

Original Article

# Effect of cleaning by whirlpool washing machine on heavy metal content and external contamination in commercially grown edible red seaweed, *Gracilaria fisheri* \*

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Received: 1 January 2019; Revised: 10 February 2020; Accepted: 17 February 2020

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## Abstract

The agarophytic seaweed, *Gracilaria fisheri* is used as food and also for agar extraction. This study investigated the use of a novel cleaning method to improve the quality of seaweed products. Dried seaweed was sampled from seaweed farms in Pattani province, southern Thailand during the dry and wet seasons and from seaweed sold in souvenir shops in the area, to determine the level of contamination and its quality and appearance before and after the cleaning process. A whirlpool washing machine was used for cleaning. The concentrations of the heavy metals, Mn, Fe, Cr, Cd, Zn, Ni, Cu, and Pb were investigated in the seaweed, and in the seawater and sediment in which it was grown. The sensory and external qualities were evaluated by 30 testers. The results showed that the concentrations of heavy metals in the seaweed were similar to those in the seawater and that most of the external contamination in the seaweed during the dry and wet seasons, as well as in the purchased samples was from mollusks and epiphytes. Nine cycles of cleaning in the washing machine produced the lowest heavy metal concentrations and external contamination in the dried *G. fisheri*. The sensory evaluation showed that the color, smell, texture, and extent of contamination were superior in the seaweed subjected to nine washing cycles. The cleaning method adopted in this study for *G. fisheri* was able to effectively reduce both the heavy metal concentrations by scraping off the cell wall, and cleaning the seaweed using a washing machine is therefore recommended prior to preparing the product for sale.

**Keywords:** *Gracilaria fisheri*, edible seaweed, heavy metal, contamination, post-harvest

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## 1. Introduction

Seaweeds have a low calorie content and are rich in soluble dietary fiber, proteins, minerals, vitamins, antioxidants, phytochemicals and polyunsaturated fatty acids (Abdallah, 2012; Kongkiattikajorn & Pongdam, 2006; Mohamaed, Matanjun, Hashim, Rahman & Mustapha, 2012).

*Gracilaria* spp. is the largest source from which agar is extracted, contributing about 60 % of world agar production (Ahmad, Surif, Ramli, Yahya, Nor & Bekbayeva, 2011; Buriyo and Kivaisi, 2003; Yeong, Phang, Reddy & Khalid, 2014) and is also an edible seaweed which contains high amounts of protein, vitamins, and minerals essential for human nutrition. Seaweeds can be consumed fresh or as dried or processed products and used as food ingredients, commonly thickening agents in gelatinous dishes or as ingredients in dishes typical of coastal areas (Norziah and Ching, 1999). Among the various species, *Gracilaria fisheri* is that most commonly used for the commercial extraction of agar (Chirapart, Munkit & Lewmanomont, 2006). Cultivation

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\*Peer-reviewed paper selected from  
The 8<sup>th</sup> International Fisheries Symposium 2018

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of *Gracilaria* has been carried out using thallus propagation, tissue culture and via explants (Dawes, Rojas, & Robledo, 1998; Yeong *et al.*, 2014).

In Thailand, *Gracilaria fisheri* is used as both a fresh vegetable and as dried products (Benjama & Masniyom, 2012). In the traditional processing of seaweed for sale, it is sun-dried for several days and then wrapped in paper or put in plastic bags to be sold. However, this traditional process reduces the quality of the seaweed for consumption because the dried plant material is difficult to cook and contamination can also occur from soil, sewage, animals, or other plants which are collected along with the seaweed. This problem also affects the quality of the product and leads to it being rejected by buyers. Therefore, seaweed products must be carefully monitored to avoid contaminants affecting the quality of the food in which they are used, or making it unsafe.

However, there has been little previous research published about post-harvest methods used in preparing *G. fisheri* and the study described in this paper investigated a novel cleaning method which could be used to improve the quality of seaweed products. In addition, seaweeds are considered to be a bio-indicator for marine environmental contamination due to their high capacity to accumulate heavy metals (Almela, Clemente, Velez & Montoro, 2006). Therefore, the study also investigated the micro-nutrient contents and heavy metal accumulations in *G. fisheri* collected during the dry and wet seasons.

