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Chemical, physical, and sensory properties of various fish skin gelatin fortified taro flour - seaweed based analogue rice

[1]Darmanto, Y.S., [1]Putut, H.R., [2,*]Susanti, S. and [1]Fahmi, A.S.

[1]*Department of Fisheries, Faculty of Fisheries and Marine Sciences, Diponegoro University Semarang, Indonesia*
[2]*Food Technology, Department of Agriculture, Faculty of Animal Science and Agriculture, Diponegoro University Semarang, Indonesia*

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Abstract

Analogue rice is an artificial rice that can be made from non-rice materials such as sorghum, mocaf, and other additional materials. The advantage of analogue rice is not only because it is shaped like rice grains, but also because its nutritional composition can be designed using various raw materials to to enhance the desired functional properties. This study aimed to get more insight into the influence of various fish skin gelatin (FSG) fortification on the chemical and sensory properties of Taro (*Colocasia esculenta*)-Seaweed (*Eucheuma cottoni*) flour-based analogue rice (TSAR). TSAR was fortified with four species of fish's skin-derived gelatin (catfish, milkfish, snapper and stingray) and *Glyceryl monostearate* (GMS) as a control. The treatments in this study were five treatments of TSAR (each repeated four times). The results showed the addition of various FSG had significant effects towards the chemical properties of TSAR such as amino acid composition, proximate (moisture, protein, ash, fat and carbohydrate content), water isotherm sorption (mono and multi-layer water), time spent cooking and water absorption. In the sensory properties, although various FSG fortifications had no effect on colour and aroma, they had a significant effect on the appearance and taste of TSAR. Fortification of Snapper skin gelatin showed the best result both on the chemical and sensory properties of TSAR. Thus, the fortification of FSG ameliorated the quality of TSAR.

1. Introduction

Although rice is mostly an Indonesian staple food which is rich in carbohydrates and energy, some people with health-specified conditions need an alternative rice occasionally. Nowadays, analogue rice has been an alternative food substitute for rice to make it can be used as a diversified food product. Analogue rice is made in the industry by utilizing food ingredients that grow abundantly in Indonesia such as sago, sorghum and corn (Budijanto *et al.,* 2015). In terms of taste and cooking method, Analogue rice is not much different from ordinary rice, but the colour is not as white as rice. In addition, unlike ordinary rice, analogue rice is more durable and does not need to be washed when it is cooked.

Analogue rice was created to meet personal nutritional needs and can be adapted to the health conditions of consumers. The nutritional content and composition of the ingredients can be adjusted to the local raw materials of the region. Likewise, carbohydrate sources can be obtained from cassava flour, sweet potatoes, taro, arrowroot, canna and sago palm sugar (Hendrawan *et al.,* 2015). Sources of protein can be added from soy flour, kidney beans, or other types of beans and fibre can be obtained from bran (Salgado *et al.,* 2002). In the previous study, high fibre and protein analogue rice made from taro and seaweed flour which is fortified by fish bone collagen was produced (Darmanto, *et al*., 2017). Fortification with collagen-derived fish bones showed significantly increased protein content of taro-seaweed based analogue rice (TSAR).

As a result, the current study fortified various fish skin gelatin (FSG) to TSAR and then assessed the chemical, physical, and sensory properties. Gelatin is known as one of protein protein-derivative products which is contained in bone and skin (Tkaczewska *et al.,* 2018). Gelatin is a biopolymer (organic polymer) derived from collagen derived from cattle, pork and fish skin. Gelatin is produced from collagen through a long chemical and thermal treatment. About 98–99% of the

*Corresponding author.
Email: *sitisusanti@live.undip.ac.id*

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content in gelatin is protein or amino acids, such as glycine, while the rest is water and small amounts of vitamins and minerals. Unfortunately, as the main ingredient, skin evenly has become a by-product in the meat and fish business and it has been underutilized. Skin is better than bone due to the protein level of its yield (Rawdkuen *et al.,* 2013). Through gelatin fortification, TSAR can be expected to be a good quality functional product which not only rich in fibre but also in protein content.

