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Mineral availability, dietary fiber contents, and short-chain fatty acid fermentation products of *Caulerpa lentillifera* and *Kappaphycus alvarezii* seaweeds

James David S. Alcantara[1*], Nancy Lazaro-Llanos[2],

[1] *Department of Science and Technology – Food and Nutrition Research Institute, General Santos Avenue, Bicutan, Taguig City, Philippines*
[2] *Chemistry Department, De La Salle University – Manila, 2401 Taft Avenue, Manila, Philippines*

*Author to whom correspondence should be addressed; email: jdalcantara19@gmail.com

ABSTRACT

Seaweeds like *Caulerpa lentillifera* and *Kappaphycus alvarezii* are used as main ingredients in Filipino delicacies. There is a need to investigate the mineral availability, dietary fiber contents, and short-chain fatty acid fermentation products of these Philippine seaweeds to assess its nutritional and health benefits. Results show that *C. lentillifera* contain higher levels of iron, zinc, and calcium (430.93 ± 1.46, 1.09 ± 0.19, and 988.44 ± 5.07 mg/100g, respectively) compared with *K. alvarezii* (11.34 ± 0.14, 0.29 ± 0.01, and 262.98 ± 2.83 mg/100g, respectively). The availability of calcium was shown to be higher in both seaweeds (94.10 - 96.45 %Ca) than iron and zinc availabilities. Both samples exhibited high amounts of dietary fibers. The soluble, insoluble, and total dietary fiber contents of *K. alvarezii* (16.73 ± 0.03, 42.24 ± 1.04, and 58.97 ± 1.01 g/100g, respectively) were higher than that of *C. lentillifera* (3.50 ± 0.41 g/100g for soluble, 27.17 ± 0.09 g/100g for insoluble, 30.67 ± 0.32 g/100g for total dietary fiber). *In vitro* fermentation using human fecal inoculum afforded short chain fatty acids (SCFA). The SCFA content analysis using HPLC showed that *K. alvarezii* produced propionate (35.53 ± 2.00 g/100g) and butyrate (3.19 ± 0.16 g/100g) SCFAs while *C. lentillifera* only produced propionate (15.92 ± 0.45 g/100g) SCFA suggesting the potential of these seaweeds for the prevention of some diseases.

Keywords: *Caulerpa lentillifera; Kappaphycus alvarezii; mineral availability; dietary fiber; short chain fatty acid*

INTRODUCTION

Philippine seaweeds are highly diversified among the flora of the Asia-Pacific regions. There are more than 800 species of seaweeds such as *Kappaphycus*, *Eucheuma*, *Gracilaria* spp. and *Caulerpa lentillifera*. There are also several species of seaweeds with economic importance such as *Sargassum*, *Poryphyra*, and *Halymenia* spp. Seaweed farming is now one of the emerging industries in the

Philippines particularly in the coastal areas of Southern Philippines. Seaweeds in the Philippines contributed for about 34% to the total fisheries production, with Regions IV-B, IX and ARMM as to the major producers. The increase in production can be attributed to high market demand, better price, and nice weather conditions that encourage the farmers to expand their areas for seaweed culture (Fisheries Commodity Roadmap, 2010). Considered as vital components of the marine ecosystem, seaweeds are living renewable resources with potential food and therapeutic applications (Kumar, et al., 2011). In the past decade, these marine algae have been widely consumed in Asian countries and have been utilized as sources of phycocolloids, thickening and gelling agents in the food industry (Ruperez and Calixto, 2001). As a valuable source of fiber, macrominerals, trace minerals, proteins, polyunsaturated fatty acids, and important bioactive compounds, seaweeds are therefore recommended as meal supplements to meet the daily intake of essential minerals and trace elements (Ortiz, et al., 2006). Seaweeds are also sought for their potential in controlling antimicrobial, antitumor, anticoagulant, and cytotoxic activities (Sabina et. al, 2005). Overall, seaweeds have gained recognition for the health benefits it provides to humans and animals (Fleurence, 1999).