## 2. Materials and Methods

### 2.1 Sample location and seaweed source

Dried seaweed was collected under three different conditions: directly from *Gracilaria* farms located in the Tunyounglulo sub-district, Mueang district, Pattani province, during both the dry and wet seasons, and purchased from local shops in Pattani province. The samples of dried seaweed from the farms were produced from newly harvested *Gracilaria* collected from the cultivation ponds, then sun-dried for a few days. Samples were collected separately in the dry and wet seasons. The samples of dried seaweed purchased from local shops consisted of ten samples selected randomly from souvenir shops in the district. All the samples were kept in plastic bags before analysis was carried out.

Water and sediment from the cultivation ponds were also collected during the dry and wet seasons to determine the metal and other chemical contents. The samples of the surface sediments were collected from between 0 and 5 cm depth, using a plastic scoop. After collection the samples were packed in pouches and frozen in order to prevent changes in the chemical composition of the sediment (Sakan, Dodevic, Manojlovic & Predrag, 2007). Before analysis, the samples were dried in an oven and ground before analysis by fractionation and to determine the elemental composition.

### 2.2 Contaminant determination

The samples of dried *G. fisheri* were weighed and any external contaminants were isolated, identified and removed. The contaminants were classified as: mollusks, epiphytes, sediment and salt, and others. The separated contaminants were weighed and were calculated as:

$$\text{contaminant (\%)} = \frac{\text{weight of contaminant in seaweed}}{\text{weight of total seaweed material}} \times 100.$$

The moisture content of the seaweed samples was ascertained according to the method of the Association of Official Analytical Chemists (2000). The remainder of the samples was then used in investigating the cleaning process.

### 2.6 Element and metal content determination

The analysis of the concentrations of the chemical elements (hereafter, *elements*), Ca, Mg, Cu, Mn, Zn, Fe, Ni, Cr, Cd, Pb, K and Na was conducted on the pooled seaweed samples from the three conditions (dry-season farmed, wet-season farmed, sold in shops), the pooled water samples, and in the pooled sediment samples, according to the method of the Association of Official Analytical Chemists (2000). Atomic Absorption Spectrophotometry (AAS; AAnalyst 100, Perkin Elmer) was used to determine the concentrations of Ca, Mg, Cu, Mn, Zn, Fe, Ni, Cr, Cd, and Pb. For the concentrations of K and Na in the seaweed and in the sediment, the analysis was performed using the flame photometric method. The elemental contents of the seaweed and in the sediment samples were extracted using 1 g of dried and ground sample in a 150 mL Pyrex beaker to which, 10 mL HNO<sub>3</sub> was added and the sample allowed to soak thoroughly. Then 3 mL of 60 % HClO<sub>4</sub> was added and heat applied gently via a hot plate until frothing ceased and the HNO<sub>3</sub> was almost evaporated. After cooling, 10 mL of HCl (1+1) was added to the solution which was then filtered with microfiber filters into a 50-mL volumetric flask before analysis.

AAS was also used to determine the concentrations of Ca, Mg, Cu, Mn, Zn, Fe, Ni, Cr, Cd Pb, K and Na in the water samples. A 100-mL water sample was placed in a 250 mL beaker and the pH of the sample was adjusted to 2.5 with HCl. A quantity of 2.5 mL of ammonium pyrrolidine dithiocarbamate was then added to and mixed with the sample, following which, 10 mL methyl iso-butyl ketone was added and the specimen vigorously shaken for 1 minute before the extraction. The instrument was calibrated against a standard solution which was prepared in the same manner as the samples and the calibration curve was established from the average of each standard before and after reading the samples against analytical blanks which were treated in a similar manner to the specimens. The elemental composition of the sediment was determined based on blended sample from each pond separately.

### 2.4 Cleaning method

Samples consisting of 500 g of dried seaweed were placed in nylon cleaning bags, which were then put into the chamber of a whirlpool washing machine (Panasonic, capacity:10 kg). For each wash cycle three bags of seaweed were put into the machine which was then filled with filtered freshwater. The cleaning cycle was set at 15 min at the *medium* setting. The water was drained and refilled after each of cycle. The number of washing cycles was varied over four levels: zero, three, six and nine cycles. After washing, the seaweed was spun to remove the water and spread out in a hot air oven at 60 °C for drying. The cleaned and dried seaweed was packed in plastic bags for color testing, sensory investigation and determination of the heavy metal content. A

part of each sample was pressed and sent to a herbarium for measurement of a cross-section of the cell wall under a light microscope with a measuring function. The color of the seaweed was measured using a ColorFlex portable spectrophotometer (HunterLab, MiniScan model EZ 4500L). The results were recorded as the  $L^*a^*b^*$  values, where the  $L^*$  value indicates the level of lightness or darkness, the  $a^*$  value indicates the redness or greenness, and the  $b^*$  value indicates yellowness or blueness.