2. Materials and methods

2.1 Extraction of fish skin gelatin

The processing of FSG followed the method described by Darmanto *et al.* (2010). Gelatin was extracted from catfish, milkfish, snapper and stingray fish skin. The skin of the fish was first cleaned in cold water. After that, the fish skin was deproteinized and demineralized by soaking it in 4% HCl for four days and changing it every 24 hrs. The fish skin was then dried for 48 hrs in a cabinet dryer. To manufacture gelatin from skin fish, the dried fish skin was ground and sieved.

2.2 Preparation of taro-seaweed-based analogue rice

The preparation of TSAR has followed the method described by Lekahena *et al.* (2014). Ball milling was used to grind taro flour, seaweed flour and gelatin into nano-sized particles. The analogue rice was prepared in the second stage with a mixture of 500 g Taro flour, 25 g gelatin from each fish skin, 10 g GMS, and 125 cc water extruded at 70°C (Mishra *et al*., 2012).

2.3 Analysis of chemical and physical properties of taro-seaweed-based analogue rice

2.3.1 Proximate analysis

Protein, fat, and ash contents were estimated by the AOAC (2005) method, while carbohydrate content was estimated by the difference method (Khan *et al.,* 2019). The moisture content was first determined using a dry oven at 105°C for 3 hrs. The protein concentration was determined using the Kjeldahl method, which includes three stages (destructive, distillation, and titration). The fat content was determined using the soxhlet method, which involved dissolving the samples in n-hexane solution for 24 hrs. The ash content of the samples was determined using the furnace method, which involved heating them to 550°C for 24 hrs. After all of the components (moisture, fat, protein, and ash) were calculated, the carbohydrate content was calculated using the difference technique, which is based on the total number of components (100%) plus moisture and protein, fat, crude fibre and ash.

2.3.2 Crude fibre

The method demonstrated by BSN was used to calculate crude fibre (1992). Fits, 2 g of sample was placed in a 500 mL Erlenmeyer flask, then 50 mL of 1.25% H_2SO_4 solution was added, and the mixture was heated for 30 mins. After the sample was heated, 50 mL of 3.25% NaOH was added and the mixture was simmered for 30 mins. The solution in the Erlenmeyer flask was then filtered using a Buchner funnel filled with weighed filter paper. As many as 1.25% H_2SO_4, hot water, and 96% ethanol were used to wash the precipitate on the filter paper. The filter paper and its contents were then placed in a crucible, dried in a 105°C furnace, and weighed until a consistent weight was reached.

2.3.3 Dietary fibre

An enzymatic approach was employed to determine the amount of dietary fibre, as described by Asp *et al.* (1992). The sample was initially extracted for 15 mins at room temperature with petroleum ether solvent. Then, 1 g of sample (W) was placed in an Erlenmeyer flask and dissolved in 25 mL of 0.1 M sodium phosphate pH6 buffer solution. This was followed by the addition of 0.1 mL of termamyl, covering it with aluminum foil and incubating it at 100°C for 15 mins, then lifting and cooling it before adding 20 mL of aquadest and adjusting the pH to 1.5 with 4 M HCl. The next stage was to add 100 mg of pepsin, incubate at 40°C for 60 mins while agitating, then add 20 ml of aquadest and adjust the pH to 6.8. The pH was set to 4.5 with HCl after adding 100 mg of pancreatin and incubating at 40°C and agitating for 60 mins. The sample was then filtered using a dry crucible that had been weighed, washed twice with 10 mL 95% ethanol, twice with 10 mL acetone, and 100 mL warm 95% ethanol, and precipitated for 1 hr. The precipitate was filtered through a dry crucible and washed twice in 78% ethanol and twice in acetone. The crucible was then dried for 12 hrs at 105°C and weighed (D), then placed in the muffle furnace for 5 hrs at 525°C and weighed again until a steady weight was obtained.

