Characteristic of Taro (*Colocasia esculenta*) and Seaweed (*Eucheuma cottoni*) Based Analogue Rice Fortified with Fishes Bone Collagen (A Promising Anti-Diabetic Functional Food)

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Abstract: Recently, approximately 9.1 million people of 237.56 million of Indonesian population suffer diabetes. Such condition was caused by high rice consumption of most Indonesian people. It has been known that rice contains low amylose, high calorie and possesses hyperglycemic properties. We formulated Taro and Seaweed flour based Analogue Rice (AR) that fortified by various Fishes Bone Collagen (FBC) in order to provide an alternative healthy and balanced diet. AR was characterized about its nutritional composition such are proximate analyses, water, dietary fiber and amylose content. Furthermore, its morphological structure was analyzed by using scanning electron microscopy while the level of consumer preferences was performed by hedonic test. Fortification by using various FBC into the AR was significantly different in physical and chemical properties thereby affecting the flavor, aroma and texture. Moreover, addition of milkfish bones collagen into AR was more preferably than others. Taro (*Colocasia esculenta*) and seaweed (*Eucheuma cottoni*) based AR fortified with various FBC showed the different food fiber and amylose contents. This founding was expected as new avenue in functional food discovery especially in the treatment and prevention of diabetic diseases.

Key words: Analogue rice, taro, seaweed, fishes bone collagen, treatment

INTRODUCTION

Currently, it is estimated that of 237.56 million of Indonesia's population, more or less 9.1 million people suffer from diabetes. Such condition has a correlation to the misunderstanding about food nutrition, inappropriate method in food selection, low purchasing power, culture, diet and people education level. In the other hand, rice as an Indonesian staple food possesses the high level of carbohydrate, sugar and calories are not recommended to the diabetic. Therefore, Analog Rice (AR) to replace the rice consumption in diabetic must be contemplated.

In tropical country, tuber has been known as an important source of carbohydrates. Indonesia as one of tropical country has abundant tubers production. One of them is taro (*Colocasia esculenta*), a tropical tuber crop contains 70-80 % starch and rich in gums (mucilage). Small sizes of taro's starch granules make it a highly digestible which is advantaged for various foods (Kaushal *et al.*, 2015).

Viewed from the aspect of nutrition, food is not adequate merely with the low level of carbohydrate, sugar and calories but it should be envisaged dietary fiber. *Eucheuma cottoni* is one of seaweed families that contain high level of dietary fiber with a composition of 27.8% of water, 8.6% of fat, 3% of fiber and 22:25% of ash (Ask and Azanza, 2002). It also contains enzymes, nucleic acids, amino acids, vitamins and minerals such as nitrogen, calcium, selenium and iron, magnesium and sodium (Anggadiredja *et al.*, 2008). Seaweed contains many soluble fibers that are difficult to be digested, able to slow down the gastric emptiness and maintain the satiety. Farmer (2009) stated that seaweed in the form of extract can lower the blood sugar, cholesterol, anti-microbial, anti-inflammatory, antipyretic, antiglesic and anti-coagulant. Seaweeds produce the carrageen which serves as a food additive, stabilizer and thickener, gelling in industries such as gel, meat canning, bread, ice cream or cheese (Carthew, 2002).

AR is the artificial rice made of taro flour and seaweed using granulation technology and heat and cold extrusion and made in the rice-like shape. It contains low carbohydrate and is rich in fiber. To complete its nutrition, it needs to add with the protein components. Fish Bone Collagen (FBC) is protein processed by deproteinization and demineralization into alanine amino acids, glycine,

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arginine, proline and hydroxyproline (Tongchan *et al.*, 2009). Darmanto *et al.* (2010) explained that the collagen added into myofibril-based food will enhance the gel strength and water holding capacity and change the position of monolayer and multilayer water. If heated, collagen changes into gelatin that has a number of physical and chemical properties resembling the collagen (Darmanto *et al.*, 2010). Further, Darmanto *et al.* (2014) explained that if the gelatin is added into the nugget, it will improve the gel strength, water holding capacity, folding test and water activities. Through this study, we formulated Taro and Seaweed flour based Analogue Rice (AR) that fortified by various Fishes Bone Collagen (FBC) in order to provide an alternative healthy and balanced diet (FCC, 1975).

