

Improvement of the nutritive quality of Sargassum powder through Aspergillus niger, Saccharomyces cerevisiae, and Lactobacillus spp. fermentations

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Abstract. This study aimed to evaluate the nutritional quality of *Sargassum* powder separately fermented by *Aspergillus niger, Lactobacillus* spp., and *Saccharomyces cerevisiae*. *Sargassum* sp. was collected from Sabutung Island, Pangkep Regency. The seaweed was washed using freshwater, spread on trays and sundried for 48 h until it reached a constant weight. The dried seaweeds were finely ground and passed through a fine meshed sieve to ensure homogeneity, and used as raw material for fermentation. The dried seaweed powder to seawater in the ratio of 1:9 (seaweed: seawater) was taken in the fermenter vessel. Each 10 mL of *A. niger, Lactobacillus* spp., and *S. cerevisiae* was inoculated at a concentration of 3.10 x 10⁸. Fermentation of *Sargassum* powder resulted in a significant increase in crude protein and a significant decrease in the levels of carbohydrate and crude lipid. With regards to anti nutritional factor, decreased total polyphenol, phytic acid, tannin and saponin were observed. Likewise, mineral contents such as Mg, Ca, and Fe increased after fermentation except Ca content of the *Lactobacillus* spp. fermented *Sargassum* powder. Fermentation with *A. niger* resulted in the highest crude protein, Mg, Ca, and the lowest carbohydrate and phytic acid content compared to other microbial types.

Key Words: microbial fermentation, seaweed, nutritional level, protein, anti-nutritional factor.

Introduction. Nutrition plays an important role to achieve an efficient aquaculture production as it brings great influences not only to the production costs but also to the fish health, growth and production of waste (Bhosale et al 2010). To produce nutritious and cost-effective diets, the specific nutritional requirement of a particular fish should be known before the formulation of any fish feed. However, in intensive aquaculture systems, commercial diets are quite expensive due to the inclusion of high priced fishmeal and fish oil, which are recognized as the best source of protein and lipid for most fish species (Davidson et al 2016). Fishmeal and fish oil are one of the most important ingredients for aquafeeds, however, both are a limited resource. Decreased availability and the increased price of the fish meal and fish oil has stimulated the search for sustainable alternatives for aquaculture feeds to replace fishmeal with readily available inexpensive plant sources.

A number of plant-derived proteins have been evaluated for partial or total replacement of fishmeal in diets for a number of different species, with the aim not only of testing the nutritional value of fish products but also of taking into consideration their eventual effects on fish health. Due to good performance and fish quality, the suitability of plant-derived protein has been shown for many carnivorous fish (Palmegiano et al 2007). Many plant-derived protein sources are fairly rich in protein and contain favorable essential amino acid profiles, but they are deficient in one or more essential amino acids (Soltan et al 2008), high fibre content, high anti-nutritional factors such as saponin, phytic acid, oxalate, tannin, etc. (Makkar & Becker 1999; Francis et al 2001), and these compounds interfere with food utilization and negatively affect fish growth. Furthermore, it is well recognized that high dietary level of plant proteins (> 40% of total protein) for

partial replacement of fish meal reduces feed efficiency and growth performances (Refstie et al 2000).

In freshwater omnivores, such as tilapia (*Oreochromis* sp.) and channel catfish (*Ictalurus punctatus*), the fish meal can be completely replaced by plant protein sources such as soybean meal (Lovell 1998). Due to the limited supplies and the high price of fishmeal, other alternative sources of protein must be considered. Plant proteins are generally cheaper per unit of nutrient than animal protein. Relatively, few plant protein sources have been used in fish feeds, because fish require high levels of dietary protein. Commercial aquaculture feeds for grow-out contain 25 to 45% crude protein. Thus, only high protein content plant feedstuffs, such as oilseed residues, are used in fish feed. The most commonly utilized by feed manufacturers are soybean meal, peanut meal, and cottonseed meal (Naylor et al 2009). Soy protein is considered the best plant protein source for meeting the essential amino acid requirements of tilapia and other fish species commercially grown. It is highly digestible by fish and the digestion coefficients are comparable or higher than fishmeal protein (Alceste & Jory 2000). Finding novel sustainable protein sources has become a major drive in the aquaculture sector in order to reduce dependency on fish meal as the main protein component in aqua feeds.