2.3.4 Amino acid composition

The content of amino acids in gelatins was measured using a Eurospher 100-5 C18 High Performance Liquid Chromatography system using a 4.6 × 250 mm Waters column. Each sample weighed less than 2.5 g and was homogeneously hydrolyzed with 15 mL 6 N HCl at 110℃. The hydrolysis was tested in an autoclave for 12 hrs at 110°C. After that, 6 N NaOH was added until the mixture was neutral. Using 50.0 mL distilled water, 2.5 mL of 40% lead acetate and 1 mL of 15% oxalic acid were added to the solution. The 3 mL solution was filtered with a millex 0.45 m filter before being injected

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with 30 mL HPLC, as described in the Waters-501 Instruction Manual (1991).

2.3.5 Water sorption isotherm

The water sorption isotherm was created by plotting the moisture content of myofibrils during dehydration against water activity. Each isotherm was divided into three portions, each delineated by M1 and M2 multilayers, as well as above M2 capillary. The Brunauer *et al*. (1968) method was used to determine the monolayer water (M1) (1938). Bull's approach was used to determine the Multilayer water (M2) (1944).

2.3.6 Cooking time

The cooking time of analogue rice was determined using the method used by Noviasari *et al.* (2013). In a rice cooker, analogue rice was cooked. The ratio of rice to water is one-to-one. The water is heated first. After the water has boiled, analogue rice is placed in the rice cooker. Cooking time is measured from the moment analogue rice is placed in the rice cooker until the time the rice is ready.

2.3.7 Water absorption

The water absorption of analogue rice was determined using the method developed by Yudanti *et al.* (2015). Water absorption is measured by weighing 25 g of analogue rice and soaking it in warm water (75°C) for 5 mins. Analogue rice was emptied after soaking. Analogue rice was weighed after it had been drained to see how much weight it had gained during the soaking procedure.

2.4 Statistical analysis

The chemical (proximate, crude fibre and dietary fibre) also physical (water sorption isotherm, cooking time and water absorption) were analyzed using parametric analysis of variance (ANOVA) test at a significance level of ≤0.05. The test was continued with the Duncan Multiple Range Test (DMRT) to find the mean value of the difference. Data analysis on the hedonic test used the non-parametric Kruskal Wallis test followed by the Mann-Whitney test. Meanwhile, the amino acid content was analyzed by descriptive test.

3. Results and discussion

3.1 Chemical characteristics of taro-seaweed-based analogue rice fortified fish skin gelatin

The addition of gelatin to analogue rice resulted in a significant ($p<0.05$) increase in protein and ash content, as shown in Table 1. However, the water and carbohydrate content of analogue rice was reduced, while the fat content remained unchanged. Gelatin, which contains numerous amino acids and minerals, contributes to the composition of analogueous rice, resulting in an increase in protein and ash content (Siburian *et al.,* 2020).

Because catfish (freshwater), milkfish (brackish water), snapper (seawater), and stingray (deep-sea water) have varied habitats, environments, and feeding habits, the proximate content of various forms of fish skin gelatin varies. Fish gelatin from Sin Croaker and Shortfin Scad has distinct proximates, according to Cheow *et al.* (2007).

The results showed that analogue rice without gelatin contained 3.41% of crude fibre, whereas analogue rice fortified by gelatin contained between 3.44–3.50% of crude fibre. The fortification of gelatin from various types of fish skin to analogue rice does not affect the amount of crude fibre content, because crude fibre is not dependent on the gelatin but depends on the seaweed and taro flour. According to Agusman *et al.* (2014), the higher the concentration of seaweed, the higher the percentage of crude fibre will be. Analogue rice fortified by *Gracillaria verucosa* seaweed and modified cassava flour has a crude fibre content between 3.35–4.65% (Spiraliga *et al.*, 2017). The results showed that analogue rice without gelatin contained 8.20% of dietary fibre, whereas analogue rice fortified by gelatin from various types of fish skin contained between 8.25–8.32% of

Table 1. Proximate analysis, crude, and dietary fibre of TSAR fortified FSG.

