

Characteristic of analog rice made from arrowroot (*Maranta arundinaceae*) and seaweed (*Gracilaria verrucosa*) flour fortified with fish collagen

¹Darmanto, Y.S., ^{1,*}Kurniasih, R.A., ¹Romadhon, R., ¹Riyadi, P.H. and ²Anggraeni, N.

¹Department of Fish Product Technology, Faculty of Fisheries and Marine Science, Diponegoro University, Semarang, Central Java Province 50275, Indonesia

²Department of Food Technology, Faculty of Science and Technology, National University Karangturi, Semarang, Indonesia

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Abstract

Analog rice is not sufficient if it only contains low sugar, low GI, and high fibre, but also needs to be fortified with other nutritional components. Fish collagen can be used for analog rice fortification because it contains amino acids. This study aimed to determine the effect of adding collagen from various types of fish scales on the characteristics of analog rice made from arrowroot and seaweed flour. The materials in this study were collagen from various types of fish scales (tilapia, milkfish, red snapper, and goldband goatfish), arrowroot flour, and seaweed flour. The parameters analyzed were proximate analysis, amino acid content, crude fibre, dietary fibre, cooking time, hedonic evaluation, and morphology observation of analog rice. The results showed that the fortification of collagen from various types of fish scales produced analog rice with significantly different ($p < 0.05$) characteristics. The fortification of collagen from various types of fish scales can improve the proximate, amino acid content, and cooking time of analog rice. However, collagen fortification did not provide a significant difference ($p > 0.05$) to the hedonic value (except for aroma parameters), crude fibre, and dietary fibre content in analog rice. Analog rice produced in this study contained higher nutrition than milled rice. Analog rice in this study contained 8.45 to 13.54% of lipid, 8.45 to 13.54% of protein, 22.4 to 23.90% of crude fibre, and 1.67 to 1.73% of dietary fibre. Therefore, it can be used as a substitute for milled rice and as functional food for people.

1. Introduction

Statistics Indonesia (2018) reported that the total rice consumption in Indonesia reaches 111.58 per capita per year. Rice is the staple food of the Indonesian population. Meanwhile, Kaur *et al.* (2016) reported that white rice boiled has a relatively high glycemic index (GI) is 24 to 160 with white bread as a reference. This is one of the reasons why Indonesia ranks 7th as the country with the highest number of diabetics globally in 2019. Diabetics in Indonesia in 2019 reached 10.7% of the total population of Indonesia (Ministry of Health Republic of Indonesia, 2020). Therefore, it is necessary to think of alternatives for non-rice food replacement, such as analog rice.

Analog rice is a grain shaped like rice made from non-rice food ingredients, such as cassava and grains as a source of carbohydrates. The analog rice can be made using hot and cold extrusion technology. One of the alternatives for raw materials in the production of analog

rice is the arrowroot because it has a lower GI than white rice, which is 32 (Priatama *et al.*, 2019). Nevertheless, Kumalasari *et al.* (2012) reported that arrowroot flour contains low fibre, 13.7% of dietary fibre, which consists of 8.7% of insoluble dietary fibre and 5% of soluble dietary fibre. Thus, the production of analog rice from arrowroot flour can be combined with seaweed flour in order to increase the fibre content of analog rice. *Gracilaria verrucosa* is a type of red seaweed that can be used for food with high fibre content. Based on a study by Jayasinghe *et al.* (2019), the total dietary fibre in *Gracilaria verrucosa* is 60%. Debbarama *et al.* (2016) investigated soluble dietary fibre that could reduce glycemic response and cholesterol. Meanwhile, insoluble dietary fibre can increase the bulking effect on faeces and play a role in maintaining body weight and cardiovascular and gastrointestinal functions.

Food is not sufficient if it only contains low sugar, low calories, low GI, and high fibre for the daily diet but

*Corresponding author.

