Antioxidant properties and fatty acid analysis in *Gracilaria fisheri.*

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Abstract

Red seaweeds (*Gracilaria fisheri*) are widely distributed in the East Coast of Peninsular Malaysia. It contains 94% saturated fatty acids, mainly palmitic acid and 6% of odd number fatty acid (C_{17}) from the total unsaturated fatty acids. A free radical scavenging activity test with 1,1 diphenyl-2-picrylhydrazl (DPPH) was used to evaluate the anti-oxidative properties of *Gracilaria fisheri*. The tests showed positive result with about 78% of inhibition as compared to 96% inhibition by ascorbic acid. This seaweed species can be used as an alternative food source for animal feeding with complete nutritional properties.

Keywords: Odd number fatty acid (C_{17}); Radical scavenging activity; Antioxidant activity

Introduction

Seaweeds belong to three main taxonomic groups, Chlorophyta, Phaeophyta and Rhodophyta (Santelices and Doty, 1989; Critchley, 1993) and *Porphyra, Gelidium, Eucheuma* and *Gracilaria* are some of the major edible and commercial types of red seaweeds. *Gracilaria fisheri* belong to family Gracilariaceae and comprises more than 150 species (Bird & McLachlan, 1984). In Malaysia, 13 species of *Gracilaria* have been described and generally found in the west cost of Peninsular Malaysia (Gan, 2003).

In food manufacturing, seaweeds have been developed as raw or semi-processed food products especially in Japan, Korea and China (Mabeau & Fluerence, 1995). In Asia, seaweeds have been used for centuries in the preparation of salads, soups and also low calorie foods (Jimenez-Escrig & Sanchez-Muniz, 2000). Currently of interest in the field of nutritional sciences is the presence of antioxidant substances in fresh and processed foods.

In recent years, fatty acids (FAs) in marine algae have aroused considerable interest among researchers because these marine plants can produce C_{18} and C_{20} polyunsaturated acids (PUFAs; Kayama, *et al.*, 1989). These FAs are essential for nutrition of many animals, including humans (Uki, *et al.*, 1986), and are of interest in biotechnology, in food chain studies and in cosmetics (Servel, *et al.*, 1994). Common fatty acids of animal and plant origin consists of even-numbered linear chains of 16 to 22 carbon atoms, with zero to six double bonds of the *cis* configurations; PUFAs have methylene-interrupted double bond systems in general. However, there are countless exceptions, especially in the plant kingdom. Fatty acids can be both odd- and even numbered, with two to almost a hundred carbon atoms. Odd-numbered fatty acids can be found in seaweeds but very rare in animals (Dobson, 1998). FAs are commonly divided into two categories that include saturated and unsaturated fatty acids (Best, 2001). Several PUFAs are recognized as essential fatty acids (EFAs) in human diet, which are used to prevent nutrition-related illnesses (Dunford, 2001).

PUFA contains at least two unsaturated carbon bonds. There are three most important groups of EFA which are known as omega-6 (n-6) fatty acids, omega-3 (n-3) fatty acids and omega-9 (n-9) fatty acids. It is important to maintain an appropriate balance of n-3 and n-6 in

the diet as these two substances work together to promote health. According to University of Maryland Medical Centre (2004), n-3 and n-6 fatty acids with ratio 3:1 play a crucial role in brain function as well as normal growth and development (Anne, 2005). The n-fatty acids are used by the human body to make anti-inflammatory, anti-thrombotic substances, while n-6 fatty acids are made into substances that promote inflammation and thrombosis (Drummond and Brefere, 2001). Furthermore, n-3 fatty acids may be protective against prostate cancer, thrombotic infarctions in women, immunoglobulin, nephropathy and cognitive decline.

Based on previous study, *Gracilaria* sp. was reported to contain Eicosapentanoic acid (EPA) and Docosahexaenoic acid (DHA) that are important to promote cardiovascular health and prevent coronary heart disease for human (Sarah, 2004). DHA is sometimes referred as "brain oil" and this element contain about 60 percent fats while the EPA element is useful for human to repair cell membrane, enabling the cells to obtain optimum nutrition and expel harmful waste products (Sarah, 2004). A primary function of EFAs is a production of prostaglandins, which regulate body functions such as heart rate, blood pressure, blood clotting, fertility, conception and play a role in immune function by regulating inflammation and encouraging fighting infection (Rotella, 2004).