Parameters (%)	Various FSG				
	Control	Catfish	Milkfish	Snapper	String Ray
Protein	03.56±0.02[a]	07.98±0.01[e]	07.55±0.03[b]	07.91±0.11[d]	07.81±0.02[c]
Moisture	10.17±0.01[a]	06.81±0.02[e]	06.23±0.13[b]	08.11±0.01[d]	05.61±0.02[c]
Fat	01.28±0.03[a]	01.46±0.01[e]	01.25±0.07[b]	01.61±0.01[d]	01.19±0.01[c]
Ash	03.81±0.02[a]	07.83±0.01[e]	09.11±0.02[b]	07.91±0.02[d]	09.41±0.01[c]
Carbohydrate	82.02±0.01[a]	78.21±0.02[e]	80.47±0.11[b]	79.41±0.01[d]	80.41±0.01[c]
Crude Fibre	03.41±0.11[a]	03.44±0.02[b]	03.48±0.12[bc]	03.50±0.02[c]	03.46±0.03[d]
Dietary Fibre	08.20±0.02[a]	08.25±0.02[b]	08.30±0.02[c]	08.32±0.02[c]	08.29±0.02[c]

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$) between the treatment groups. Control means TSAR without FSG.

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dietary fibre. Based on the results of dietary fibre contained in analogue rice, it showed that gelatin does not affect the amount of dietary fibre. Analogue rice which contains more than 3% of dietary fibre, has qualified as a dietary fibre food source (Foschia *et al.,* 2013)

Table 2 shows that analogue rice fortified with gelatin from various fish skins can increase the content of amino acids. Gelatin from Snapper skin has a higher content of alanine, glycine and proline amino acids compared to milkfish, stingray, and catfish skin gelatine. Because each variety of fish skin gelatin has a different bonding tissue structure, energy for life activities, food habits, and environments, there are variances in the amino acids included in analogous rice. The gelatin from the skins of Red Tilapia, Walking Catfish, and Striped Catfish includes a variety of amino acids in varying levels (Jamilah *et al*., 2011). While amino acids from Giant Catfish and Calf Skin Gelatin have distinct types and amounts of amino acids. Gelatin plays an important role in increasing gel strength and viscosity and forming colloidal systems. Therefore, fortification of gelatin from various types of fish skin to analogue rice will increase the nutrients and increase the characteristics of analogue rice.

3.2 Physical characteristics of taro-seaweed-based analogue rice fortified fish skin gelatin

The quality of analogue rice is influenced by its hydration properties. Food products such as analogue rice have hydration properties that can be described by looking at the relationship between water content and water activity (a_w). Monolayer water content indicates the amount of water that can be bound to chemical groups of foodstuffs such as carbohydrates, proteins, and minerals through hydrogen bonds, ionic-dipole and inter-pole (dipole).

Water absorption isotherm is a graph of the relationship between moisture content and water activity (a_w) per g of material, which is limited by monolayer and multilayer water. Water bound in a processed material will affect how much moisture content and a_w of the material, so it will affect the taste and durability of food. The presence of water in a material will affect its texture, taste, colour changes, and damage. Bound water in a processed material will determine the value of moisture content and aw, so it will affect the taste and durability of food. Thus, as shown in Table 3, there was a link between a_w and moisture content of analogue rice supplemented with various fish skin gelatine.

Analogue rice enriched with fish skin gelatin was seen to have a lower water content value than analogue control rice (Table 3). Meanwhile, based on Table 3, the water content of monolayer and multilayer analogue rice enriched with fish skin gelatin appears to be larger than that of analogue control rice. The greater the a_w monolayer, the more multilayer water forms, resulting in a higher aw value.

The cooking time of analogue rice supplemented

Table 2. Amino acids profile of TSAR fortified FSG.