MATERIALS AND METHODS

The materials of study consisted of taro, seaweed and collagen from various types of fish bones. Taro was obtained from Semarang City and the seaweed *Eucheuma Cottoni* was obtained from Jepara regency, Central Java Province. Four types of fish bones were catfish, milkfish, snapper and ray fish, taken from TPI (Fish Auction Place) in Semarang.

Preparation of taro and seaweed flour: Taro flour was processed according to the procedure which described earlier (Cahyadi *et al.*, 2012). Briefly, production of taro flour was carried out through a number of phases such as washing, stripping the skin, cutting, soaking in 0.05% metabisulfite for 30 min, drying, smoothing and sieving. In the other hand, production of seaweed flour was performed in several stages such as washing, soaking in KOH 8% within 6 h, drying in an oven of 55°C for 2 days, milling and sieving (Afriwanti, 2008).

Preparation of various fish bone collagen: Various fish bone used in this study derived from several fishes such as catfish, milkfish, snapper and ray fish. The procedure of collagen production as described earlier (Darmanto *et al.*, 2010). Briefly, making of collagen was conducted through several steps: washing, deproteinization, demineralization, drying, milling and sieving.

Production of analog rice: First of all, collagen, taro and seaweed flour were grinded become a nano-particle by using ball milling device (Lekahena *et al.*, 2014). Analog rice was produced according to the food formulation as through taro flour (500 g), seaweed flour (25 g) and each collagen (25 g), 10 g of glyceryl mono stearate (10 g) and

water (125 cc). Mixed formula was steamed for 15 min and then it was formed through the granulation and extrusion technique at temperature of 70°C (Mishra *et al.*, 2012).

Experimental analyses: The proximate analysis was conducted according to the procedure established by AOAC (2007). Dietary fiber was calculated according to the method established by Johannson *et al.* (1983). Dietary fiber is a complex carbohydrate derived from plant tissues that cannot be digested by digestive enzymes. Therefore, the measurement of dietary fiber was done by enumeration the weight of soluble and insoluble fiber.

The hedonic test of rice analogue was conducted by 30 trained panelists to assess the preference towards color, aroma and the stickiness of taste of the super rice analogue using the scale of 1-5 (Meilgaard *et al.*, 1999).

Amylose is an important parameter in determining the hedonic test on the rice texture both on the hardness and on the adhesiveness. Analysis was measured with the color in tenstitasusing spectrophotometer (Masniawati *et al.*, 2013).

The analysis on the size of the collagen Nano particle with Scanning Electron Microscopy (SEM) used the 10.000x magnification at a voltage of 20 kV. The source of electrons was emitted to the sample to scan the surface of the sample and then the gold metal as a conductor would reflect the electrons to a detector on SEM microscope. Furthermore, the scanning results would be passed by the detector to the monitor (Ruiz-Gutierrez *et al.*, 2012).

Statistical analysis: One-way ANOVA was used to identify the effect of different FGW levels. For post-hoc multiple comparisons of group differences, Duncan's multiple range testwas used. Statistical analyses were performed using the software package SPSS for Windows (IBM SPSS 64 Bit Version). A value of $p < 0.05$ was considered statically significant (Dawson and Trap, 2001).

RESULST AND DISCUSSION

The proximate analysis is a rough description of various elements in a food material. It includes moisture, protein, fat, ash and carbohydrates. The results showed that the addition of collagen of various types of fish bone had a significant effect ($p < 0.5$) on the results of proximate analysis on the super rice analogue. Table 1 shows the results of proximate analysis on the rice analogue.