Besides that *Gracilaria fisheri* also have high antioxidant properties which are beneficial for human health. Growing knowledge about the health promoting impact of antioxidants in everyday foods combined with the assumption that a number of common synthetic preservative may have hazardous effects (Krishnakumar & Gordon, 1996), has led to multiple investigations in the field of natural antioxidants. The current focus is toward natural antioxidants, as antioxidants in processed foods and become important in the food industry as an alternative to synthetic antioxidants (Madsen and Bertelsen, 1995). Through the process of biotechnology, red seaweed could be turned as a potential source for a new tea product containing high antioxidant and dietary source of essential fatty acids

Materials and Methods

Sample preparation and derivation

Samples of *Gracilaria fisheri* were collected from the reservoir at Brackish Water Aquaculture Research Centre in Gelang Patah, Johor, located south of Peninsular Malaysia. Samples were cultured in the cage at the reservoir. Fresh samples of algae were collected and thoroughly cleaned with distilled water to remove epiphytes, bivalves, small invertebrates and sand particles. The samples were freeze dried until constant weights were obtained. All samples were ground and kept in plastic container in -80°C until used for analysis.

Fatty acids analysis

Lipid extraction: Extraction of lipid was prepared according to methods described by Blight and Dyer (1959). Exactly, 12g of fine ground seaweeds was weighed and transferred into a clean tube. The reaction mixture comprised 72 ml of distilled water, 270 ml of chloroform: methanol (1:2) and 72 ml water (added one minute after the mixture of chloroform: methanol). The mixture was mixed for one minute and filtered to remove the insoluble parts of the sample. It was followed by centrifugation at 400 rpm for 10 min and the supernatant of the liquid phase was collected and evaporated. Residue (3.2495 g) was extracted three times with small portions of chloroform: methanol and water for phase separations and then the organic phase was collected and evaporated to dryness in vacuum and the total lipid content (1.0404 g) was weighed.

Fatty Acid Methyl Esters (FAMEs): Fatty acids were converted to methyl esters by transmethylation of lipid samples (4-5 mg) using 0.5 mL 1% sodium hydroxide (NaOH) in methanol and heated for 15 min at 55°C. Then, 1.0 mL 5% hydrochloric acid in methanol was added and heated for 15 min at 55°C (Carreau and Dubacq, 1978) and finally 0.5 mL water was added. Fatty acid methyl esters (FAMEs) were extracted by adding hexane and the organic phase was evaporated to dryness under reduced pressure.

Gas chromatography: FAMEs sample were analyzed as described by Norziah and Ching (2000) with a Hewlett-Packard (HP) 6890 Series gas chromatography (GC) equipped with fused silica capillary column Durabond-225 (30 m x 0.251 mm, film thickness 0.15 µm) and an FID detector. The GC was connected to an HP integrator model 3396A. The carrier gas was helium. The run method was through a temperature gradient from 110°C up to 210°C with an increase rate of 8°C min^{-1} and total run time of 40 min. The detector was set to operate at 260°C. Data were collected and manipulated using HP integrator. Identification of fatty acids in the samples was performed by making comparison with chromatograms of fatty acids standards (C_{13} – C_{20} fatty acids) from Supelco Chemical and the concentration of the fatty acids was expressed in relative percentages.

Antioxidant activity: DDPH free radical scavenging activity method was carried out as described by Lourens, *et al.(* 2004) with minor modification. Stock solutions of crude extract was prepared in methanol and diluted to different concentrations in a 96-well microtiter plate. Then DDPH solution (100 µM) was added to each well. The absorbance was measured at wavelength 550 nm after 30 minutes incubation at ambient temperature. The solutions were freshly prepared and stored at darkness. All determination was performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994):

$$\% \text{ inhibition} = [(A_{C(0)} - A_{A(t)}/A_{C(0)}] \times 100,$$

where, $A_{C(0)}$ is a the absorbance of the control at t = 0 min
$A_{A(t)}$ is the absorbance of the antioxidant at t = 30 min.

Results and Discussion

Antioxidant capacity

DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants (Oyaizu, 1986). DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares, *et al.,* 1997). The antioxidant capacity of *Gracilaria fisheri* showed strong inhibition of about 78% while the ascorbic acid posses the inhibition of 96% at 1 mg/mL (Figure 1).

