucosidase inhibitory activity.

## II. METHODS

### A. Materials and Equipment

Materials used in rice analog making were *R. mucronata* mangrove fruits, dried *E. cottonii* seaweed, cassava flour and corn flour. *R. mucronata* mangrove fruits used were the old ones (yellowish green to brownish yellow with 15-25 cm length), obtained from Banyuwangi region, Banyuwangi district, East Java. Mangrove fruits were made into flour using a method from Ref. [21]. Dried *E. cottonii* seaweed was obtained from Pasar Besar Malang and processed into flour using a method from Ref [14]. Cassava flour and cornflour were obtained from Pasar Besar Malang. Materials used for inhibitory activity assay were  $\alpha$ -glucosidase enzyme (Megazyme, England), p-Nitrophenyl  $\alpha$ -glucopyranoside (PNPG) substrate

(Megazyme), dimethyl sulfoxide (DMSO), pH buffer, 200 mM Na<sub>2</sub>CO<sub>3</sub>, K<sub>2</sub>HPO<sub>4</sub>, 0.2 M NaOH, 2 N HCl and acarbose.

Equipment used to prepare mangrove flour were stainless steel knife, tray, digital balance, oven, Erlenmeyer, dessicator, disc mill, 60 mesh sieve and basin. Equipment used to prepare seaweed flour were basin, blender, tray and oven. Equipment used to prepare rice analog were analytical balance, digital balance, porridge maker, disc mill, 80 mesh sieve, single screw extruder, steamer, thermometer, basin and plastic bags.

### B. Method

Research method used was experimental method. Rice analog was prepared with treatment of *R. mucronata* fruit flour addition (5%, 10%, 15%) and *E. cottonii* flour addition (0%, 3%, 5%, 7%) to tapioca flour and corn flour.

Rice analog was made using extrusion method. Rice analog preparation includes weighing according to formulation, mixing, extrusion and drying. Mixing process was done in two stages, i.e. mixing of cassava flour and mangrove flour, then added with 15% water and mixed until homogenous. Afterwards, mixing was done between corn flour and seaweed flour, then added with 30% water and mixed until homogenous. Steaming process was done for 10-15 minutes. Then, mixing was done between two steamed flours (according to composition) in warm condition (before the flours became hard when cooled). Mixing was done until the flours are mixed thoroughly. The dough was then put into a screw conveyor for 5 minutes. The purpose is to ensure that dough is mixed well and easier to control extrudate obtained from the extrusion process. Afterward, the extrusion of dough was done in the extruder at a temperature of 85-90 °C. Extrudate rice was then sun-dried at  $\pm$ 30 °C for 2 days. This process was done to reduce the water content of rice analog to reach <14%.

Parameters observed were  $\alpha$ -glucosidase inhibition activity [22], organoleptic tests, i.e. hedonic dan scoring [23], dietary fiber [24], cooking time [25], cooking loss [26], volume expansion [27], rehydration properties [28].

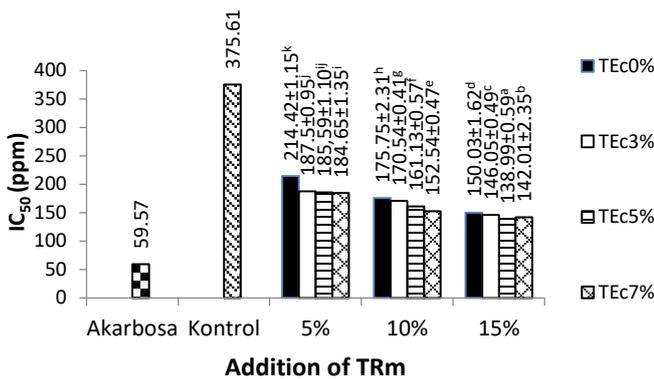
## III. RESULTS AND DISCUSSION

### A. $\alpha$ -glucosidase Inhibitory Activity of Rice Analog

The function of the  $\alpha$ -glucosidase enzyme is to break down the carbohydrate into glucose in digestion, especially in the small intestine.  $\alpha$ -glucosidase activity assay was done to compare rice analog added with *R. mucronata* mangrove fruit flour and *E. cottonii* seaweed flour, with acarbose as the positive control. Statistical analysis (Anova) result showed that addition of *R. mucronata* mangrove fruit flour and *E. cottonii* seaweed flour and interaction of both gave significant effect ( $p < 0.05$ ) on IC<sub>50</sub>. The result of the post hoc test using Duncan can be observed on Figure 1.