## 2.5 Sensory evaluation

Sensory evaluation was conducted using a questionnaire incorporating a 5-Point hedonic scale with thirty testers, ten testers of whom had previous experience of seaweed. The questionnaire investigated the general quality, color, odour, texture, and contamination of the seaweed to investigate the outcome of the cleaning of the seaweed in a whirlpool washing machine.

## 2.6 Data analysis

Data from each experiment are reported herein as mean  $\pm$  standard error (SE). The data were analyzed using one-way ANOVA to compare the means of contaminant %, elemental composition/heavy metal accumulations in the seaweed, water and sediments, the cell wall thickness, color, and the results of the sensory testing. The variance in means were analyzed for significant differences ( $p < 0.05$ ) using the Tukey HDS test.

## 3. Results and Discussion

### 3.1 Habitat characteristics and external contamination of *G. fisheri*

The red alga, *G. fisheri* used in the present study was sampled from seaweed cultured in earthen ponds which were previously used as shrimp ponds. No shrimp farming activity had been conducted in the ponds for more than twenty years and they had been used to grow seaweed for ten years. For a number of years the seaweed farming was discontinued in some ponds because disease was manifested by the plants. The cultivation ponds were sized between 0.5 and 1.4 hectares (position 6N53.183/ 101E21.191, see Figure 1). The water was supplied and changed daily by tidal flows directly from Pattani Bay. Cultivation was carried out throughout the year and some seaweed was harvested every month. The three different seaweed conditions (farmed in the dry season, farmed in the wet season and shop-purchased) were similar in quality. They were soft, pale pink or brown in color, with the surface covered with salt stains and bearing a strong fermented smell because the product was dried on a net or bamboo sheet beside the ponds for a few days only and then kept in plastic bags for sale to the local market or in souvenir shops, with a moisture content of  $18.5 \pm 4.0$  % (Figure 2 a and b). The main contaminant in the seaweed was mollusks with an average of  $23.04 \pm 5.38$  % (Figure 2c, Figure 2). The highest contamination by weight in the seaweed from different sources was from the shells of the periwinkle, *Littoraria pallescens* Pholippe, 1846 (Figure 1c) and the level of contamination was not significantly different between the

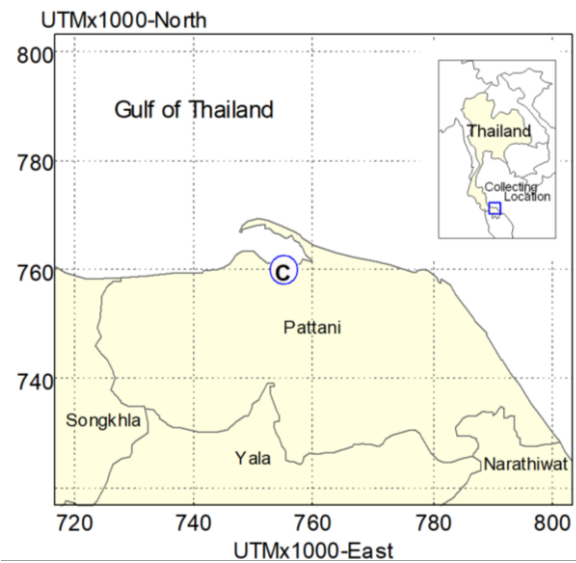


Figure 1. Map of Pattani Province and the collecting area (C)