Email: retnoayukurniasih@lecturer.undip.ac.id

needs to be added with other nutritional components such as protein. Therefore, other ingredients are needed for the nutritional fortification of analog rice. Collagen from various types of fish scales can enrich analog rice nutrition because it contains complete amino acids. Collagen is a long-chain protein composed of unique amino acids containing alanine, arginine, glycine, proline, and hydroxyproline (Liu *et al.*, 2015). Collagen could also act as a stabilizer and emulsifier (Santana *et al.*, 2011) and is also helpful as an antioxidant to brighten skin, decrease blood pressure, and increase mineral density in bones (Wang *et al.*, 2013; Sila and Bougatef, 2016). Amino acids hydroxyproline and glycine play a role in increasing the gel strength. While hydroxyproline plays a role in increasing the viscoelasticity of food (Atma and Ramdhani, 2017).

Research related to the application of collagen in food has been conducted by Darmanto *et al.* (2014), by adding collagen from 9 types of fish bones into myofibril protein-based food (thread bream fish meat). The results showed that the fortification of collagen could inhibit the decrease in gel strength and water holding capacity as well as slow down the rate of protein denaturation in myofibril protein-based food. Collagen extracted from various types of fish bones also affects the quality of food products. Furthermore, Darmanto *et al.* (2017) explained that analog rice with the fortification of collagen from the different types of fish species bone with the formulation of taro flour and seaweed (*Eucheuma cottonii*) flour can alter the physical and chemical properties, thus enable to provide it with different flavours, aromas, and textures. However, research on the fortification of collagen from various types of fish scales to analog rice made of arrowroot flour and seaweed (*Gracilaria verrucosa*) flour has never been carried out. Therefore, this study aimed to determine the effect of adding collagen from various fish scales (tilapia, milkfish, red snapper, and goldband goatfish) on analog rice's characteristics based on arrowroot seaweed flour.

2. Materials and methods

2.1 Materials

The materials used in this research were fresh arrowroot, dried seaweed, and various fish scales. Arrowroot was purchased from a local market in Semarang (Central Java Province, Indonesia), seaweed (*Gracilaria verrucosa*) was purchased from UD. Rumpun Laut Mandiri (Special Region of Yogyakarta, Indonesia), and four different fresh fish scales (tilapia, milkfish, red snapper, and goldband goatfish) were bought from a fish market in Semarang. The food additive used is glyceryl monostearate (GMS). The chemicals used in the

production of arrowroot flour, seaweed flour, and collagen are technical chemicals.

2.2 Production of arrowroot flour and seaweed flour

The production of arrowroot flour is carried out in several stages, namely washing, stripping, cutting, soaking in 0.05% metabisulfite for 30 mins, drying at 60°C, and milling. Meanwhile, the production of seaweed flour is carried out in washing, soaking in 8% KOH for 6 hrs, drying in an oven at 55°C for 2 days, and milling.

2.2 Production of various fish scales collagen

Collagen production from fish scales has been described in a previous study by Darmanto *et al.* (2014), by washing, deproteination using 0.1 M NaOH, demineralization using 0.5 M acetic acid, separation, freeze-drying, and flouring.

2.3 Production of analog rice

Analog rice production is based on the study of Darmanto *et al.* (2017). The arrowroot flour (500 g), seaweed flour (25 g), collagen (25 g), GMS (10 g), and water (125 mL) were mixed and then steamed for 15 mins. Furthermore, the dough was formed like rice using granulation and extrusion techniques at a temperature of 70°C. Finally, analog rice was dried under the sun. In this study, analog rice was also produced without the fortification of collagen as a control.

2.4 Proximate analysis

Proximate consisting of moisture, ash, protein, lipid, and carbohydrate content was analyzed using AOAC (2005) on a wet basis. Moisture and ash content was determined by the gravimetric method. Protein content was determined by the Kjeldahl method. Lipid content was determined using the Soxhlet method. Furthermore, carbohydrate content is estimated by differences.

2.5 Amino acid composition

The amino acid profile of the analog rice was evaluated using Eurospher 100-5 C18 High-Performance Liquid Chromatography (Shimadzu LC-6A, Japan) with liquid chromatography column 4.6 × 250 mm. Each sample was weighed 2.5 g and hydrolyzed with 15 mL 6 N HCl at 110°C for 12 hrs until homogeneous. It was followed by adding 6N NaOH until neutral. In the solution added 2.5 mL lead acetate 40% and 1 mL oxalic acid 15%. The solution was filtered using a Millex filter of 0.45 µm. The filtrate (25 µL) was added 475 µL of the OPAA solution, then was vortexed and incubated for 3 mins. Finally, injection with 30 µL into HPLC (AOAC, 2005).