The protein content in super rice analogue with the addition of various fish bone collagens showed a different composition. As stated by Darmanto *et al.* (2012), 14 different types of fish bone collagen had the different chemical compositions. Table 1 shows that the protein

Table 1: Proximate analysis of taro and seaweed based analog rice fortified with various fish bone collagen

Analysis (%)	Control	Stingray	Milkfish	Catfish	Snapper
Ash content	3.47±0.50[a]	9.80±0.07[b]	7.49±0.03[b]	9.05±0.06[c]	7.22±0.02[c]
Water content	10.43±0.04[e]	4.86±0.09[a]	5.29±0.08[b]	6.87±0.02[c]	7.36±0.04[d]
Protein content	3.41±0.06[a]	6.13±0.06[b]	6.04±0.06[b]	6.42±0.05[b]	6.32±0.06[b]
Fat content	1.25±0.06[b]	1.48±0.08[a]	1.57±0.02[b]	2.28±0.06[c]	1.66±0.08[b]
Carbohydrate	81.43±0.07[b]	81.28±0.10[b]	79.69±0.09[a]	75.37±0.01[a]	75.87±0.15[a]

Data were expressed as means±SD. [a-e]Means in the same row with different superscripts differ significantly (p<0.05) according to Duncan's multiple-range test

content of super rice analogue by adding collagen analogue of catfish was higher than snapper, stingray and milkfish.

The ash content in super rice analogue was at 3.470% but with the addition of collagen the content level was found at 7.220-9.800%. This was due to the differences in mineral content in each of fish bone collagens. Marsono and Atanu (2002) stated that collagen in addition to its function as the source of protein contained several mineral elements such as potassium, calcium, phosphorus, sodium and iron. This is also supported by Darmanto *et al.* (2010), stating that the addition of the collagen from tilapia bone could increase the levels of ash in view of the very high mineral content in the bones of tilapia. As shown in Table 1, ash content and protein content varied greatly caused by the difference in concentration of minerals and amino acids of each of different collagen forticant. Stated by Darmanto *et al.* (2014), the difference in protein and minerals in collagen could affect the WHC (Water Holding Capacity) and gave an effect on the water position (monolayer water and multilayer water) of a material.

Amylose content is essential in the process of making super rice analogue which will determine the functionality and the level of good taste of the rice. Amylose on rice will affect the quality as well as the consumer acceptance (Avaro *et al.*, 2009).

Figure 1 shows that the addition of collagen from the different types of fish bone had a significant effect (p<0.5) on the amylose content. The highest amylose content was found in the addition of collagen of stingrays at 19:18% while the lowest one without the addition of collagen was at 13.61%. It was caused by the high levels of ash in the rice analogue with the addition of the collagen of the stingray bone. The higher ash content could make the higher the mineral content inside it. Sarofa and Ardiansyah (2015) mentioned that the mineral content was capable of forming the crosslinks with the amylose and amylopectin in the heating process.

The amylose level of super rice analogue of taro was found in the class with the low level of amylose (10-20%). The rice with low amylose content after being cooked would produce a sticky rice. While, the one with medium level of amylose would produce the rice with the fluffy texture and other with high amylose content will produce
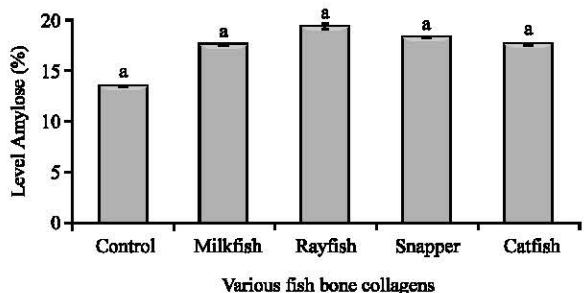

Fig. 1: Level of amylose content in taro and seaweed based analog rice with addition of various fish bone collagen. Amylose contents were expressed as percentage. Data were mean±SD of 4 replicate analyses; [a, b]Differ superscripts above the bar chart show differ significantly (p<0.05) according to Duncan's multiple-range test

dry rice (Kurniawati, 2013). Rice with the high amylose level could make the starch find difficult to experience the gelatinization and more easily combine to form a dense crystalline structure or undergo the retrogradation (Sharma *et al.*, 2008).