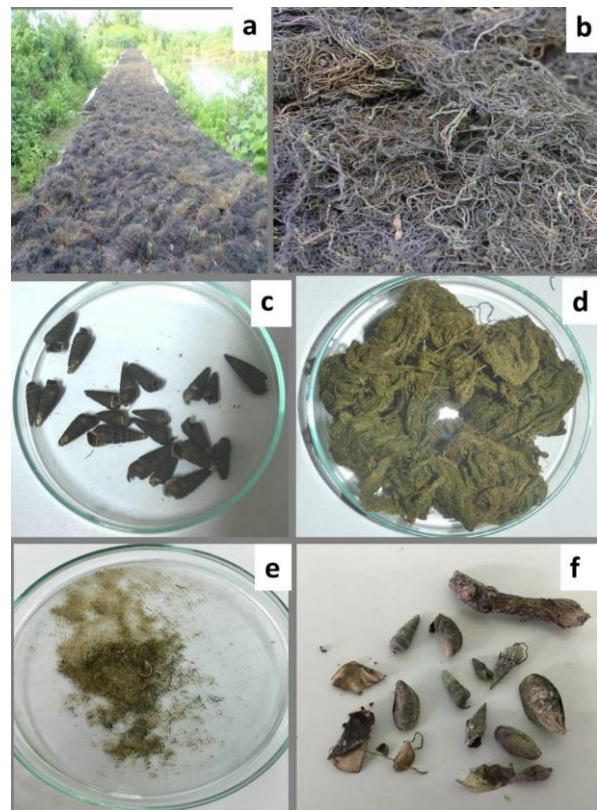


Figure 2. Characteristics of *Gracilaria fisheri* and its external contaminants: (a) drying method near the pond; (b) dry produce for selling; (c) mollusk contaminant, *Littoraria pallescens* Pholippe, 1846; (d) epiphyte contaminant, *Rhizoclonium riparium*; (e) sediment and salt contaminants; (f) other contaminants

three conditions. The second highest contamination was from epiphytes which again showed no significant differences among the sources. Epiphytic contaminants and sediments were found in the seaweed collected during both the dry and

wet season but were not found in the seaweed purchased in shops since the seaweed product had undergone a sorting process before being sold. Although, the contamination with the green alga, *Rhizoclonium riparium* (Figure 2 d) found in this study was the first reported in *Gracilaria*, a similar contamination was previously reported by Ganesan, Sahu and Eswaran, 2011 concerning the green alga, *Ulva intestinalis*, which had contaminated a *Gracilaria* farm and caused biomass reduction. Green algae can become a significant contaminant in red alga cultivation because green algae show faster growth and have higher metabolisms than red algae (Lobban & Harrison, 1994). The other contaminants found were sediment, salt dust, plant leaves, and a fragment of wood (Figure 2 e, f). The percentage of all classes of contaminants is shown in Figure 3.

### 3.3 Chemical element contents in the seaweed, water and sediment

The concentrations of the elements, Mn, Fe, Cr, Cd, Zn, Ni, Cu, and Pb in the seaweed and the water in the dry season were found to have similar patterns but those in the wet season showed a different trend. In addition, the levels of accumulation of those in the sediments were different from those in the seaweed and the water. The highest concentration of Mn was found in the seaweed collected in the dry season which was significantly higher than that in the wet season (Figure 4 a). Moreover, the accumulation of Mn in the seaweed bought in shops was significantly different from both the dry- and wet-season farmed seaweed. The Zn concentration in the seaweed from shops was significantly higher than that of the seaweed collected from the farms in the wet and in dry seasons (Figure 4 b, c). Some metals and elements such as Cu and Zn are essential for plant growth and the development of many enzymes and proteins, and their accumulation in cells depends on the environment (Hall, 2002).

The elemental concentrations in *G. fisheri* in the samples from the three conditions were different. The levels of all the elemental concentrations in the water in the dry season were higher than those in the wet season with the exception of Cu and Cd. In a previous study, all the concentrations in water of all those elements, except Cd were also reported to be higher in the summer in a coastal lagoon, than in the autumn (Salem *et al.*, 2014) and another study reported that the concentration of Cu in *G. fisheri* in the summer was higher than that in the rainy season (Benjama & Masniyom, 2012). These conflicting results may be the result of different environmental factors, with the concentrations in the wet season being affected by flood water draining into the area and causing metal concentrations in the vicinity of a river mouth to be higher than those recorded in the dry season, as well as causing variations in water temperature, salinity, light, and nutrients (Dawes, Rojas, & Robledo, 1998). It has previously been suggested that the synthesis of nutrients in seaweeds may be influenced by seasonal changes in ecological conditions (Lobban, Harrison & Duncan, 1985). The concentrations of Cu, Zn, Fe, Cd, Ni and Pb in *G. fisheri* have also been recorded as being much lower than those in *G. verrucosa* (Khaled, Hessein, Abdel-Halim & Morsy, 2014) and in addition, the concentrations of Cu, Zn, Fe, Cd, Ni, Pb and Cr in *G. fisheri* recorded in this study in both the dry and