Figure 1 shows that the average IC<sub>50</sub> value of rice analog added with *R. mucronata* mangrove fruit is 138.99-214.42

ppm. The lowest IC<sub>50</sub> value of rice analog was obtained from treatment of addition of 15% *R. mucronata* mangrove fruit flour and 5% *E. cottonii* seaweed flour. The highest IC<sub>50</sub> value of rice analog was obtained from treatment of addition of 5% *R. mucronata* mangrove fruit flour and 0% *E. cottonii* seaweed flour. The lowest IC<sub>50</sub> value of rice analog (138.99 ppm) is higher than IC<sub>50</sub> value of acarbose (59.57 ppm), but lower compared to control (375.61 ppm). This result indicates that rice analog is less effective in inhibiting α-glucosidase enzyme compared to acarbose, but more effective compared to control.



Note : different superscript notation shows significant difference p<0.05

Figure 1. IC<sub>50</sub> value of corn flour and cassava flour-based rice analog (300 g) with addition of *R. mucronata* (TRm) flour and *E. cottonii* (TEC) flour

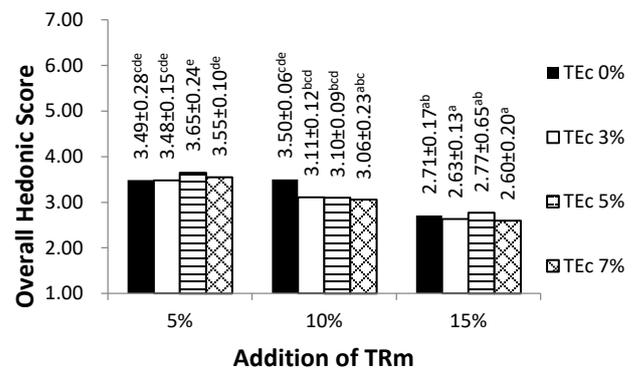
IC<sub>50</sub> value of brown rice bran extract is 441.74 mg/ml [29]. Based on this statement, IC<sub>50</sub> value of rice analog added with *R. mucronata* mangrove fruit and *E. cottonii* seaweed is lower, i.e. 138.99 ppm. Therefore, it is better in inhibiting α-glucosidase enzyme. IC<sub>50</sub> (inhibition concentration) value is concentration of sample to reduce DPPH activity about 50% [30]. Lower IC<sub>50</sub> value shows higher inhibitory activity, where IC<sub>50</sub> value lower than 50 ppm shows very strong inhibition, IC<sub>50</sub> value of 50-150 ppm shows strong inhibition and IC<sub>50</sub> value of 150-200 ppm shows moderate inhibition. The lowest IC<sub>50</sub> value of rice analog extract added with *R. mucronata* mangrove fruit and *E. cottonii* seaweed obtained in this research was 138.99 ppm. It means that the inhibitory activity of rice analog added with *R. mucronata* mangrove fruit and *E. cottonii* seaweed towards α-glucosidase enzyme is categorized as strong since the IC<sub>50</sub> value is between 100-150 ppm. This result was obtained from treatment of addition of 15% *R. mucronata* mangrove fruit flour and 5% *E. cottonii* seaweed flour.

The ability of rice analog in inhibiting α-glucosidase enzyme is by delaying the breakdown of oligosaccharides into monosaccharides, therefore, reducing sugar absorption in the body. Hyperglycemia is the condition in which glucose concentration in the blood is above normal, such

as in diabetics [31]. Inhibition of α-glucosidase enzyme can help body to overcome hyperglycemia condition because of lesser amount of monosaccharides that can be absorbed by intestine.

B. Hedonic Organoleptic Result of Rice Analog

Overall hedonic is the assessment of a panelist's preference in overall towards a certain product. There are several parameters assessed in rice analog using overall hedonic assessment, i.e. taste, colour and texture. Statistical analysis (Anova) result shows that the addition of *R. mucronata* mangrove fruit flour and *E. cottonii* seaweed flour and interaction of both gave significant effect (p<0.05) on overall hedonic score. The result of post hoc test using Duncan can be observed on Figure 2.