2.6 Crude fibre content

Crude fibre content was carried out according to AOAC (2005). The sample was treated with acid and alkaline, as occurs in the system of human digestion. First, the sample will be acidified with H_2SO_4 and heated for 30 mins. The next step was to apply a strong alkaline of 1.5 N NaOH and later reheated for 30 mins, then filtered using filter paper. The residue contained in the filter paper was washed with 1.25% H_2SO_4 , hot water, and 95% ethanol. The filter paper and its contents were dried in an oven at 105°C and weighed until constant. The crude fibre content was calculated based on the percentage ratio between the difference in weight of filter paper and the weight of the filter paper often containing the sample after drying with the sample weight.

2.7 Dietary fibre content

Analysis of dietary fibre content used an enzymatic method (AOAC 2005). The sample was extracted first with petroleum ether solvent at room temperature for 15 mins. Then 1 g of sample (W) was placed in the Erlenmeyer flask, dissolved with 25 mL of 0.1 M buffer solution of sodium phosphate pH 6. The next step was added with 0.1 mL of the terminal, covered with aluminium foil, incubated at 100°C for 15 mins, then lifted and cooled, then added 20 mL of aqua dest and pH adjusted to 1.5 with 4 M HCl. The next step was 100 mg of pepsin added, incubated at 40°C, agitated for 60 mins, then 20 mL of aquadest added with the pH adjusted to 6.8. Next, the mixture 100 mg of pancreatin was added to the mixture, incubated at 40°C, and agitated for 60 mins, and the pH was set to 4.5 with HCl. The sample was then filtered with a dry crucible that had been weighed, washed twice with 10 mL of 95% ethanol, twice with 10 mL of acetone, and 100 mL of warm 95% ethanol, and then precipitated for 1 hr. Next, the precipitate was filtered with a dry crucible and washed twice with 10 mL of 78% ethanol and twice with 10 mL of acetone. After that, the crucible was dried at 105°C for 12 hrs and weighed, then placed in the muffle furnace at 525°C for 5 hrs and weighed again until a constant weight was obtained.

2.8 Cooking time

The cooking time was determined using a method by Sukamto and Patria (2020) with a slight modification: analog rice was cooked in the rice cooker with the ratio of rice and water 1:1 (w/v). Analog rice was placed into the rice cooker after the water was boiled. Cooking time was calculated from the time the analog rice was placed into the rice cooker until the rice was cooked.

2.9 Hedonic evaluation

The hedonic evaluation of the cooked analog rice was evaluated by trained panellists to assess the preferences towards colour, aroma, taste, and appearance using a scale of 1-5. The most minor scale indicates the most unpleasant preference for analog rice.

2.10 Morphology observation

Scanning Electron Microscopy (SEM) (Jeol JSM 6510LA, Japan) was used to analyse the morphological properties. Analog rice was coated with platinum before being visualized. SEM was operated with the following standard operating parameters, high voltage of 10 kV, spot size of 40, work distance (WD) of 10 mm, and magnification of 3000x.

2.11 Statistical analysis

Parametric data on the effect of different fish scale collagen on analog rice characteristics (proximate content, crude fibre content, dietary fibre content, and cooking time) were analyzed by one-way Analysis of Variance (ANOVA). Furthermore, Tukey HSD is used for multiple comparisons between treatments. Non-parametric data were analyzed by Kruskal-Wallis and continued with Mann-Whitney test for multiple comparisons. SPSS version 16 (International Business Machines Corporation, USA) was used for statistical analysis.

3. Results and discussion

3.1 Proximate content

3.1.1 Moisture content

The moisture content of analog rice in this study ranged from 11.99 to 14.89% (Table 1). This was also confirmed by Hossain *et al.* (2016) and Rojas *et al.* (2017), the range of appropriate moisture content in rice is 14% or below, thus the rice can be stored in the long-term period. The moisture content of rice grains of 14% could prevent microbial activity.

Analog rice with milkfish scales collagen fortification had the highest moisture content (14.89%), while the lowest moisture content was analog rice control (11.99%) ($p < 0.05$). The high and low moisture content in analog rice is influenced by the quality and protein content in collagen. Collagen can bind to more water because the polar and nonpolar groups in the amino acids that make up collagen play a role in water binding (Barzideh *et al.*, 2014). Proline is an amino acid that has a greater ability to bind water than other types of amino acids (Anderson and Witter, 1982).