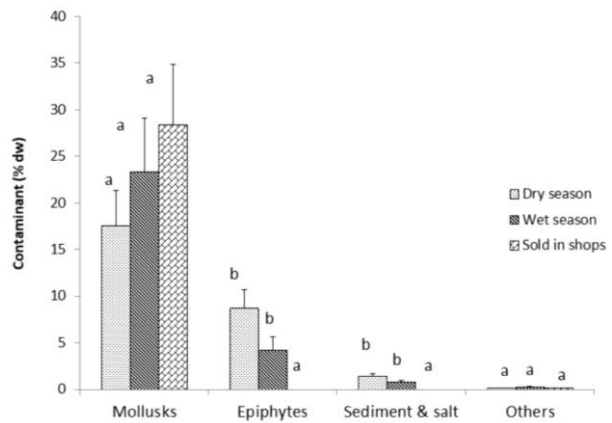


Figure 3. Concentrations of contaminants in *Gracilaria fisheri* collected from Pattani province in three different conditions. Different letters above the bars indicate significant differences within a cluster of bars ( $p < 0.05$ ).

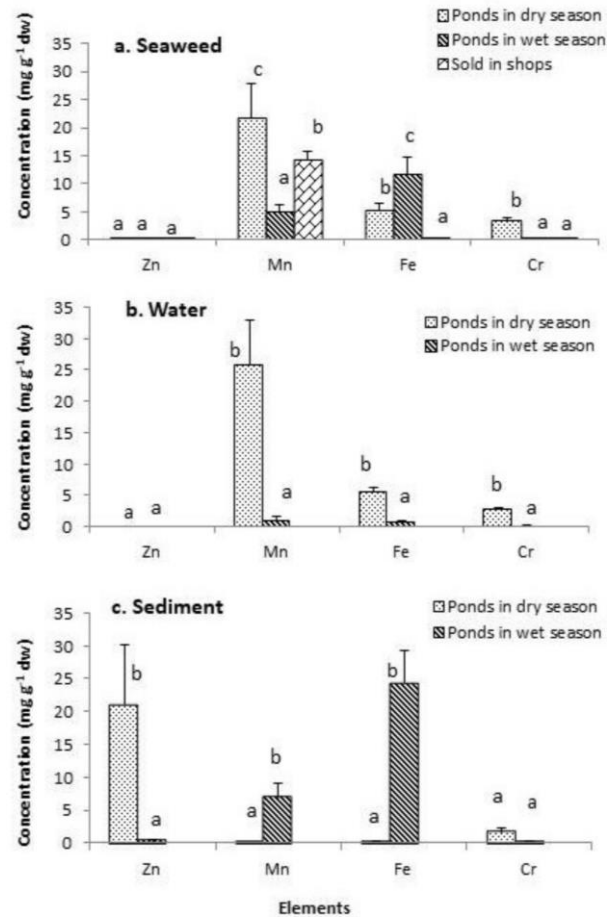


Figure 4. The concentrations of Zn, Mn, Fe and Cr in three materials: (a) seaweed, (b) water, and (c) sediment. Different letters above the bars indicate significant differences ( $p < 0.05$ ).

wet season samples were much lower than those reported in *Gracilaria gracilis* by Caliceti, Argese, Sfriso & Pavoni (2002) in samples taken in Venice.

Mn and Fe were the dominant metals in the water in comparison to the other elements. The concentrations of Mn, Fe, and Cr in the water in the dry season were significantly higher than those in the wet season (Figure 4 c) but there was no significant difference in the concentrations of Cu and Cd in the water in the two seasons. However, the concentrations of Zn, Ni, and Pb in the water in the dry season were significantly higher than those in the wet season (Figure 5 a, b). The concentrations of all the elements and heavy metals in the present study were much lower than those detected in Manchar Lake which was classified as a polluted area (Arain *et al.*, 2008). The trend of the concentrations of the elements in water was similar to those in the seaweed with those in the dry season being mostly higher than those in the wet season, with the exception of Cu and Cd. As noted above, Salem *et al.* (2014) recorded all the elements measured in water in the present study except Cd to be higher in the summer than in the autumn.

The concentrations of Mn, Zn and Fe were dominant in the sediment but the pattern varied between the seasons with Cu, Zn, Cd, and Cr being higher in the sediments in the dry season while Ni, Pb, Mn and Fe were higher in the wet season (Figures 4 c, 5 c). This result is similar to that reported by Salem *et al.* (2014) with the concentrations of elements in the sediment in the summer in a coastal lagoon generally being higher than those in the autumn, with the only exception being Pb. In the present study, no significant differences were found in the concentrations of Cu, Ni, and Pb in the sediment in the two seasons but the Cd concentration in the sediment in the dry season was significantly higher than that in the wet season.