Seaweeds are a good source of proteins, vitamins, and minerals. Seaweeds with good protein level are receiving considerable attention as novel feeds with potential nutritional benefits. *Sargassum* is a genus of brown seaweed that is widely distributed throughout Indonesia waters (Kadi 2005). More than 1000 species of *Sargassum* have been found in tropical, subtropical and temperate regions (Mattio & Payri 2009). In Southeast Asia particularly Indonesia, 300 species of *Sargassum* have been reported (Chan et al 2013). Some types of *Sargassum* sp. contain significant amounts of fats, proteins, vitamins and minerals (Wong & Cheung 2001), although their content varies greatly depending on species, location, weather, and temperature (Sanchez-Machado et al 2004). The content of ash and mineral elements in seaweed *Sargassum* sp. is very potential compared to local vegetables so it can be a source of minerals, especially Ca, P, and Fe. In addition, *Sargassum* sp. contains higher levels of vitamin C and vitamin A than some vegetables (Handayani et al 2004). However, the presence of high crude fiber and low protein content are the main issue for the low inclusion of seaweeds in aquafeeds (Sanchez-Machado et al 2004).

Uchida & Murata (2002) claimed that fermentation is a simple and cheap method, which might considerably decrease crude fiber content and increase protein value. Fermentation of seaweeds with lactic acid bacteria and yeast enhance the nutritive value by enriching protein, vitamin, mineral, essential amino acids, essential fatty acids and also improves the digestibility of seaweed-based feeds. In order to further improve the use of seaweed incorporated diets, this experiment was conducted with the aim of evaluating the nutritional compounds of <code>Sargassum</code> sp. with the potentiality of producing nutritious fish feed using fermentation process involving <code>Aspergillus niger, Saccharomyces cerevisiae, and Lactobacillus spp.</code>

Material and Method

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Experimental design. This study used completely randomized design (RAL) with 4 treatments and 5 replicates. The treatments of the research are as follows:

A = fermentation of *Sargassum* powder with yeast *S. cerevisiae*;

B = fermentation of *Sargassum* powder with yeast *A. niger*;

C = fermentation of Sargassum powder with lactic acid bacteria Lactobacillus spp.;

D = unfermented *Sargassum* (control).

Preparation of Sargassum powder. The seaweed was collected from the near-shore waters of Saugi Island, Pangkep Regency from April to June 2017. It was thoroughly washed using freshwater, spread on trays, and sundried for 48 h until it reached a constant weight. Then, the dried seaweed was finely ground and passed through a fine-meshed sieve to ensure homogeneity (2 mm), and used as raw material for fermentation.

Fermentation. The fermentation process was performed following the method of Felix & Brindo (2014) with some modifications. The dried seaweed powder to seawater in the ratio of 1:9 (seaweed: seawater) was taken in the fermenter vessel. Each 10 mL of *A. niger, Lactobacillus* spp., and *S. cerevisiae* was inoculated at a concentration of 3.10 x 10^8 . The sugar substrate, dextrose was added at the rate of 5% w/v of the base material. The fermentation was carried out till the pH reached at 4.00. The fermented *Sargassum* was collected from the fermenter and dried in a hot air oven at 60° C for 2 days. The fermented *Sargassum* is then used for proximate analysis.