Based on the study of Barzideh *et al.* (2014),

Table 1. Proximate analysis of analog rice

| Treatment | Moisture (%) | Ash (%) | Protein (%) | Lipid (%) | Carbohydrate (%) |
|-------------------|-------------------------|-------------------------|-------------------------|------------------------|-------------------------|
| Control | 11.99±0.01 ^a | 2.40±0.05 ^a | 8.45±0.06 ^a | 0.61±0.01 ^a | 76.55±0.01 ^d |
| Tilapia | 13.57±0.01 ^b | 3.07±0.01 ^d | 12.76±0.04 ^c | 0.76±0.01 ^b | 69.84±0.01 ^a |
| Milkfish | 14.89±0.01 ^c | 2.96±0.03 ^{bc} | 11.28±0.02 ^b | 0.75±0.02 ^b | 70.12±0.09 ^b |
| Red Snapper | 13.54±0.01 ^b | 3.01±0.02 ^{cd} | 13.54±0.00 ^d | 0.85±0.01 ^c | 69.06±0.04 ^a |
| Goldband Goatfish | 13.02±0.04 ^b | 2.90±0.00 ^b | 12.25±0.06 ^c | 0.76±0.01 ^b | 71.07±0.07 ^c |

Values are presented as mean±SD. Values with different superscript within the same column are significantly different ($p < 0.05$).

collagen has water-binding capacity, water absorption capacity, and water holding capacity, which can affect the moisture content of a product. Water absorption capacity is defined as the amount of moisture absorbed by dry matter at a specific humidity. The ability of collagen to absorb water is likely due to its higher hydrophilic content. The higher water holding capacity is due to peptides with a lower molecular weight with higher hydrophilic properties than larger peptides.

Darmanto *et al.* (2017) added that different types of fish bones could also cause collagen's chemical content to be added. The results showed that collagen fortification from red snapper fish bones produced analog rice with higher moisture content (7.36%) than collagen fortification from stingray (4.86%), milkfish (5.29%), and catfish (6.87%) bones. Sukamto and Patria (2020) stated that the extrusion temperature ingredients influence the moisture content in analog rice, and the drying process is carried out after the extrusion process.

3.1.2 Ash content

The ash content of analog rice ranged from 2.4 to 3.07% (Table 1). Collagen fortification from fish scales causes an increase in the ash content of analog rice. It is probably due to mineral residues from fish scales on collagen (Lopez *et al.*, 2018). According to Sockalingam *et al.* (2015), soaking fish scales in HCl solution was able to reduce most of the phosphorus and calcium, which are the constituent elements of fish scales.

The highest ash content was found in analog rice which was treated with the fortification of tilapia scales collagen (3.07%) ($p < 0.05$). However, the results of this study are lower than the results of previous studies by Darmanto *et al.* (2017), the ash content of analog rice ranged from 3.47 to 9.80%. This is because the mineral content in each collagen raw material is different, thus affecting the ash content of collagen and analog rice. Meanwhile, the ash content in analog rice control was caused by the mineral content in seaweed flour. However, the results of this study are slightly lower than the ash content of analog rice made from sago flour and red bean flour, which is 2.54% to 3.69% (Wahjuningsih *et al.*, 2020). The ash content in analog rice indicates the presence of minerals. The higher of ash content, the

greater of minerals in analog rice. The amount of minerals contained in the analog rice can be affected by the type of material used.

3.1.3 Protein content

Table 1 shows that the protein content of analog rice in this study is 8.45 to 13.54%, which was higher than milled rice, at 6.81 g/100 g (USDA, 2015). The results of this study were also higher than previous studies by Darmanto *et al.* (2017), whereby the protein content of analog rice ranged from 3.41 to 6.42%. The protein content of analog rice is highly dependent on the raw materials. For example, the fortification of collagen can increase the protein content of analog rice. However, differences in the types of raw materials used in collagen production also cause differences in the protein content of analog rice.