### 3.4 The quality of *G. fisheri* after cleaning

The results of the analysis of the elemental contents after cleaning the seaweed samples in a washing machine showed that this affected the concentrations of the various elements, with a higher number of cleaning cycles leading to successively lower concentrations of heavy metals in the seaweed, as shown in Figure 2. The seaweed samples subjected to nine washing cycles showed the lowest heavy metal contents. Generally, the heavy metals and other elements are concentrated in the cell wall because the most active absorption in a cell is performed by the cell wall of epidermis cells in the meristem region (Tupan, Herawati, Arfiati & Alanniam, 2014) and the amount of heavy metal accumulated depends on the action of the cell wall and extracellular exudates (Hall, 2002). In the present study the microscopic examination of a cross-section of *G. fisheri* showed that cleaning in the washing machine had caused scratches on the seaweed's surface (Figure 7) and this was the reason for the decrease in the heavy metal content. The cross-section of the main filament showed that the thickness of the cell wall (Table 1) was lower and reduced in parallel with increases in the number of cleaning cycles applied, a pattern which was also reflected by the decrease noted in the elemental concentrations. Without washing (zero cycles), the sediment was removed but the bark of the seaweed surface remained intact. However, after three, six or nine washing cycles, the bark on the surface of the seaweed was scratched and some areas of the cell wall were removed.

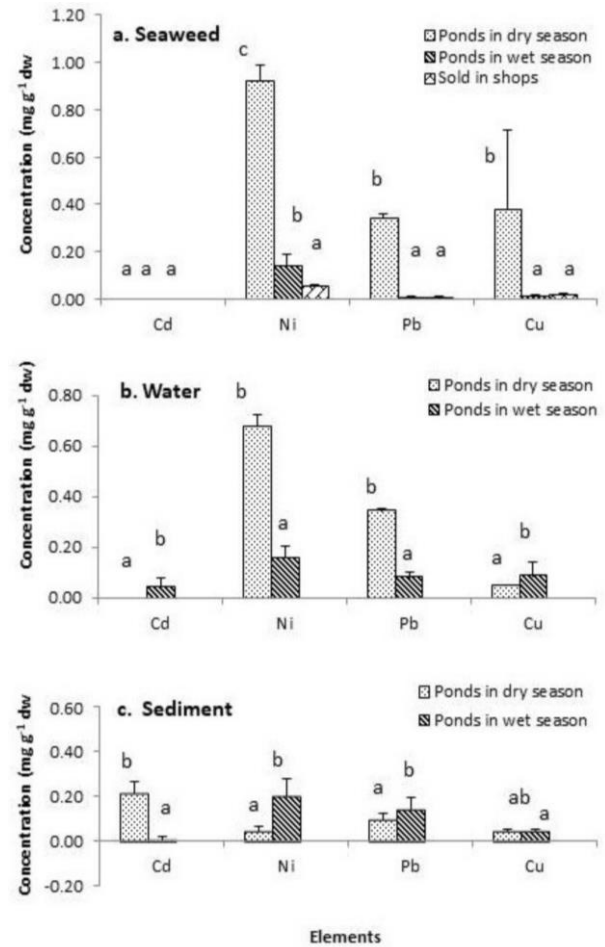


Figure 5. The concentrations of Cd, Ni, Pb and Cu in three materials: (a) seaweed, (b) water, and (c) sediment. Different letters above the bars indicate significant differences ( $p < 0.05$ ).

After cleaning, the dried seaweed was whiter in color than before cleaning with an  $L^*$  value in a range of  $33.04 \pm 3.88$  to  $5.14 \pm 3.39$  (where 0 indicates black, 0-50 indicates dull and a 51-100 indicates white). The  $L^*$  value in the present study was significantly different ( $p < 0.01$ ) among the samples subjected to different numbers of cleaning cycles and the elemental concentrations of all the metals tested decreased, in some cases significantly, as the number of cycles increased. The trend of  $L^*$  in the seaweed was similar to that of the yellow tone ( $b^*$ ) which ranged between  $7.20 \pm 2.40$  and  $14.94 \pm 1.84$  indicating a generally yellow color (positive  $b^*$  indicates a yellow tone while a negative  $b^*$  indicates a blue tone). Finally, the  $a^*$  value did not vary significantly according to the number of washing cycles, with values in the range of  $1.65 \pm 0.53$  to  $2.14 \pm 0.43$  (a positive  $a^*$  indicates red while a negative  $a^*$  indicates green). Overall, therefore, the seaweed showed a lighter redder and yellower tone when compared to the samples that had not been cleaned in the washing machine. The photosynthetic pigments in *Gracilaria* occur in the outer cortex layer (Fredericq & Norria, 1985), and cleaning, which caused damage to the outer cells,