Proximate analysis. Proximate analysis of the experimental diets was performed following AOAC (2000) procedures as follows: moisture was determined after drying the samples in an oven at 105° C for 24 h. Crude protein was determined by micro-Kjeldahl digestion (N x 6.25) and distillation after acid digestion using a Kjeltec 1026 Distilling Unit together with a Tecator Digestion System (Tecator, Sweden). Ash was determined by incineration at 550° C for 12 h in a Muffle furnace to constant weight. Crude lipid was determined by Soxhlet extraction with diethyl ether at $40\text{-}60^{\circ}$ C for 7-8 h, while crude fibre content was determined as loss on ignition of dried lipid-free residues after digestion with 1.25% H₂SO₄ and 1.25% NaOH.

Anti-nutritional analysis. The anti-nutritional compounds such as phytic acid, tannins, total polyphenol, oxalates, and cyanogenic glycoside were measured in the present study. Phytic acid was determined using the procedure described by AOAC (2000), while tannins were determined by folin-denis spectrophotometric following the method of Pearson (1991). Total polyphenol was measured according to the Folin-Ciocalteau method (Slinkard & Singleton 1977), while ion chromatography method described by Holmes & Kennedy (2000) was used for oxalate determination.

Mineral analysis. The minerals were analysed from the solution obtained by first dryashing the raw and fermented samples as described by AOAC (2000). The mineral constituents such as Ca, K, Mg, and Na were digested with H_2SO_4 -Se and analysed using an atomic absorption spectrophotometer (Analyst-400), while Fe, Cu, Zn, and Cd were digested with H_2CIO_4 -HNO₃ 3:5 and analysed separately using an atomic absorption spectrophotometer (Analyst 400).

Data analysis. Data obtained were analysed using analysis of variance (ANOVA) at 5% test level, followed by Tukey HSD Test at 5% test level using IBM SPSS 21, while organoleptic test data was descriptively analysed.

Results and Discussion. Fermentation is a technique that can change complex compounds such as proteins, carbohydrates, lipids and other organic materials into simpler compounds. Fermentation results can lead to an improvement in the nutritional value of a product, which in turn is expected to alter the basic properties of a product such as improved digestibility and the preferred taste and flavor (Wu et al 2016). In the present study, fermentation of Sargassum powder using three types of microorganisms showed that fermentation with A. niger resulted in the highest protein content (p < 0.05) compared to other fermentation agents (Figure 1). Following fermentation, the protein of A. niger fermented Sargassum increased from 7.5 to 10.1%. This result was similar with the previous findings in other plant-based ingredients such as cassava (Azoulay et al 1980), sesame seed meal (Mukhopadhyay & Ray 1999), palm kernel meal (Ng & Chen 2002), and soybean meal (Mukherjee et al 2016; Yuan et al 2017). It is well established that protein-rich fish diet is more growth promoting (Kaushik 2000; Refstei et al 2001). Significant increases in protein contents of fermented *Sargassum* powder over the control (non-fermented) Sargassum powder are highly appealing to speculate the growthpromoting effects on the fish. Comparable results have been reported by Raimbault et al (1998), when they cultured cassava peels with Aspergillus sp. The authors reported several fold increase in the protein contents. Such protein enrichment has been reported for barley, wheat, cassava and dehydrated beet pulp following fermentation (Duru & Uma 2003).

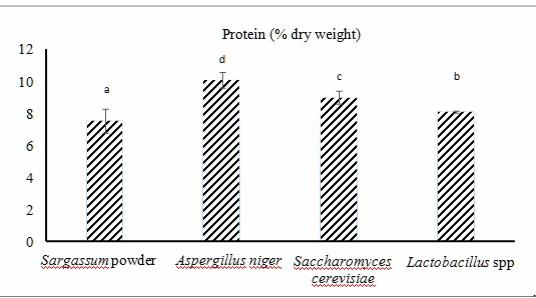


Figure 1. Protein content of *Sargassum* powder before and after fermentation.