Analog rice with collagen fortification from red snapper scales had the highest protein in this research ($p < 0.05$). The presence of protein in analog rice can also reduce the glycemic response because protein can prolong the flow rate of food in the stomach so that the absorption and digestion rate in the small intestine becomes slower (Alsaffar, 2011). Therefore, this condition will indirectly be an advantage of analog rice as a functional food.

3.1.4 Lipid content

Analog rice produced in this study contained 0.61 to 0.85% of lipid (Table 1). This lipid content was higher than the lipid content of the milled rice, which was 0.55 g/100 g (USDA, 2015). Sukamto and Patria (2020) explained that the lipid contained in analog rice could be caused by adding oil to the dough before extrusion. The use of oil in the analog rice production process can improve the smoothness of analog rice's surface.

The results also showed that the fortification of collagen was able to increase the lipid content of analog rice, whereas the fortification of collagen from red snapper scales caused a higher lipid content compared to other analog rice. Furthermore, the high lipid content in analog rice has a positive correlation with protein content. According to Barzideh *et al.* (2014), collagen

has an oil absorption capacity. The difference in oil absorption capacity in each type of material can be caused by the presence of polypeptides with different molecular masses and the presence of polar and non-polar side groups that can bind hydrocarbon side chains to oil (Sridaran *et al.*, 2012). Similar results were also shown by Darmanto *et al.* (2017) that collagen from various types of fish bones causes an increased lipid content in analog rice.

3.1.5 Carbohydrate content

Analog rice in this study had a carbohydrate content of 69.06 to 76.55% (Table 1). Thus, the carbohydrate content of analog rice in this study was relatively high. This was influenced by the type of flour used as a carbohydrate source (Nugraheni *et al.*, 2020). However, the carbohydrate content in this study was lower than the carbohydrate content in rice, which was 77.69% (Pudjihastuti *et al.*, 2018).

Based on Table 1, it can be seen that collagen fortification gave a significant difference in the carbohydrate content of analog rice ($p < 0.05$). This is presumably because carbohydrate content was calculated based on carbohydrate by contrast. Thus, carbohydrate content was strongly influenced by moisture, ash, protein, and lipid content. Therefore, with the increase in nutrient content, the carbohydrate content will decrease.

3.2 Amino acid

The results showed that collagen was able to increase the amount of each amino acid in analog rice (Table 2). The amino acid profile is known to be dominated by glycine. The highest value of glycine was shown by analog rice with the fortification of goldband goatfish scales collagen in 18,270.19 mg/kg. The lowest was at analog rice without the fortification of fish scales collagen (control) of 4,572.68 mg/kg. Then the highest value of proline was also shown by analog rice with the fortification of tilapia scales collagen that is equal to 10,179.02 mg/kg, while the lowest was at analog rice without the fortification of fish scales collagen (control) of 3,046.67 mg/kg. Thus, collagen consists of amino acids with a repetitive Gly-X-Y, each of which is glycine, proline, and hydroxyproline (Wu *et al.*, 2019).

Based on Table 2, it can be seen that arginine is a type of essential amino acid that is widely contained in analog rice as a result of this study. Newsholme *et al.* (2010) explained that arginine, leucine, and glutamine play a role in decreasing glucose content in the blood by affecting insulin secretion from beta cells of pancreatic. Meanwhile, the ratio between arginine and lysine can affect cholesterol content in the blood. The higher the arginine to lysine ratio, the higher the ability to decrease cholesterol content (Damasceno *et al.*, 2001). Leucine effectively reduces atherosclerosis by reducing systemic inflammation and plasma lipid profile (Zhao *et al.*, 2016).