Table 1. Cell-wall thickness and color of *Gracilaria fisheri* after cleaning using a whirlpool washing machine for different numbers of cycles

Number of washing cycles	Cell wall thickness ( $\mu\text{m}$ )	Color		
		L*	a*	b*
0 cycle	12.38 $\pm$ 1.44 <sup>a</sup>	33.04 $\pm$ 3.88 <sup>a</sup>	1.65 $\pm$ 0.53 <sup>a</sup>	7.20 $\pm$ 2.40 <sup>a</sup>
3 cycles	10.86 $\pm$ 1.68 <sup>a</sup>	41.55 $\pm$ 3.43 <sup>b</sup>	1.93 $\pm$ 0.34 <sup>a</sup>	10.53 $\pm$ 2.13 <sup>b</sup>
6 cycles	8.77 $\pm$ 1.86 <sup>b</sup>	43.74 $\pm$ 3.47 <sup>bc</sup>	2.07 $\pm$ 0.55 <sup>a</sup>	14.40 $\pm$ 2.69 <sup>c</sup>
9 cycles	6.72 $\pm$ 0.76 <sup>c</sup>	45.14 $\pm$ 3.39 <sup>c</sup>	2.14 $\pm$ 0.43 <sup>a</sup>	14.94 $\pm$ 1.84 <sup>c</sup>

Note: Different superscripts indicate significant differences within a column ( $p < 0.05$ ).

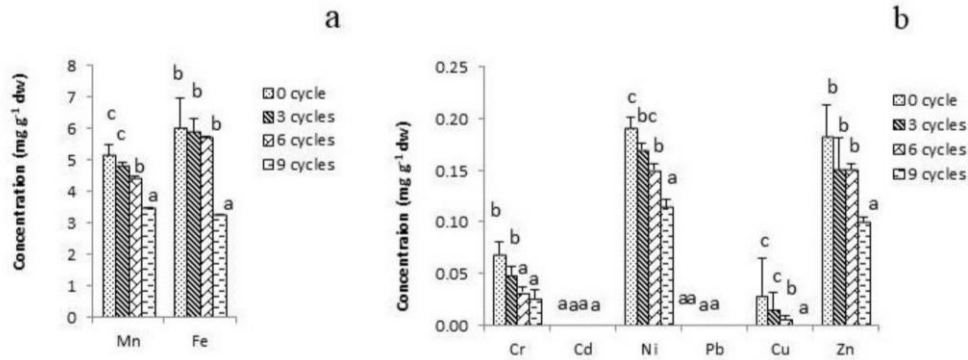


Figure 6. The concentrations of elements after cleaning using whirlpool washing machine for various numbers of washing cycles: (a) Mn and Fe (b) Cr, Cd, Ni, Pb, Cu and Zn. Different letters above the bars indicate significant differences ( $p < 0.05$ ).

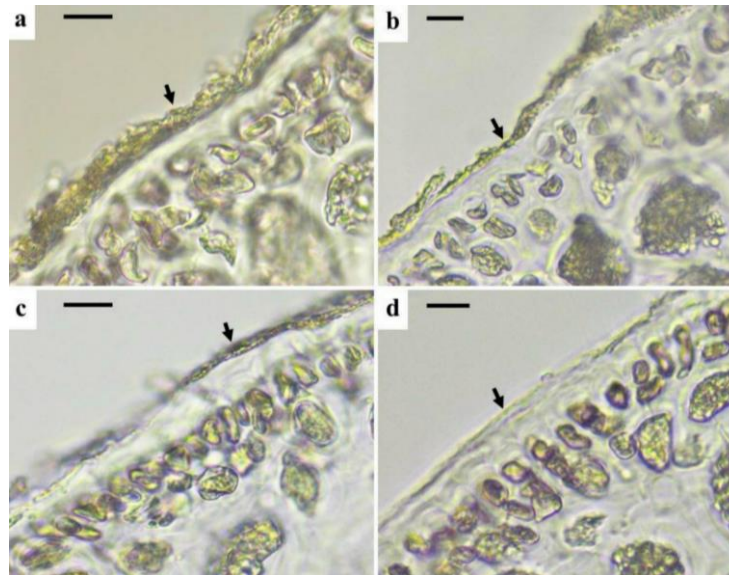


Figure 7. The cell wall thickness after cleaning in a whirlpool washing machine for different numbers of cycles (arrow head, scale bar = 15  $\mu\text{m}$ ): (a) 0 cycles, (b) 3 cycles, (c) 6 cycles and (d) 9 cycles