S. cerevisiae fermented Sargassum powder experienced a significant reduction (p < 0.05) in lipid contents, in which the lipid decreased from 1.33% to 0.49% (Figure 2). However, lipid contents recorded in the present study were higher than those reported in Sargassum polycystum, Padina australis, and Turbinaria conoides (Santoso et al 2006), and in Sargassum sp. (Tamayo & Del Rosario 2014). A significant decrease in carbohydrate contents was also observed in A. niger fermented Sargassum powder from 50.7 to 29.1% (Figure 3). This result may be rationalised as these two compounds were the source of energy for maintenance and production of A. niger. The energy was released into the air as an energy loss. Kusumaningrum et al (2012) reported that the decrease of lipid content was due to rich of the glucose content of Sargassum that may induce growth of yeast biomass, resulting in increased production of lipase enzymes to overhaul rough lipids. Foods that contain lots of lipids are not good for fish health because it will more easily oxidize and produce unpleasant smells. Decreased carbohydrates contents probably have been reported on sorghum (Chavan et al 1988) and on pearl millet (Osman 2011). Decreased in carbohydrates content is probably due to the utilization by microorganisms. Starch represents a low cost and easily available feed ingredient. Thus, the percentage of a starch source is desired to be increased in fish However, the ability of certain fish species to hydrolyze (digest) complex carbohydrates is limited due to weak amylolytic activity in their digestive tracts. Starch digestion decreases as the proportion of dietary starch is increased (Hasan 2001; Chen et al 2006).

One of the major growth promoting factors in the fish diet is protein. Protein is required to maintain normal body functions, and the defficiency of this nutrient can affect the protein synthesis and lead to the reduction in weight and other symptoms to the fish, while carbohydrates are an excellent source of energy and carbon in feed formulations (Craig & Helfrich 2002). They can be easily distinguished from the other energy-yielding nutrients in terms of their abundance and low price. The dietary inclusion level and appropriate source of carbohydrate are decided based on a protein sparing without any adverse effect on growth and physiology of the fish. Decreased carbohydrate contents may be assumed a decrease in crude fibre from *Sargassum* powder, since crude fibre is part of unstable carbohydrates, which can be separated by a nitrogen-free extract (BETN) comprising mainly starch, by analysis simple chemistry (IOM 2001). Decreased crude fiber content is caused by the reshuffling of complex substances in *Sargassum* into simpler compounds performed by *A. niger*, as well as by other types of microorganisms, including *S. cerevisiae* and *Lactobacillus* spp.

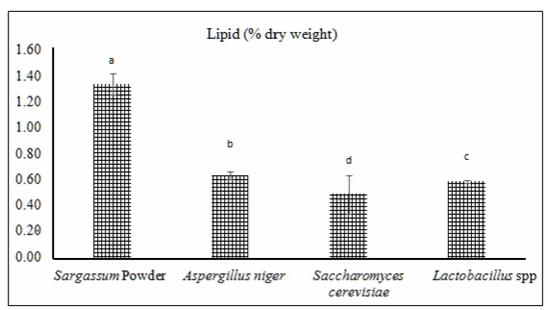


Figure 2. Lipid content of *Sargassum* powder before and after fermentation.

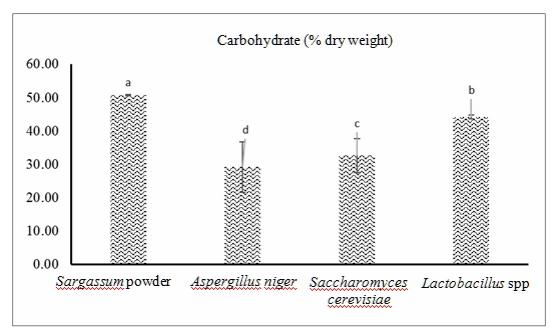


Figure 3. Carbohydrate content of Sargassum powder before and after fermentation.