Table 2. Amino acid analysis of analog rice

| Amino Acid | Analog Rice (mg/kg) | | | | |
|---|---------------------|-----------|----------|-------------|-------------------|
| | Control | Tilapia | Milkfish | Red Snapper | Goldband Goatfish |
| Essential amino acids (EAA) | | | | | |
| L-Arginine | 6379.86 | 10639.25 | 8682.89 | 8116.51 | 10988.23 |
| L-Leucine | 4068.64 | 5441.41 | 4417.95 | 5003.73 | 5540.97 |
| L-Phenylalanine | 3880.35 | 5514.89 | 4238.79 | 3616.15 | 4947.17 |
| L-Threonine | 3009.09 | 4798.08 | 3586.10 | 3916.86 | 4794.24 |
| L-Valine | 2854.65 | 4105.01 | 3274.86 | 3716.83 | 4075.64 |
| L-Isoleucine | 2063.13 | 2807.57 | 2390.91 | 2437.57 | 2729.68 |
| L-Lysine | 1633.55 | 3992.14 | 2964.50 | 4461.08 | 3601.17 |
| L-Histidine | 1223.68 | 1457.16 | 1099.66 | 1101.51 | 1418.96 |
| Total EAA | 25112.95 | 38755.51 | 30655.66 | 32370.24 | 38096.06 |
| Non-essential amino acids (NEAA) | | | | | |
| L-Glutamic Acid | 6711.82 | 13550.81 | 9825.99 | 13527.32 | 11026.84 |
| L-Aspartic Acid | 5685.13 | 9855.17 | 7012.23 | 9610.02 | 7464.62 |
| L-Glycine | 4572.68 | 18163.73 | 14036.27 | 15220.80 | 18270.19 |
| L-Alanine | 3169.91 | 9160.29 | 6626.43 | 9674.08 | 8760.27 |
| L-Serine | 3570.91 | 5674.02 | 4616.16 | 4603.00 | 5705.67 |
| L-Proline | 3046.67 | 10179.02 | 7942.12 | 9006.91 | 9316.87 |
| L-Tyrosine | 1603.49 | 1749.30 | 1377.08 | 1173.34 | 1709.33 |
| Total NEAA | 28360.61 | 68332.34 | 51436.28 | 62815.47 | 62253.79 |
| Total Amino Acids | 53473.56 | 107087.85 | 82091.94 | 95185.71 | 100349.85 |

From functioning for health, several types of amino acids can also affect the characteristics of a food product. For example, proline contained in collagen plays a role in gel formation with hydroxyproline. The higher proline in a food, the stringer gel formed (Gomez-Guillen *et al.*, 2011). Amino acids could also affect the flavour of a food product. For example, glycine and alanine provide a sweet taste, while glutamic acid and aspartic acid cause an umami taste (Kong *et al.*, 2012).

3.3 Crude fibre and dietary fibre

Table 3 shows that analog rice with the fortification of collagen from various types of fish scales did not give a significant difference between crude fibre and dietary fibre ($p>0.05$). This is because the seaweed flour (*Gracilaria verrucosa*) used in the production of analog rice has the same amount in each treatment. In fortification, collagen does not contain crude fibre and dietary fibre. Madhu *et al.* (2017) reported that seaweed contains cellulose, hemicellulose, pectin, and lignin which are crude fibres that cannot be digested by enzymes.

The crude fibre content of analog rice was relatively high (at 22.4 to 23.90%), while the natural fibre content of the milled rice was about 0.12% (Sukamto and Patria, 2020). Meanwhile, the dietary fibre content of analog rice was about 1.67 to 1.73%. The Codex Alimentarius (2009) recommended that food can be declared a source of fibre if it contains dietary fibre of at least 3% per serving or 10% of the daily reference value per serving and to be high fibre if it has more than 6% of dietary fibre (Foschia *et al.*, 2013).

The dietary fibre cannot be digested, and it will be fermented slowly by the colon's microflora. This phenomenon is called resistant starch. The slow digestion process can slow down glucose absorption in the blood to control the glycemic response and provide prolonged satiety (Ashraf *et al.*, 2012). Sources of resistant starch in analog rice in this study can be derived from the arrowroot flour and seaweed flour used.

Additionally, the amount of fibre in the food can affect the glycemic response. The higher the fibre content in the food, the lower of glycemic response.

Soluble dietary fibre can also slow down the digestive process in the intestines, provide prolonged satiety, and inhibit the appearance of glucose in the blood. Thus, it can maintain blood sugar stability (Alsaffar, 2011). Capuano (2017) added that dietary fibre could also inhibit lipid absorption in the gastrointestinal tract. Therefore, rice that contains high nutritional fibre has a low glycemic index and can reduce the glycemic response. Thus, analog rice with high fibre content can be a functional food alternative for people with diabetes and hypercholesterolemia.