The antioxidant reactions involve multiple steps including the initiation, propagation, branching and termination of free radicals. The antioxidants which inhibit or retard the formulation of free radicals from their unstable precursors (initiation) are called the "preventive" antioxidants, and those which interrupt the radical chain reaction (propagation and branching) are the "chain-breaking" antioxidants (Ou, *et al.*, 2001).

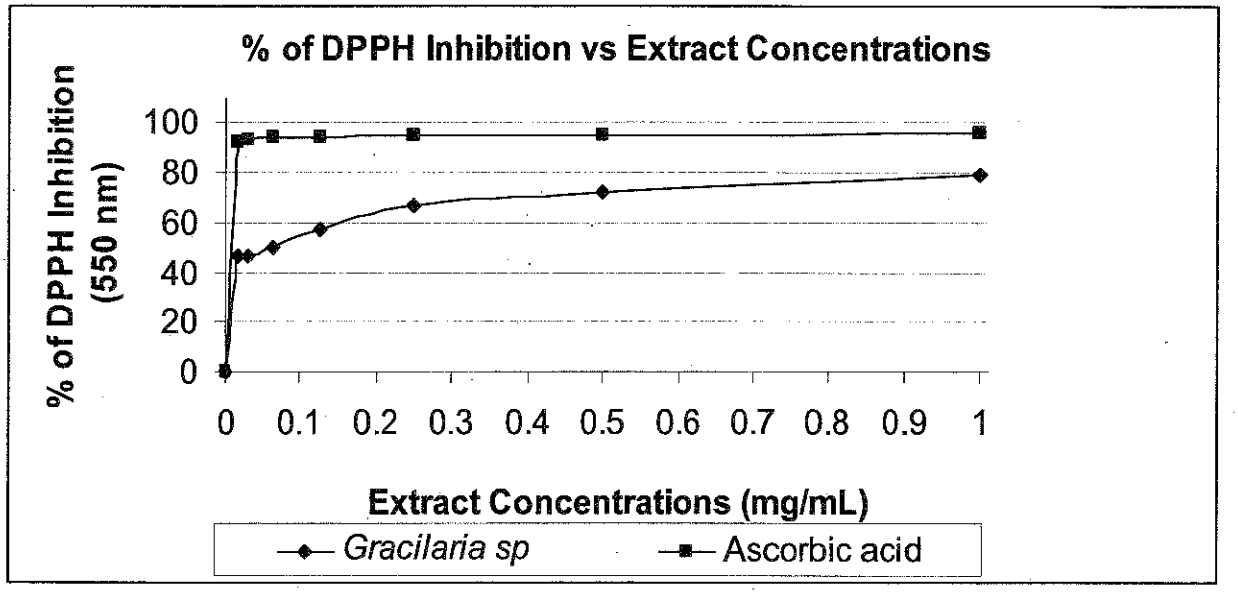

Figure 1: Percentage of DPPH inhibition against extract concentrations.

Fatty acids analysis

Gas chromatograms of fatty acids of *Gracilaria fisheri* is shown in Figure 2. Only seven fatty acids can be identified based on the fatty acids standards used. These fatty acids are myristic acid (14:0), palmitic acid (16:0), heptadecanoic acid (17:0), stearic acid (18:0), linolenic acid (18:3) and arachidic acid (20:0). These samples were identified by comparison with chromatograms of fatty acids standards (C_{13}-C_{20}) which are presented in Figure 2. Palmitic acid (16:0) is the most abundant fatty acids present in the *Gracilaria fisheri* samples studied (Table 1). The total content of palmitic acid was 78% and contained a higher composition of saturated fatty acids (95%) from the fatty acids identified.