The use of *Lactobacillus* spp. as a fermentation agent for *Sargassum* powder showed a distinct trend for moisture, in which *Lactobacillus* spp. significantly reduced moisture contents following fermentation (Figure 4). The main reasons are the lower moisture, reciprocal translocation of nutrients and organic matter loss by microbial fermentation. Ash contents of fermented and non-fermented *Sargassum* powder found in the level of 27-34% (Figure 5). The results were in consistent with the finding of Tamayo & Rosario (2014) in *Sargassum* sp. (27.11%) and Sanchez-Machado et al (2004) in *Laminaria ochroleuca* (29.47%). However, ash contents found in this study were much lower than that reported in *S. polycystum* (38%) (Matanjun et al 2009). Generally, the ash content of brown seaweeds is much higher than those of terrestrial vegetables other than spinach (Sanchez-Machado et al 2004). Increased ash contents of fermented *Sargassum* powder following fermentation were due to the elevated mineral availability of the substrate. The ash content of the *Sargassum* powder often correlates with the amount of mineral content in the *Sargassum* powder (Dani et al 2005). Ash content is the indication of the amount of mineral elements present in the bioprotein produced after the fermentation

process. This increase may be also due to contribution by fermentation microorganisms in the breakdown of the organic components of the *Sargassum* powder during fermentation (Oseni & Ekperigin 2007). According to Craig & Helfrich (2002), the minimum amount of ash content required for fish is about 8.5%. Hence, the ash contents found in all fermented *Sargassum* powder were above the minimum requirement for fish.

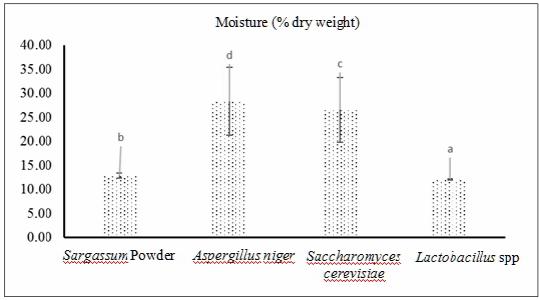


Figure 4. Moisture content of *Sargassum* powder before and after fermentation.

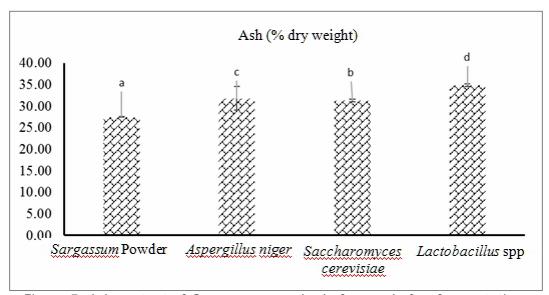


Figure 5. Ash content of *Sargassum* powder before and after fermentation.

The contents of different anti-nutritional factors concentrated in fermented and nonfermented Sargassum powder are shown in Table 1. Among different anti-nutritional factors, phytic acid occurred within the range of 17.88-22.35 mg g⁻¹. Sargassum powder fermented with A. niger had lowest amounts of phytic acid (17.88 mg g⁻¹) followed by those fermented with S. cerevisiae (19.49 mg g⁻¹), while the highest was observed in non-fermented Sargassum powder (22.35 mg g⁻¹). Significant differences (p < 0.05) in tannin contents were recorded among fermented and non-fermented Sargassum powder. Tannin content (mg g⁻¹) of all Sargassum powder ranged from 0.65 to 0.90 mg g⁻¹, being minimum for Sargassum powder fermented with Lactobacillus spp (0.65 mg g⁻¹), followed by those fermented with A. niger (0.66 mg g⁻¹).