No	Amino Acid (ppm)	Various FSG				
		Control	Catfish	Milkfish	Snapper	String Ray
1	L-Histidine	1196.52	780.45	785.42	7261.22	640.18
2	L-Serine	3355.33	3652.24	3245.80	3456.76	3168.74
3	L-Arginine	2441.93	3816.17	3650.20	3702.18	3474.05
4	Glycine	2613.50	5021.60	5454.76	5496.20	5104.10
5	L-Aspartic Acid	4754.64	6245.16	6712.60	5679.18	6756.27
6	L-Glutamic Acid	7022.38	8664.27	10446.20	8906.10	9045.16
7	L-Threonine	2443.90	2465.65	2301.15	2469.78	2451.24
8	L-Alanine	3613.53	3786.26	3845.62	4058.06	4458.37
9	L-Proline	3179.61	3249.22	3675.18	4256.54	3672.65
10	L-Cysteine	216.27	176.24	< 156.25	< 167.40	< 170.46
11	L-Lysine	2170.76	2676.27	2974.05	2358.70	2840.72
12	L-Tyrosine	1730.37	1342.76	1240.53	1146.72	1205.62
13	L-Methionine	1043.70	8041.15	845.60	784.05	760.06
14	L-Valine	2721.52	2710.56	2647.70	2715.16	2760.56
15	L-Isoleucine	1841.94	1476.70	1746.54	1368.40	1648.67
16	L-Leucine	6617.22	3377.50	3646.62	3368.70	3564.76
17	L-Phenylalanine	2776.36	2579.40	2406.76	2680.78	2349.20
18	Tryptophane	477.25	660.20	704.86	670.12	694.18

Control means TSAR without FSG.

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with fish skin gelatin was longer than the cooking time of analogue rice without fish skin gelatin (Table 4). When compared to other treatments, analogue rice enriched with snapper fish skin gelatin had the largest water content, as well as monolayer and multilayer a_w. As a result, this analogue rice had the highest % water absorption (Table 4).

Table 3. Water sorption isotherm of TSAR fortified FSG.

Various FSG	Monolayer water		Multilayer water	
	Moisture	a_w	Moisture	a_w
Control	6.36	0.12	15.53	0.62
Catfish	9.52	0.13	18.70	0.62
Milkfish	11.16	0.16	18.19	0.63
Snapper	11.90	0.19	19.65	0.64
String Ray	11.28	0.18	18.90	0.64

a_w: Water activity of samples in some indicated moisture (g/100 g of sample). Control means TSAR without FSG.

Analogue rice's cooked time and water absorption are essential physical characteristics. Table 4 shows the results of analogue rice analysis reinforced with gelatin from several types of fish skin.

The results showed that the cooked time was directly proportional to the water absorption. Therefore, the longer it is cooked, the greater the %age of water absorption will be. The cooked time of analogue rice without fortification of gelatin was 14.33 mins, while the cooked time of analogue rice fortified by gelatin was between 15.17–15.29 mins. Furthermore, the water absorption of analogue rice without fortification of gelatin was 183.8%, whereas the water absorption of analogue rice fortified by gelatin was between 185.87–201.40%. When further examined, each type of gelatin (catfish, milkfish, snapper and stingray) spent 15 mins to cook, but the water absorption was between 185.87–201.40%. According to Noviasari *et al.* (2013), the cooked time of analogue rice is between 20 – 30 mins, while according to Agusman *et al*. (2014), the cooked time of analogue rice is 13 mins. The difference in cooked time is because of the analogue rice composition. Gelatin has a sufficient amino acid composition, seaweed contains many elements of minerals and tuber flour has carbohydrate content, thus causing differences in cooked time and water absorption of analogue rice.

Table 4. Cooking time and water absorption of TSAR fortifed FSG.