may have led to a loss of pigments, thus causing the b\* value to increase, and the seaweed to exhibit a yellower color. The a\* value of the seaweed is related to phycobilin, with phycoerythrins being the most abundant phycobiliproteins in many red algae, and are also associated with the chlorophyll in Rhodophyta. Isami and Osman (2016) noted that the pigment contents varied seasonally in algal species as well as the physical and chemical parameters, and that nutrients, pH and dissolved oxygen all have positive impacts on phycobilin while the temperature has a negative impact on photosynthetic pigments, which also respond to exposure to low salinity. Therefore, in the present study, the different number of

cleaning cycles which were carried out with filtered fresh water had no differential effect on the loss of phycobilin.

### 3.5 Sensory evaluation analysis

The thirty testers who were employed in the sensory tests rated the sample subjected to nine washing cycles most highly in all the areas on which they were asked to give an opinion, viz taste, smell, odour and texture and agreed that washing reduced the amount of external contamination (Table 2). It was found that washing with several cycles tended to make the dried seaweed less stiff because washing with

Table 2. The 5-scale acceptance score of sensory evaluation of *Gracilaria fisheri* after cleaning in a whirlpool washing machine for different numbers of cycles

Number of washing cycles	Color	Odor	Texture	Contamination	Overall acceptance
0 cycle	3.23±0.94 <sup>ab</sup>	2.67±1.15 <sup>a</sup>	3.23±1.19 <sup>a</sup>	2.23±1.36 <sup>a</sup>	2.50±0.68 <sup>a</sup>
3 cycles	3.17±0.94 <sup>a</sup>	2.53±1.01 <sup>a</sup>	3.30±0.88 <sup>a</sup>	2.50±1.20 <sup>ab</sup>	2.83±0.65 <sup>ab</sup>
6 cycles	3.27±0.94 <sup>ab</sup>	2.53±1.07 <sup>a</sup>	3.27±0.74 <sup>a</sup>	2.63±1.27 <sup>ab</sup>	2.97±0.56 <sup>b</sup>
9 cycles	3.77±0.94 <sup>b</sup>	2.97±1.27 <sup>a</sup>	3.63±0.93 <sup>a</sup>	3.10±1.12 <sup>b</sup>	3.20±0.41 <sup>b</sup>

Note: Different superscripts indicate significant differences within a column ( $p < 0.05$ ).

freshwater caused the salt on the surface of the seaweed skin to completely disappear. After nine washing cycles the seaweed had a moisture content of 12 - 28 % and, the texture of the seaweed was softer than after three or six cycles due to the greater number of washes.

#### 4. Conclusions

The cleaning method used in this research was able to reduce the amount of external contamination and the concentrations of heavy metals in *Gracilaria fisheri* with some of the differences being significant. Analysis of the elemental concentrations in the seaweed, the water, and the sediment indicated that the concentrations in the seaweed were similar to those in the water even though the seaweed was grown from the bottom of the pond. The fluctuations in the elemental concentrations were influenced by both the environmental aspects of pond cultivation and the processing of the product. While generally consumers prefer clean products, the removal of external contaminants by washing may cause a loss of seaweed surface which may reduce its appeal to consumers. The concentrations of Cu, Mn, Ni, Pb, and Cr in the seaweed were higher in the dry season than in the wet season while those of Zn, Fe, and Cd were higher in the wet season. Therefore the amount of heavy metals consumed in seaweed is dependent on the season in which it is harvested. Moreover, in order to prevent contamination of dried *G. fisheri*, hand sorting and then washing around ten times is recommended before drying in order to provide a safe material for use as a cooking ingredient.

#### Acknowledgements

This study was financially supported by the Halal Food Science Center, Department of Food Sciences, Faculty of Science and Technology, Prince of Songkla University, Pattani, Thailand.

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