3.4 Cooking time

Cooking time is the time needed to cook analog rice until it is consumable. The cooking time of the analog rice in this study ranged from 12.52 to 16.51 mins (Table 3). Analog rice from this study has a relatively long cooking time than rice noodles produced by extrusion technology, between 5 to 9 mins (Yogeshwari *et al.*, 2018). The cooking time of analog rice was influenced by gelatinization temperature whereby the higher gelatinization was influenced by the chemical components of flour (Sukamto and Patria, 2020). As amylopectin has less ability to bind water than amylose, any rice with higher amylose content is more difficult to gelatinize (Sharma *et al.*, 2008).

Based on Table 3, it can be seen that the fortification of collagen from different types of fish scale caused significantly different cooking times ($p<0.05$). Furthermore, it had a longer cooking time than analog rice without the fortification of fish scales collagen (control) caused by the difference in protein content in each analog rice. Those with high protein content have a relatively long cooking time. According to Sumardiono *et al.* (2020), the increase in cooking time is caused by the concentration of added protein, thus the time needed to reach the degree of gelatinization increases. The addition of protein tends to affect the peak temperature of gelatinization, therefore, the attainment of the gelatinization phase will be longer. This is because the presence of protein will prevent the entry of water into the starch granules when water is meant to make gelatinized starch, thus, requiring a longer gelatinization time.

Table 3. Crude fibre, dietary fibre, and cooking time analysis of analog rice

| Treatment | Crude fibre (%) | Dietary fibre (%) | Cooking time (min) |
|-------------------|-------------------------|------------------------|-------------------------|
| Control | 22.40±0.02 ^a | 1.67±0.08 ^a | 12.52±0.03 ^a |
| Tilapia | 23.90±0.04 ^a | 1.72±0.04 ^a | 16.51±0.06 ^d |
| Milkfish | 22.82±0.08 ^a | 1.69±0.06 ^a | 15.09±0.02 ^c |
| Red Snapper | 23.04±0.02 ^a | 1.70±0.03 ^a | 16.31±0.03 ^d |
| Goldband Goatfish | 22.76±0.06 ^a | 1.73±0.04 ^a | 13.47±0.08 ^b |

Values are presented as mean±SD. Values with different superscript within the same column are significantly different ($p<0.05$).

3.5 Hedonic evaluation

Based on the hedonic evaluation results (Table 4), it was known that the fortification of collagen from different fish scales did not affect the panellists preference for the colour, taste, and appearance parameters for the cooked analog rice produced from arrowroot and seaweed flour ($p>0.05$). This is presumably due to the concentration of raw materials and additives from four treatments having the same amount. The taste of a food can come from the ingredients nature or because other substances are added during production processing. The utilization of arrowroot flour dominates the comparison of composition in analog rice production compared to seaweed flour and fish scales collagen. Analog rice in this study has the same colour, yellowish-white. According to Sukamto and Patria (2020), colour and taste are essential attributes that determine food acceptance by consumers that are consequently influenced by the ingredients. Good analog rice has the most similar appearance to milled rice.

Based on the hedonic evaluation results of the aroma parameters, it can be seen that collagen fortification increased the panellist's preference for analog rice. A distinctive aroma is produced by analog rice during the steaming process and this aroma lasts as long as the rice is still hot. The specific aroma of analog rice can be attributed to the fishy compounds contained in seaweed and collagen. Cho *et al.* (2015) reported that seaweed and fish contain the compound 6,10-dimethyl-5,9-undecid-2-one, and geranyl acetone gives the fish a specific aroma. Collagen produced from tilapia scales contains off-odour compounds, such as methional which is similar to the odour of baked potato, dimethyl trisulfide, and dimethyl tetrasulfide, which gives off sulfurous odours. Degradation of methionine pada tilapia scales results in the presence of methional in the resulting collagen.

The amino acids contained in fish scale collagen also affect the aroma of analog rice. Gunlu and Gunlu (2014) reported that glycine, alanine, and glutamic acid can affect the taste and flavour of foods. These three types of amino acids can increase the sensory value of food, but

in excessive amounts, they can reduce the sensory value of food. Meanwhile, analog rice fortified with collagen from milkfish scales contained lower glycine, alanine, and glutamic acid than analog rice fortified with collagen from tilapia, red snapper, and goldband goatfish scales. Therefore, the analog rice fortified with collagen from milkfish scales has a more favourable aroma.