Table 1 Anti-nutritional contents of fermented and non-fermented sargassum powder

Types of Sargassum powder	Phytic acid (mg g ⁻¹)	Total polyphenols (mg g ⁻¹)	Tannin (mg g⁻¹)	Oxalates (mg g ⁻¹)	Saponin (mg g ⁻¹)
Non fermented	22.35 ± 0.39^{a}	0.90 ± 0.06^{a}	5.50 ± 0.24^{a}	0.16 ± 0.01^{a}	1.52 ± 0.05^{a}
Sargassum powder					
Sargassum powder	17.88 ± 0.38^{d}	0.66 ± 0.04^{c}	3.24 ± 0.43^{b}	0.16 ± 0.01^{b}	1.22 ± 0.03^{b}
fermented with A. niger	_				
Sargassum powder	19.49±0.27 ^c	0.70 ± 0.05^{c}	3.20 ± 0.36^{c}	0.16 ± 0.01^{b}	1.22±0.03 ^b
fermented with					
S. cerevisiae	L.	L.	_	I.	
Sargassum powder	19.83±0.45 ^b	0.65 ± 0.06^{b}	3.20 ± 0.43^{c}	0.13 ± 0.01^{b}	1.21 ± 0.04^{c}
fermented with					
<i>Lactobacillus</i> spp.					

Data are expressed as mean \pm SE, standard error; values with same superscripts indicate no significant difference (p > 0.05).

Decreased phytic acid contents as result of microbial fermentation were observed in the present study. However, different fermentation agent resulted in different levels of phytic acid, in which A. niger fermented Sargassum powder had lowest levels of phytic acid. Reduced level of phytic acid may give benefits because phytate can also inhibit digestibility by chelating with calcium binding with the substrate or proteolytic enzyme. Limited studies are available regarding the phytate content of Sargassum. The variations in phytate content may be attributed to differences in milling extraction rate, genotype, and environmental effects. Likewise, significant differences in the tannin content were seen among fermented Sargassum powder. The reduction of phytic acid and tannin may be as a result of an increase in the production of alpha-galactosidase by the microorganisms used as starter culture during fermentation. Rodríguez Couto (2008) and Schons et al (2012) reported that fermentation and enzyme and combination of fermentation-enzyme treatments were effective in diminishing tannin and phytate in sorghum flour as observed by Kayode et al (2007) who attribute the reduction in phytate by lactic acid bacteria and yeasts because of their ability to produce enzymes during fermentation. Fermentation with A. niger was more effective to eliminate phytic acid, resulting in a protein source for feed with highly available P (Ilyas et al 1995) as well as Zn (Hirabayashi et al 1998).

The study revealed that total polyphenol contents were significantly lower in fermented *Sargassum* powder than in non-fermented *Sargassum* powder. Total polyphenol contents calculated as catechin in grains of fermented *Sargassum* powder was 3.20 mg catechin/g d.m. while in non-fermented *Sargassum* powder it was 5.50 mg catechin/g d.m. However, total polyphenol contents found in this study were lower compared to *S. classifolium* (1.08 mg g⁻¹), *S. binderi* (1.14 mg g⁻¹), and *S. dublicatum* (1.82 mg g⁻¹) (Bambang et al 2013). Variation in total polyphenols might be due to the different election of reference standards used in each study. Besides, the nature of samples in each study might also affect the phenolic acid values.

Fermentation did not significantly change oxalates contents of *Sargassum* powder (0.16%). The oxalate contents of *Sargassum* powder were lower compared to other plant protein sources such as rice (1.76), maize (3.71), and wheat (5.36) (Olukemi et al 2016). The data suggested that fermentation using these three types of microbes did not have any positive effect on the reduction of oxalate content in *Sargassum* powder. Akindahunsi & Salawu (2005) described that oxalates, like phytates, bind minerals like Ca and Mg and interfere with their metabolism. The bioavailability of the essential nutrients in plant foods could be reduced by the presence in these plants of some antinutritional factors such as oxalates and cyanogenic glycosides. However, due to lower oxalate contents of *Sargassum* powder, its use as a source of protein for fish feed is still recommended in the present study.