Seaweeds contain large amounts of polysaccharides, most of which cannot be digested by humans without the required enzymes for degradation. These polysaccharides can therefore be regarded as excellent sources of dietary soluble fibers (Lahaye, 1991), which can lower the risk of cardiovascular diseases, obesity, and constipation (Baghurst, et al., 1998). Dietary fiber comes from the family of carbohydrates that cannot be digested in the small intestine but can be further fermented in the colon into short chain fatty acids (SCFA) such as acetate, propionate, and butyrate. SCFAs contribute 6.3–8.4 kJ/g (1.5–2.0 kcal/g) dietary fiber (Nutrition Recommendations, 1990), enhance water absorption in the colon, and can help prevent constipation. Propionate SCFAs have been shown to inhibit the activity of the enzyme β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) reductase, the limiting enzyme for cholesterol synthesis. Dietary fibers have the ability to bind with bile acids and prevent their reabsorption in the liver, inhibiting cholesterol synthesis (FNRI, 2000). Butyrate SCFAs have been shown to enhance cell differentiation preventing tumor formation in the colon (Bourquin et al., 1992). The viscous and fibrous nature of dietary fibers can control the release of glucose with time in the blood, thus helping in the proper control and management of diabetes mellitus and obesity.

Due to their marine habitat, seaweeds have higher concentrations of minerals, as well as higher contents of trace and ultra-trace elements that are essential for human nutrition compared with terrestrial vegetables (Bocanegra, et al., 2009). Therefore, they can be considered as alternative sources of minerals, trace, and ultra-trace elements. Calcium, for example, can accumulate in seaweeds at a higher level compared with terrestrial plants. Their calcium content may be as high as 7% of the dry weight and up to 25 to 34% in the chalky seaweed lithothamnion. Seaweed consumption may be beneficial to expectant mothers, adolescents, and elderly who are at risk of calcium deficiency (Burtin, 2003). However, their nutrient compositions vary depending on the maturity, species, habitats, and environmental conditions (Ito and Hori, 1989). A cursory survey of the literature showed no studies have been conducted on the mineral availability, dietary fiber contents and short chain fatty acid composition of *C. lentillifera* and *K. alvarezii* from the Philippines. This work reports the nutrient composition, *in vitro* mineral availability, dietary fiber, and short chain fatty acid fermentation products of *C. lentillifera* and *K. alvarezii*.

MATERIALS AND METHODS

Material Preparation

Caulerpa lentillifera and *Kappaphycus alvarezii* samples were purchased from the wet markets of Metro Manila on April 2016. The fresh samples were authenticated at the Botany Department of the

National Museum of the Philippines. The samples were washed with tap water, rinsed with distilled water, and stored in the freezer before subjected to lyophilization. After lyophilization, the samples were ground into fine powders using a household grinder then stored in airtight containers.

Figure 1. *Caulerpa lentillifera* (left) and *Kappaphycus alvarezii* (right)

Analytical Methods

The proximate analysis and the total, soluble, and insoluble dietary fiber contents of the seaweed samples were determined using the Association of Official Analytical Chemists methods (AOAC, 2012). The phytic acid content of the samples was determined using the AOAC method (AOAC, 1986) while the tannic acid content was also determined following the procedure described by Earn et. al. (1981).

In vitro mineral availability

The total mineral (Fe, Ca, and Zn) availability in the small intestine was measured by using the *in vitro* method involving pepsin-pancreatin-bile digestion (Trinidad et al. 1996). Freeze-dried seaweed samples were weighed in 250-mL Erlenmeyer flask. The samples were homogenized and the pH was adjusted to 2.0 using 6N HCl. Pepsin-HCl solution was added and the resulting solution was incubated for three hours at 37°C in a water bath shaker (100rpm). From the digested sample solution, twenty grams were aliquoted and 5mL of pancreatin-bile solution was added. The pH was adjusted to 7.5 using 0.5M KOH. Four times the volume of the KOH used was equivalent to the volume of 0.5M $NaHCO_3$ in 100mL dialysis solution. Another 20g of pepsin-digested sample solution was weighed in a dialysis bag plus 5mL pancreatin-bile solution. The dialysis bag was immersed in the bicarbonate solution. The mixture was incubated for a total of 12 hrs at the same condition as the pepsin digestion. The dialysates were collected every 3 hrs, replacing with 100mL of double deionized water. The dialysates were analyzed for mineral contents using AAS. Dialyzable mineral was read in an Atomic Absorption Spectrometer (Varian AA 240FS, Australia) and was used as a measure of mineral availability for absorption in the small intestine. This method simulated the amount of mineral released that can be potentially absorbed in the small intestine.