3.6 Morphology observation

The results of the morphology observation showed that analog rice without the fortification of collagen or control (Figure 1a) and analog rice with the fortification of collagen from milkfish scales (Figure 1c) there were more cavities on the surface of analog rice. Meanwhile, analog rice with the fortification of collagen from tilapia (Figure 1b), red snapper (Figure 1d), and goldband goatfish (Figure 1e) scales have a denser surface. The difference in the morphology of the analog rice particles in this study is closely related to the quality of the collagen used. It was evident that the absence and presence of collagen fortification from milkfish scales produced analog rice with lower protein content than analog rice with the fortification of collagen from tilapia, red snapper, and goldband goatfish scales. Mishra *et al.* (2012) explained that starch and protein can interact and undergo gelatinization during the heating process using an extruder machine in the production of analog rice. The interaction between starch and protein will produce

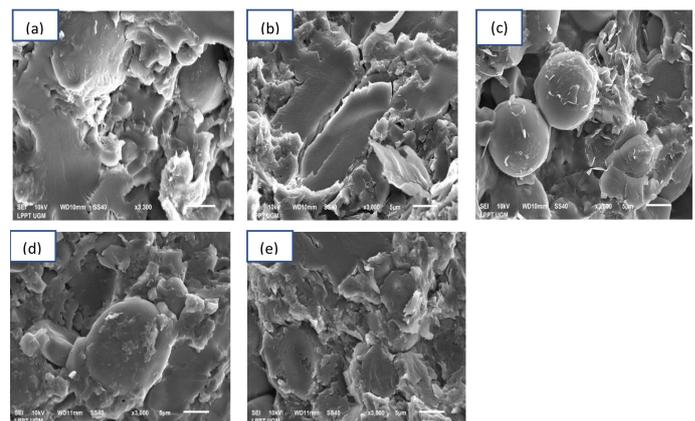


Figure 1. Morphology observation of analog rice: without collagen fortified (a), with fortified collagen from tilapia scales (b), milkfish scales (c), red snapper scales (d), and goldband goatfish scales (e)

Table 4. Hedonic evaluation of the analog rice cooked

| Treatment | Parameters | | | |
|-------------------|------------------------|-------------------------|------------------------|------------------------|
| | Colour | Aroma | Taste | Appearance |
| Control | 3.81±0.57 ^a | 2.94±0.39 ^a | 3.78±0.59 ^a | 3.45±0.49 ^a |
| Tilapia | 3.70±0.59 ^a | 2.96±0.41 ^a | 3.40±0.61 ^a | 3.51±0.46 ^a |
| Milkfish | 3.85±0.63 ^a | 3.45±0.41 ^b | 3.75±0.92 ^a | 3.57±0.17 ^a |
| Red Snapper | 3.81±0.33 ^a | 3.25±0.67 ^{ab} | 3.55±0.66 ^a | 3.55±0.28 ^a |
| Goldband Goatfish | 3.78±0.46 ^a | 3.01±0.39 ^{ab} | 3.84±0.71 ^a | 3.54±0.44 ^a |

Values are presented as mean±SD. Values with different superscript within the same column are significantly different ($p<0.05$).

a compact and denser particle.

The morphological differences in analog rice in this study can also be influenced by glycine and proline content in collagen, where analog rice without fortified collagen and with fortified collagen from milkfish scales has lower content than analog rice with fortified collagen from tilapia, red snapper, and goldband goatfish scales. As collagen has the ability to bind water and retain water in the gel matrix (Darmanto *et al.*, 2014), it is able to retain moisture in analog rice formulas. Meanwhile, water can act as a plasticizer for starch, reduce viscosity and mechanical energy, and inhibit the formation of bubbles during the gelatinization process. Bubbles formed can rupture due to heat and pressure during the extrusion process, resulting in cavities in the product after extrusion (Sukanto and Patria, 2020).

4. Conclusion

The research results showed that the fish scales collagen fortified have significant differences ($p < 0.05$) in nutrition, characteristic, and hedonic value of the analog rice based on arrowroot flour and seaweed flour. The fortification of collagen from various fish scales can improve the proximate, amino acid content, and cooking time of analog rice. However, collagen fortification was not significantly different ($p > 0.05$) from the hedonic value (except for aroma parameters), crude fibre, and dietary fibre content.

Conflict of interest

The authors state no conflict of interest.

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