The seaweed *E. cottoni* is rich in dietary fiber derived from the cell wall composed of several types of carbohydrates such as cellulose, hemicellulose, pectin and lignin polymer non-carbohidrate such as lignine polymer, a number of gums and musilase (Poedjiadi, 1994). The results of the analysis on the dietary fiber of super rice analogue is presented in Fig. 2. Figure 2 shows that the addition of collagen from some types of fish bone has given a significantly different effect (p<0.05) on the content of dietary fiber on the super rice analogue. The highest level of dietary fiber was obtained in collagen of snapper at 18:46% while the lowest one was found at 16.76% without any addition of collagen. Kurniawati (2013) stated that rice analogue commonly has a fiber content of food of 13.3%. The level of the dietary fiber will bring the effect on the activity of digestive enzymes. Additionally, it will provide the longer satiety and inhibit digestion in the gastrointestinal (Sadek *et al.*, 2016).

Scanning Electron Microscopy (SEM) is one of the methods to observe the size and morphology of a material. Principally, the mechanism of SEM is to lead the electrons to the very small particles. The results of SEM analysis on the super rice analogue are shown in Fig. 3.

As shown in Fig. 3, the rice analogue with the addition of collagen of milkfish had an evenly homogeneous granule size. With the addition of the collagen of milkfish bone, it was seen a smooth surface with a little indentation in the form of lumps and some of pores. Similarly, with the addition of collagen of catfish bone, the surface was smooth with some of pores. While, the addition of collagen of snapper bone, the surface had some lumps and few pores.

Zaidar *et al.* (2013) stated that the mor phology with a slight indentation could be caused by the addition of seaweed flour that was less homogenous. However, with
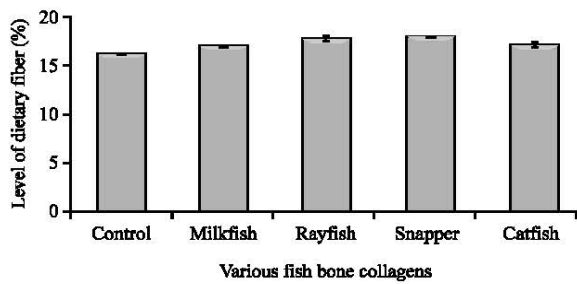

Fig. 2: Level of dietary fiber content in taro and seaweed based analog rice with addition of various fish bone collagen. Fiber contents were expressed as %. Data were mean±SD of 4 replicate analyses

the addition of granulecollagen on the super rice analogue it could create a smooth and even surface. Thipayarat and Leelayuthsoontorn (2005) stated that the increase of pore size and the thickness of the sponge texture were due to the increasing temperature in the heating process.

The hedonic analysis is the analysis of consumer preferences towards color, aroma, texture and the entirety of super rice analogue. The results of hedonic analysis on the super rice analogue are presented in Table 2. Table 2 shows that the collagen fortification did not give any effects on the color of the super rice analogue. The results of the research showed that the color of the super rice analogue was in the range of 3.70-3.85, indicating that the panelists preferred the brownish white super rice analogue. Sastro *et al.* (2012) mentioned that the color is one of the important indicators determining the factors of food acceptance by consumers.