In vitro fermentation using human fecal inoculum

Dietary fiber from the seaweed samples were fermented *in vitro* using human fecal inoculum (McBurney and Thompson, 1987). Five replicates of one-gram freeze-dried samples of *K. alvarezii* and *C. lentillifera* were weighed in Wheaton bottles. Each bottle was added with 40mL of fermentation media containing buffer solution of NaH_2PO_4 and KH_2PO_4, 0.1mL of 0.1% resazurin solution, and 2mL of reducing solution (mixture of 1.25g of cysteine-HCl dissolved in

100mL of deionized water plus 1.25g of Na_2S and 50 pellets of KOH dissolved in 100mL of deionized water). The bottles were flushed with CO_2 gas using rubber tubing until the solution becomes colorless. The bottles were sealed with a rubber stopper and were crimped with aluminum caps and stored at 4°C overnight. The bottles were then placed in a water bath for 1-2 hours at 37°C. A 10-mL fecal inoculum (1:15 dilution of fresh feces from a human volunteer who hasn't taken any antibiotics for the past six months) was added into each bottle and the mixture incubated for 24 hours at 37°C. The fermented digest was filtered and read in a high-performance liquid chromatography (Shimadzu LC-10A, Shimadzu Corp., Tokyo, Japan) to measure short chain fatty acids against a volatile acid standard mixture of acetate, propionate, and butyrate (SUPELCO, Philadelphia, USA). No internal standards were used.

Statistical Analysis

The results were expressed as Mean ± Standard Deviation (SD). Statistical analysis was done using Student's T-Test to determine significant differences between treatments. Aside from the *in vitro* fermentation analysis, which used five replicates, all analyses were done in triplicate measurements.

RESULTS AND DISCUSSION

Proximate Composition of Caulerpa lentillifera and Kappaphycus alvarezii

The proximate composition of the samples is shown in Table 1 as dry basis. *C. lentillifera* showed lower moisture content (5.76 ± 0.06 g/100g) compared with *K. alvarezii* (7.17 ± 0.23 g/100g). The former showed higher ash content (26.57 ± 0.57 g/100g) than that of the latter (19.93 ± 0.71 g/100g). The results were in agreement with those reported in other species i.e., *Himanthalia elongate* (26.78 g/100g), *Laminaria ochroleuca* (29.47 g/100g), and *Poryphera* sp. (19.07 g/100g). Generally, the ash content of seaweeds is higher than that of terrestrial vegetables apart from spinach and other vegetables (Ratana-arporn and Chirapart, 2006). This might indicate that the total mineral content of seaweeds is higher than that of terrestrial plants. Both the samples showed low fat content (0.75 ± 0.04 g/100g and 0.84 ± 0.01) indicating that these seaweeds having less than 4% fat are not considered a good source of crude lipid (Herbetreau et al., 1997). The protein content of *C. lentillifera* was found to be higher (5.10 ± 0.05 g/100g) than that of *K. alvarezii* (4.11 ± 0.25 g/100g). The carbohydrate contents of both samples (*C. lentillifera*: 61.82 ± 0.64 g/100g; *K. alvarezii:* 67.97 ± 1.13 g/100g) are high, indicating that these seaweeds contain substantial amounts of dietary fibers and other types of carbohydrates.

Table 1. Proximate Composition (dry weight basis) of *Caulerpa lentillifera* and *Kappaphycus alvarezii*

Samples	Moisture g / 100g	Ash g / 100g	Fat g / 100g	Protein g / 100g	Total Carbohydrates g / 100g
C. lentillifera	5.76 ± 0.06^{b}	26.57 ± 0.57^{a}	0.75 ± 0.04^{b}	5.10 ± 0.05^{a}	61.82 ± 0.64^{b}
K. alvarezii	7.17 ± 0.23^{a}	19.93 ± 0.71^{b}	0.84 ± 0.01^{a}	4.11 ± 0.25^{b}	67.97 ± 1.13^{a}

[ab] Denotes significant differences between samples at $p < 0.05$.