Note : different superscript notation shows significant difference p<0.05

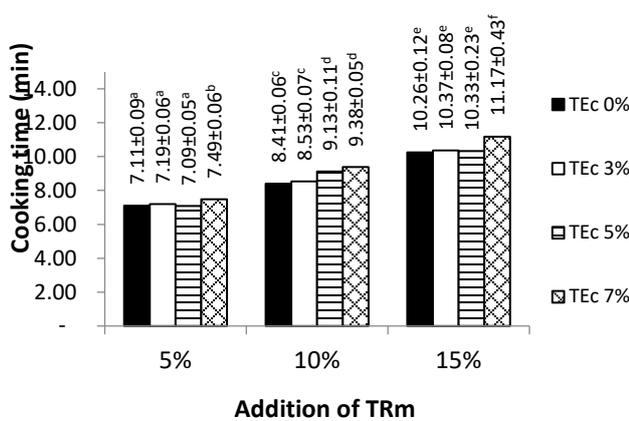
Figure 2. Overall hedonic score (1 = extremely dislike; 7 = extremely like) of cornflour and cassava flour-based rice analog (300 g) with addition of *R. mucronata* (TRm) flour and *E. cottonii* (TEC) flour

Figure 2 shows that the average hedonic score of rice analog is about 2.6-3.65, which means that the acceptance of panelists is between slightly dislike to neutral. The best result was obtained from cassava flour and corn flour-based rice analog added with 5% *R. mucronata* mangrove fruit flour and 5% *E. cottonii* seaweed flour, which is 3.65, and categorized as slightly dislike. On the contrary, the lowest hedonic score was obtained from rice analog added with 15% *R. mucronata* mangrove fruit flour and 7% *E. cottonii* seaweed flour, i.e. 2.60. This overall hedonic score is lower compared to a previous research of rice analog made from cassava flour and *E. cottonii* seaweed flour [34], which obtained an overall acceptance score of 4.17 to 5.23, categorized as neutral to like. The low acceptance score might be caused by the addition of *R. mucronata* fruit flour which has slightly astringent taste due to its tannin content [10].

C. Cooking Time of Rice Analog

Statistical analysis (Anova) result shows that that addition of *R. mucronata* mangrove fruit flour and *E. cottonii* seaweed flour and interaction of both gave significant

effect ( $p < 0.05$ ) on cooking time of rice analog. Result of post hoc test using Duncan can be observed on Figure 3.



Note : different superscript notation shows significant difference  $p < 0.05$

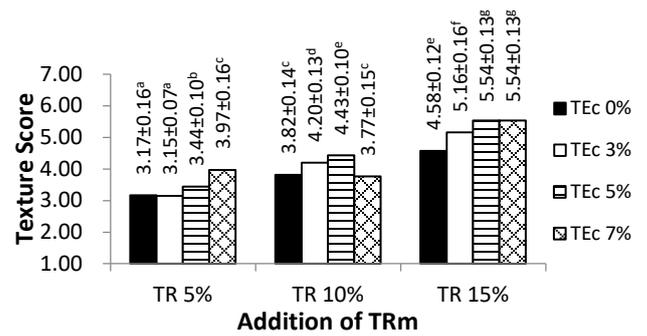
**Figure 3.** Cooking time of corn flour and cassava flour-based rice analog (300 g) with addition of *R. mucronata* (TRm) flour and *E. cottonii* (TEc) flour

Figure 3 shows that average cooking time of corn-based and cassava flour-based analog added with *R. mucronata* mangrove fruit flour and *E. cottonii* seaweed flour is about 7.09 minutes to 11.17 minutes. The shortest cooking time is obtained from rice analog added with 5% *R. mucronata* mangrove fruit flour and 5% *E. cottonii* seaweed flour, whereas the longest cooking time is obtained from rice analog added with 15% *R. mucronata* mangrove fruit flour and 7% *E. cottonii* seaweed flour.

Cooking time of rice analog made from white corn is 4.06 minutes [32]. On the other hand, the shortest cooking time of rice analog added with *R. mucronata* is 7.09 minutes. Longer the cooking time of rice analog indicates the longer time required by rice analog to be fully cooked. The higher the concentration of *R. mucronata* added the longer the cooking time. It is because higher concentration of *R. mucronata* added makes rice analog obtained becomes more compact. Thus, affecting the rice cooking process.

**D. Texture Organoleptic Result of Rice Analog**

Scoring of texture is done to give assessment on texture of a food product with score from 1 to 7, where 1 = extremely not sticky, 2 = not sticky, 3 = slightly sticky 4 = sticky, 5 = slightly stickier, 6 = very sticky, 7 = extremely sticky. Statistical analysis (Anova) result shows that the addition of *R. mucronata* mangrove fruit flour and *E. cottonii* seaweed flour and interaction of both gave significant effect ( $p < 0.05$ ) on texture scoring of rice analog. Result of the post hoc test using Duncan can be observed in Figure 4.