Various FSG	Cooking Time (Min)	Water Absorption (%)
Control	14.33±0.57^a	18.80±0.29^a
Catfish	15.17±0.57^b	200.40±0.24^c
Milkfish	15.20±0.15^b	185.87±0.07^b
Snapper	15.29±0.57^b	201.40±0.22^d
String Ray	15.27±0.58^b	199.72±0.08^b

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$) between the treatment groups. Control means TSAR without FSG.

The results showed that analogue rice without gelatin contained 3.41% crude fibre, whereas analogue rice fortified by gelatin contained between 3.44–3.50% crude fibre. The fortification of gelatin from various types of fish skin to analogue rice does not affect the amount of crude fibre content, because crude fibre is not dependent on the gelatin but depends on the seaweed and taro flour. According to Agusman *et al.* (2014), the higher the concentration of seaweed, the higher the percentage of crude fibre will be. Spiraliga *et al.* (2017) said that analogue rice fortified by *Gracillaria verucosa* seaweed and modified cassava flour has crude fibre content between 3.35–4.65%. The results showed that analogue rice without gelatin contained 8.20% of dietary fibre, whereas analogue rice fortified by gelatin from various types of fish skin contained between 8.25–8.32% of dietary fibre. Based on the results of dietary fibre contained in analogue rice, it showed that gelatin does not affect the amount of dietary fibre. According to Foschia *et al.* (2013) analogue rice which contained more than 3% of dietary fibre, has qualified as a dietary fibre food source.

3.3 Hedonic characteristics of taro-seaweed-based analogue rice fortified fish skin gelatin

The acceptance test employed was the hedonic test, which included colour, scent, appearance and taste. Table 5 shows the results of the hedonic test.

The hedonic test result for analogue rice colour was 3.40–3.45 with a somewhat brownish tint. Although the hue is slightly brownish, it is not yet preferred; however, analogue rice has a chance to become as popular as brown rice. According to Agusman *et al.* (2014), colour is one of the parameters for consumer acceptance. The colour changes occur when taro flour and gelatin are mixed followed by the heating process. According to Yuwono and Zulfiah (2015), the colour changes to slightly browned by the Maillard reaction between carbohydrates and proteins. Because the aroma of analogue rice is dominated by taro, while gelatin is neutral, there was no difference in the aroma of analogue rice, which was around 3.50–3.54. The particular aroma of analogue rice is caused by flour as its raw material, according to Korompis *et al.* (2016), but there is no absolute uniformity of view about the product's aroma.

The appearance value of gelatin fortified on analogue rice demonstrated that there is a difference in appearance value between each type of fish skin gelatin. This is due to the fact that each gelatin has a unique

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RESEARCH PAPER

combination of amino acids and minerals that make up the gelatin. Consumers prefer imitation rice with a solid texture, hardness, and lack of brittleness, according to Budi *et al*. (2013). Collagen and gelatin can help Myofibril protein-based products have more gel strength and viscosity (Darmanto *et al*., 2012, 2014; Darmanto *et al*., 2017).

Table 5. Hedonic of TSAR fortified FSG.

Treatment	Hedonic Attributes			
	Color	Aroma	Morphology	Taste
Catfish	3.45±0.06	3.51±0.01	3.54±0.01	3.20±0.07[a]
Milkfish	3.43±0.02	3.52±0.03	3.47±0.05	3.48±0.05[a]
Snapper	3.43±0.02	3.54±0.07	3.58±0.06	3.60±0.04[b]
String Ray	3.41±0.01	3.51±0.03	3.57±0.04	3.59±0.07[b]

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$) between the treatment groups. Control means TSAR without FSG.

4. Conclusion

Based on the results of this study, it is concluded that the fortification of gelatin from various types of fish skin on taro and seaweed flour-based analogue rice can improve its nutrients (higher in protein and ash content), and produce a better taste and durability. In general, the fortification of gelatin from Snapper fish skin on analogue rice had better results compared to other fish skin gelatin.

Conflict of interest

The authors declare no conflict of interest.

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RESEARCH PAPER

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