Dietary fiber content of Caulerpa lentillifera and Kappaphycus alvarezii

The seaweed samples were found to contain soluble and insoluble dietary fibers (Figure 2). *K. alvarezii* was found to have higher soluble dietary fiber (16.73 ± 0.03 g/100g) than that of *C. lentillifera* (3.50 ± 0.41 g/100g) suggesting that *K. alvarezii* may play a role in the prevention, reduction, and treatment of some chronic diseases such as coronary heart diseases and cancer due to its high soluble dietary fiber content (Trinidad et al., 2006). The soluble dietary fiber content found in *K. alvarezii* also suggests that it can be further fermented to produce short chain fatty acids (Wong et al., 2006). The high insoluble fiber content in both samples also suggests that they are fibrous in nature. In terms of physiological functions, the high content of insoluble dietary fiber in both seaweed samples suggest potential use to relieve constipation and diverticular disease (Potty 1996), since seaweeds and insoluble dietary fiber have excellent water-holding capability (Slavin, 2013).

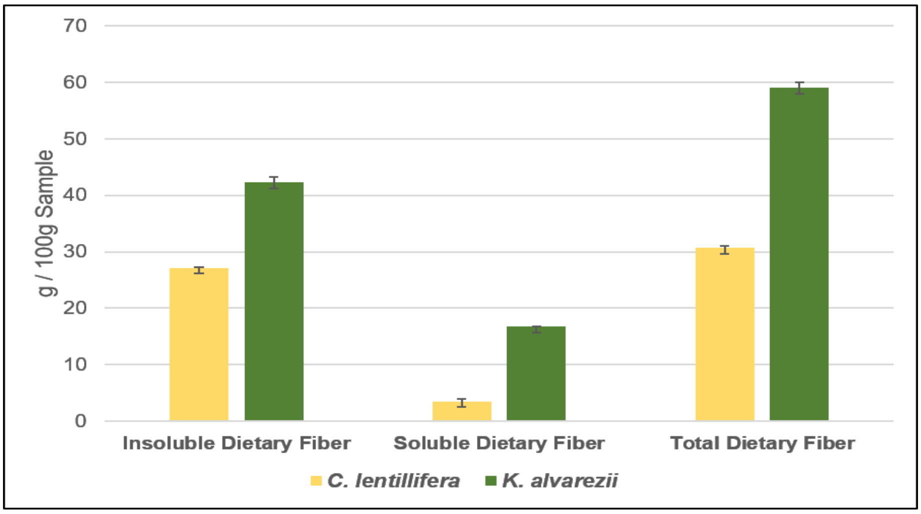

Figure 2. Dietary Fiber contents (dry weight basis) of *C. lentillifera* and *K. alvarezii*

In vitro fermentation of dietary fiber from Caulerpa lentillifera and Kappaphycus alvarezii

In vitro fermentation of human fecal inoculum has been used to analyze the rate and extent of fermentation (McBurney and Thompson, 1987). The method can be used to model the human colon, resulting to short chain fatty acid (SCFA) molar ratios similar to those seen through the *in vivo* fermentation method. It demonstrates that fecal materials, which are collected from individuals at different times of the year, provide a sufficiently uniform source of microorganisms. As a result, fermentation values such as fiber digestibility and gas or SCFA production can be compared between studies. The method also exhibits the differences in fermentability of different fiber sources. Pectin, for example, underwent fermentation in the shortest amount of time, followed by psyllium gum, tragacanth gum, and cellulose (McBurney and Thompson, 1987). The present study, however, does not consider it an objective to determine the rate and extent of fermentation of the different test foods. The researchers have only determined the SCFA produced after 24 hours of fermentation that mimics fermentation in the colon.