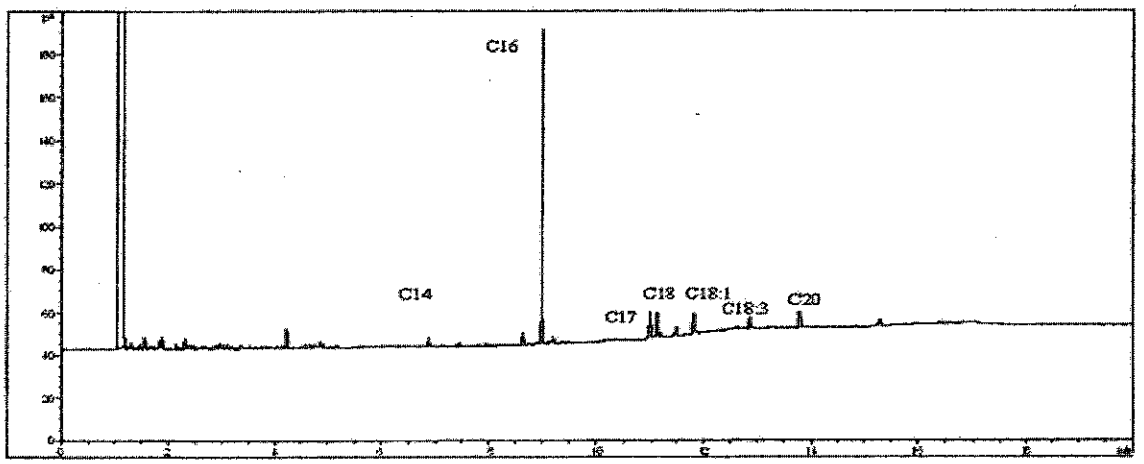

Figure 2: Fatty acid profiles of *G. fisherii*

From this study, the saturated fatty acids were found to be more abundant than the unsaturated ones. Oleic acid (18:1) and linolenic acid (18:3) were the unsaturated fatty acids present in *Gracilaria fisheri* and the others are the saturated fatty acids. However, Norziah and Ching (2000) reported that in another red seaweed species, *Gracilaria changii,* had

higher composition of unsaturated fatty acids (74%), mainly the omega fatty acids such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexanoic acid (DHA, 22:6n-3) which are apparently important to human health.

Types of fatty acids in Gracilaria fisheri

Palmitic acid (16:0) is one of the most common saturated fatty acids found in animals and plants. Its chemical formula is $CH_3(CH_2)_{14}COOH$ and as its name suggests, the acid is found in palm oil and also in butter, cheese, milk and meat. In this study, the seaweed contained 78% palmitic acid which is the first fatty acid produced during lipogenesis (Table 1).

Two percent of myristic acid, also known as tetradecanoic acid (14:0) was present in the *Gracilaria fisheri* studied. It is also a common saturated fatty acid which is found in dairy products with the chemical formula: $CH_3(CH_2)_{12}COOH$.

Table 1: Composition of fatty acids in *Gracilaria fisheri*

Fatty acids	Total fatty acid content (%)
Myristic acid (14:0)	2
Palmitic acid (16:0)	78
Heptadecanoic acid (17:0)	6
Stearic acid (18:0)	1
Oleic acid (18:1)	2
Linolenic acid (18:3)	3
Arachidic acid (20:0)	8

Heptadecanoic acid (17:0) is an odd-numbered fatty acid which is found in some marine organisms and is also very rare in mammals. In the red seaweed sample studied the content of this acid was low with only 6% of the total fatty acids. The chemical structure is $CH_3(CH_2)_{15}COOH$.

Stearic acid, also referred to as octadecanoic acid (18:0) accounted for about 1% of the total fatty acid identified. It is a waxy solid with a chemical formula $CH_3(CH_2)_{16}COOH$. Stearic acid, obtainable from many animals and vegetable fats, is used in making candles, soaps, plastics, oil pastel and cosmetics.

The monosaturated fatty acid, oleic acid (18:1) found in various animal and vegetables sources was also present in the red seaweed and contributed 2% of the total. It has the formula $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$. The saturated form is stearic acid and is used in Lorenzo's oil.

Linolenic acid (18:3) is a polyunsaturated fatty acid with three double bonds and is known as omega-3-fatty acid with the chemical formula: $CH_3(CH_2CH=CH_3(CH_2)_7COOH$. In the seaweed samples tested this fatty acid only contributes 3% from the total fatty acids.

The other rather significant component obtained was arachidic acid (20:0) representing 8% of the total fatty acids. This saturated acid is also referred to as eicosanoic acid and is normally found in peanut oil. Its chemical formula is $CH_3(CH_2)_{18}COOH$.

Conclusion

From this study, it can be concluded that the red seaweed, *Gracilaria fisheri* contained significant concentration of antioxidants and fatty acids that are known to be beneficial for human nutrition and health. The successful determination of the presence of odd number fatty acid (C_{17}) and Omega-3 acid in *Gracilaria fisheri* is an important discovery as the red seaweed can be a potential source of dietary essential fatty acids.

Acknowledgements

We would like to express our gratitude to the Ministry of Higher Education of Malaysia for the research grant provided through Universiti Malaysia Terengganu (Vote: 59060) and to all members of Biotechnology Laboratory for their help and support.

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