Significant differences were observed in tannin contents of fermented and nonfermented Sargassum powder. Nevertheless, the tannin content obtained from this study is much lower than the results of several previous studies of several types of Sargassum such as S. muticum (Moorthi & Balasubramanian 2015), and S. ilicifolium (Waghmode & Kumbar 2015). A similar trend was also observed in saponin content, in which Lactobacillus spp. fermented Sargassum was found to be lowest (1.21 mg g^{-1}) compared with A. niger (1.22 mg g^{-1}) and S. cerevisiae (1.22 mg g^{-1}), while the highest saponin contents were found in non-fermented sargassum powder (1.52 mg g^{-1}).

Macromineral contents of fermented and non-fermented *Sargassum* powder are shown in Table 2. High Mg concentrations were found in *A. niger* and *S. cerevisiae* fermented *Sargassum* powder. Likewise, high Ca content (p < 0.05) was also observed in fermented *A. niger Sargassum* powder, while no differences of Na and K contents were observed in all types of *Sargassum* powder. Significant effects of fermentation (p < 0.05) were also recorded in Fe content, however, no differences were recorded in Zn, Cu and Cd contents (Table 3).

The macromineral contents of fermented *Sargassum* powder were higher than those found in *S. wightii* (Kannan 2014). This may be due to leaching of soluble minerals into the processing water during the period of the fermentation. In addition, fermenting microorganisms might have used it for metabolic activities as reported by Osman (2011). Thus, Ca and Fe contents of fermenting *Sargassum* powder could be an indication that certain organisms utilize them for their growth and metabolism (Hasannia et al 2006). Minerals in many plant-based feed ingredients may change their chemical form during and after processing such as fermentation, or interaction with other compounds. Hence, the solubilities of the mineral can increase or decrease depending on the type of processing method. However, due to the low protein content of *Sargassum* powder, the interaction between minerals and proteins may not occur (Santoso et al 2006).

Table 2 Macromineral contents of fermented and non-fermented *Sargassum* powder

Types of Sargassum powder	Mg	Ca	Na	К
Non-fermented Sargassum	58.57 ± 0.65^{a}	17.71 ± 0.34^{a}	9.47 ± 0.27^{a}	16.81 ± 0.26^{a}
powder				
Fermented Sargassum	61.14±0.59 ^b	18.83 ± 0.38^{b}	9.44 ± 0.28^{a}	16.87 ± 0.33^a
powder with <i>A. niger</i>				
Fermented Sargassum	60.57 ± 0.43^{b}	18.33 ± 0.26^{ab}	9.41 ± 0.30^{a}	16.71 ± 0.31^{a}
powder with S. cerevisiae				
Fermented Sargassum	60.00 ± 0.31^{ab}	17.07 ± 0.36^a	9.39 ± 0.25^{a}	16.71 ± 0.24^{a}
powder with Lactobacillus spp.				

Data are expressed as mean \pm SE, standard error; values with same superscripts indicate no significant difference (p > 0.05).

Table 3 Micromineral contents of fermented and non-fermented *Sargassum* powder

Types of Sargassum powder	Fe	Zn	Cu	Cd
Non-fermented Sargassum	0.49 ± 0.01^{a}	0.39 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
powder				
Fermented Sargassum	0.54 ± 0.01^{b}	0.39 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
powder with A. niger				
Fermented Sargassum	0.54 ± 0.02^{b}	0.39 ± 0.02	0.03 ± 0.01	0.02 ± 0.01
powder with S. cerevisiae				
Fermented Sargassum powder	0.54 ± 0.01^{b}	0.39 ± 0.02	0.03 ± 0.01	0.02 ± 0.01
with Lactobacillus spp.				

Data are expressed as mean \pm SE, standard error; values with same superscripts indicate no significant difference (p > 0.05).

Conclusions. This research suggested that microbial fermentation may increase the nutritional values of *Sargassum* powder by increasing protein and mineral contents and decreasing anti-nutritional contents. Therefore, microbial fermentation can make *Sargassum* a potential source of protein and contribute to maintain the availability of feed to support the sustainability of aquaculture.

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