Due to their total dietary fiber contents, it was expected that the two samples would produce SCFAs (den Besten et al., 2013). After being subjected to *in vitro* fermentation, both samples produced propionate SCFAs (Table 2) but no acetate SCFAs were found. *K. alvarezii* produced significant amounts of propionate (35.53 ± 2.00 g/100g) than butyrate (3.19 ± 0.16 g/100g). The production of propionate suggests the potential of these seaweeds in preventing cardiovascular diseases. *C. lentillifera* did not produce as much SCFA compared with *K. alvarezii* because it does not contain enough soluble dietary fiber that can be further fermented in the colon. Most of its dietary fiber contents were insoluble dietary fibers, which are less fermentable due to its fibrous structure (Chen et al., 2019). There are certain types of fibers that are resistant to bacterial fermentation and can therefore cause reduced levels in SCFA content.

Table 2. Short Chain Fatty Acid content after fermentation (dry weight basis) from *Caulerpa lentillifera* and *Kappaphycus alvarezii*

Sample	Total SCFA (g / 100g)	Acetate SCFA (g / 100g)	Propionate SCFA (g / 100g)	Butyrate SCFA (g / 100g)
C. lentillifera	15.92 ± 0.45^{c}	N.D.	15.92 ± 0.45^{c}	N.D.
K. alvarezii	38.15 ± 0.36^{b}	N.D.	35.53 ± 2.00^{b}	3.19 ± 0.16^{a}

^{abc} Denote significant differences between samples at $p < 0.05$.
N.D. – Not Detected

The high levels of propionates found in both samples suggest protective potential for the risk of cardiovascular diseases and it also suggests being a potent inhibitor of the enzyme β -hydroxy- β -methylglutaryl-CoA (HMG-CoA) reductase, the limiting enzyme for cholesterol synthesis. Statin type of drugs is a strong HMG-CoA reductase inhibitor and has shown to reduce plasma cholesterol by up to 30% in hypercholesterolemia patients. Furthermore, this current work showed that only *K. alvarezii* produced butyrate SCFA (3.19±0.16 g/100g). The butyrate SCFA, a main end product of microbial fermentation of dietary fibers in the human intestine, plays an important role in the maintenance of intestinal homeostasis and overall health status. The effects exerted by butyrates are multiple and involve several distinct mechanisms of action. Its well-known epigenetic mechanism, through the inhibition of histone deacetylase, results in the regulation of gene expression and in the control of cell fate. At the intestinal level, butyrate SCFA exerts multiple effects such as the prevention and inhibition of colonic carcinogenesis, the improvement of inflammation epithelial defense barrier, and the modulation of visceral sensitivity and intestinal motility (Canani et al., 2011). Butyrate SCFA is also of particular interest because it is the preferred fuel of the colonic cells (Roediger, 1982) and is supposed to be involved in the protective effects of fiber against colon cancer (Kelly, 2015). The results shown in this current work suggest health benefits of consuming the seaweeds due to its capabilities to produce beneficial SCFAs.

Mineral content and mineral availability

Seaweeds contain a major constituent of 8 to 40% essential minerals and trace elements, both of which are considered vital for human nutrition (Ruperez & Calixto, 2002). Since seaweeds are valuable sources of minerals compared with terrestrial plants, the mineral content and *in vitro* mineral availability of *C. lentillifera* and *K. alvarezii* were evaluated through atomic absorption spectrophotometry. The mineral content (Table 3) and % mineral availability (Table 4) of the samples show that *C. lentillifera* contained high amounts of iron, zinc, and calcium (430.93±1.46, 1.09±0.19, and 988.44±5.07 mg/100g, respectively) compared with *K. alvarezii* (11.34±0.14, 0.29±0.01, and 262.98±2.83 mg/100g, respectively). Calcium was found to be the abundant mineral in both seaweeds. In terms of availability, this study show that *K. alvarezii* is found to have a higher iron availability (11.72±1.60%) under conditions in the small intestine compared with *C. lentillifera* (0.78±0.02%) despite the latter's higher iron content. The finding is attributed to the observed higher phytic and tannic acid contents (Figure 3) of *C. lentillifera* (247.98±1.42 mg/100g and 172.95±1.48 mg/100g, respectively) compared with *K. alvarezii* (43.13 ± 0.70 mg/100g and 105.22±2.08 mg/100g, respectively). The result is in conjunction with the findings of Gillooly et al. (1984), and Hurrell (2003), in which phytic and tannic acids were shown to be potent inhibitors of iron absorption in the small intestine. Out of the three minerals investigated calcium exhibited the highest amount in terms of content and % availability (988.44±5.07 mg/100g Ca, 96.45±2.32% availability for *C. lentillifera* and 262.98±2.83 mg/100g Ca, 94.10±2.53% availability for *K. alvarezii*). This may explain why the availability of iron and zinc are relatively low compared with calcium. This difference in availability can be explained by the mineral-mineral interaction, in which one mineral may dominate during its release for potential absorption. Calcium interacts with other minerals, and the simultaneous intake of calcium with iron can decrease iron absorption (Cook, 1990). Calcium interferes with iron absorption in the process of interaction that occurs in the lumen (Lynch, 1997). Results show that both seaweeds can supplement mineral absorption in the small intestine due to its availability.