The average assessment of the panelist towards the aroma of the super rice analogue was in the range of 2.94-3. Meanwhile, the most favored one without any collagen given among was in the value of 3:45. The results of the assessment on the texture showed that the collagen fortification from the different types of fish bone did not give any effects on the texture of the superrice analogue. Based on the data obtained, the average values
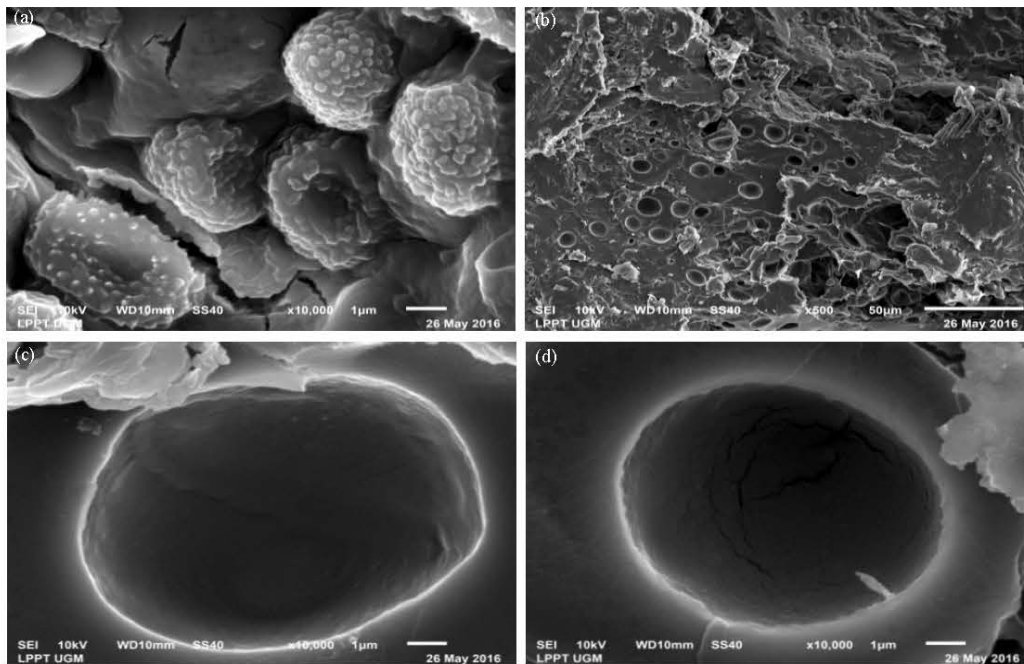

Fig. 3: Morphological appearance of taro and seaweed based analog rice with addition of various fish bone collagen by Scanning Electron Microscopy (SEM) analysis; a) Control; b) Milkfish collagen; c) Catfish collagen and d) Snapper collagen

Table 2: Hedonic test of taro and seaweed based analog rice fortified with various fish bone collagen

Various fish bone collagen	Parameters			
	Color	Aroma	Texture	Entirety
Control	3.81±0.57[a]	3.45±0.41[b]	3.78±0.59[a]	3.51±0.46[a]
Milkfish	3.85±0.63[a]	2.94±0.39[a]	3.75±0.92[a]	3.67±0.17[b]
Rayfish	3.70±0.59[a]	2.96±0.41[a]	3.40±0.61[a]	3.45±0.49[a]
Snapper	3.81±0.33[a]	3.25±0.67[ab]	3.55±0.66[a]	3.55±0.28[a]
Catfish	3.78±0.46[a]	3.01±0.39[ab]	3.84±0.71[a]	3.54±0.44[a]

Data were expressed as means±SD. [a-b]Means in the same row with different superscripts differ significantly (p<0.05) according to Duncan's Multiple-Range test

of preference towards the texture of rice analogue were in the range of 3.55-3.84% indicating the preferences of the panelists towards the texture of super rice analogue.

Overall, the sensory of the rice analogue showed that the collagen fortification had significant effects on the entirety of the sensory of the super rice analogue. The average value of preference towards the entirety of the taro flour and seaweed based super rice analogue were in the range of 3.51-3.67% indicating that overall the panelists preferred the super rice analogue.

CONCLUSION

Based on the results of the research, it showed that the super rice analogue with the addition of the collagen from the different types of fish species with the formulation of taro flour and seaweed flour can alter the physical and chemical properties; thus enable to provide it the different flavor, aroma and texture. The panelists preferred the super rice analogue with the addition of the collagen of milkfish bone rather than any other fish. The addition of collagen of different types of fish bones will produce the different food fiber contentsand amylose on super rice analogue.

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