Note : different superscript notation shows significant difference  $p < 0.05$

**Figure 4.** Texture score of corn flour and cassava flour-based rice analog with addition of *R. mucronata* (TRm) flour and *E. cottonii* (TEc) flour

Figure 4 shows that average scoring for texture of corn based and cassava flour-based analog added with *R. mucronata* mangrove fruit flour and *E. cottonii* seaweed flour is about 3.15-5.54, which shows that rice analog was perceived as slightly sticky to slightly stickier, in terms of texture, by the panelists. The highest scoring value was obtained from rice analog added with 15% *R. mucronata* mangrove fruit flour and 7% *E. cottonii* seaweed flour, with score of 5.65, which shows that the texture was slightly stickier.

**E. Selected Rice Analog**

Based on the overall hedonic test result, the selected rice analog formulation was rice analog added with 5% *R. mucronata* mangrove fruit flour and 5% *E. cottonii* seaweed flour, with score that shows slightly like by the panelists. The physicochemical characteristics of selected rice analog can be seen in Table 1.

**Table 1.** Physicochemical characteristics of selected rice

Parameter	Selecte d analog rice	Rice from black mangrove [33] <sup>1</sup>	Rice from mocaf and E.cottonii [34] <sup>2</sup>	Rice from mocaf and corn starch [35] <sup>3</sup>
Yield	64.06%	81.94%	99.00%	-
Rehydration	40.37%	-	-	155.06%
Expansion	44.16%	-	-	142.58%
Cooking Time	7.09 min	-	-	12.45 min
Cooking Loss	16.86%	-	-	0.85%
Moisture	9.47%	13.48%	8.76%	8%
Starch	70.99%	67.59%	-	84.86%
IC <sub>50</sub> (ppm)	185.59	-	-	-
Protein	3.24%	3.57%	0.86%	1.88%
Fiber	9.71%	8.16%	49.76%	-
- Soluble	1.8%	-	-	-
- Insoluble	7.88%	-	-	-

Table 1 shows that best formulation of rice analog from black mangrove flour and sago flour added with chitosan [33] was made using 70% black mangrove flour, 30% sago flour, added with 0.5% chitosan. This formulation has yield of 81.94%, moisture content of 13.48%, starch content of 67.59%, protein content of 3.57% and dietary fiber of 8.16%. Moreover, the best formulation of rice analog from mocaf flour and *E. cottonii* flour [34] was the one added with 5% *E. cottonii* flour. This formulation has yield of 99.00%, moisture content of 8.76%, protein content of 0.86%, fat content of 0.15%, ash content of 1.96% and dietary fiber of 49.76%. Moisture content of rice analog obtained has already fulfilled the standard of SNI 6128: 2015, i.e. less than 14%. Another research of rice analog made from mocaf flour, corn starch, CMC (*Carboxy Methyl Cellulose*) and okara flour [35] shows that the best rice analog was obtained from the addition of 1.5% CMC and 5% okara flour. This formulation has a moisture content of 8.00%, starch content of 84.86%, the volume of expansion of 142.58%, rehydration of 155.06%, the cooking time of 12.45 minutes, cooking loss of 0.85% and protein content of 1.88%.

#### IV. CONCLUSION

Higher amount of *R. mucronata* mangrove fruit flour and *E. cottonii* seaweed flour into rice analog increases the inhibitory activity towards  $\alpha$ -glucosidase enzyme but decreases the panelists' acceptance. The selected rice analog is corn flour based and cassava flour-based rice analog added with 5% *R. mucronata* mangrove fruit flour and 5% *E. cottonii* seaweed flour.

The selected rice analog has yield of  $64.06 \pm 0.47\%$ , rehydration of  $40.37 \pm 0.84\%$ , volume of expansion of  $44.16 \pm 0.83\%$ , cooking time of  $7.09 \pm 0.05$  minutes, cooking loss of  $16.86 \pm 0.53\%$ , moisture content of  $9.47 \pm 0.19\%$ , starch content of  $70.99 \pm 0.72\%$ , protein content of 3.24%, soluble dietary fiber content of 1.83%, insoluble dietary fiber content of 7.88%,  $IC_{50}$  value of 185.59 ppm, overall hedonic score of 3.65 (slightly dislike-neutral) and texture score of 3.44 (slightly sticky).

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