Table 3. Total mineral content (dry weight basis) of *Caulerpa lentillifera* and *Kappaphycus alvarezii*

Sample	Iron content (mg / 100g)	Zinc (mg / 100g)	Calcium (mg / 100g)
C. lentillifera	430.93 ± 1.46[a]	1.09 ± 0.19[a]	988.44 ± 5.07[a]
K. alvarezii	11.34 ± 0.14[b]	0.29 ± 0.01[b]	262.98 ± 2.83[b]

[ab] Denotes significant differences between samples at $p < 0.05$.

Table 4. Percent mineral availability (dry weight basis) from *Caulerpa lentillifera* and *Kappaphycus alvarezii*

Sample	Iron (% availability)	Calcium (% availability)	Zinc (% availability)
C. lentillifera	0.78 ± 0.02[b]	94.10 ± 2.53[c]	15.61 ± 0.27[a]
K. alvarezii	11.72 ± 1.60[a]	96.45 ± 2.32[c]	9.53 ± 0.97[b]

[ab] Denote significant differences between samples at $p < 0.05$.
[c] Denotes insignificant difference between samples at $p < 0.05$.

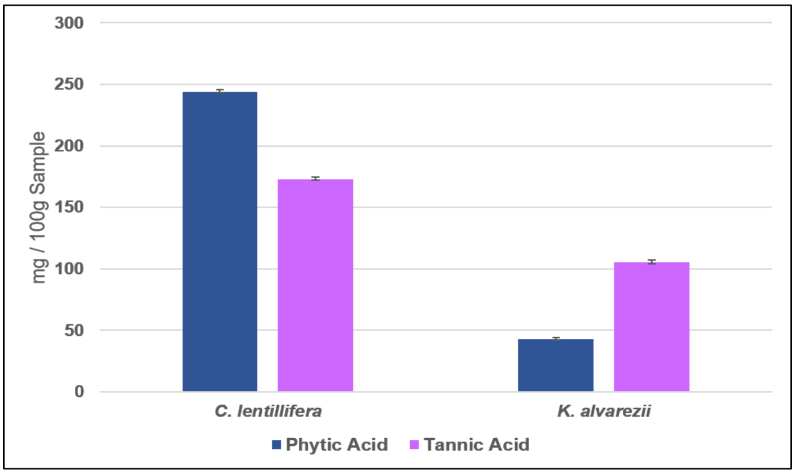

Figure 3. Phytic and Tannic Acid contents (dry weight basis) of *C. lentillifera* and *K. alvarezii*

CONCLUSION

The freeze-dried samples of *Caulerpa lentillifera* and *Kappaphycus alvarezii* contained low moisture, protein, and fat contents. On the other hand, they are found to be high in ash and total carbohydrate contents, as well as in minerals, dietary fiber, and short chain fatty acid (after *in vitro* fermentation). Out of the minerals investigated, calcium was found to have the highest content and % availability. Both seaweeds, which are fermentable in the colon, can also produce propionate SCFAs. In the case of *K. alvarezii*, it can produce both propionate and butyrate SFCAs. However, potential contaminants in the samples, especially heavy metals, must be investigated for safety assessment. Food development of the samples may also be conducted for the improvement of the mineral availability of iron and zinc. It is also recommended to analyze for the presence of other chelators as it might affect mineral availability. Further studies are needed to validate the physiological functions of dietary fiber and short chain fatty acid contents in the selected seaweed samples.

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