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DPPH RADICAL SCAVENGING ACTIVITY EFFECT OF EDIBLE SEAWEEDS FOR NOODLES

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ABSTRACT

The 1- diphenyl 2-picrylhyorazyl (DPPH) scavenging activity from ethanol extracts of four edible alga, *Porphyra tenera*, *Undaria pinnatifida*, *Sargassum fusiforme* and *Enteromorpha linza* were evaluated. DPPH scavenging activity was analysed according to the method of Brand-Williams et al. *S. fusiforme* was showed the highest inhibition activity of DPPH among four alga. DPPH scavenging activities of *S. fusiforme* was evaluated 69.3% at 8.0 mg/ml. DPPH scavenging activity of *P. tenera* was evaluated 27.0% at 8.0 mg/ml. *U. pinnatifida* and *E. linza* were showed similar inhibition of DPPH scavenging activity. According to results of DPPH assay per seaweed, inhibitory activities were in the order of *S. fusiforme* > *P. tenera* > *E. linza* > *U. pinnatifida*. The DPPH scavenging activities of dough which was instant noodles mixed with *P. tenera* and 3.5% salt were 18.6% at 8.0 mg/ml. After boiling for 5.0 minutes, DPPH scavenging activity of mixed instant noodles with extracts of *S. fusiforme* was evaluated 54.0% at 8.0 mg/ml.

KEYWORDS: 1, 1- diphenyl 2-picrylhyorazyl (DPPH), noodle, *Porphyra tenera*, *Sargassum fusiforme*, *Undaria pinnatifida*.

INTRODUCTION

People have interest in maintaining good health and an excellent body figure. The research and exploration of natural antioxidants has been rising in recent years. They have become more careful in the food they choose to consume, looking for food with a high nutritional value, bioactive compounds and antioxidant capacity.[1] This increased attention is driven by several trends in the food industry. Many studies have been conducted with regard to free radicals, oxidative stress and antioxidant activity of food, giving antioxidants a prominent benetifical role, but recently many authors have questioned their importance, whilst trying to understand the mechanisms behind oxidative stress.[2-3] There is great number of methods for determination of antioxidant capacity of foods and beverages based on different principles. The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method is rapid, simple, accurate and inexpensive assay for measuring the ability of different compounds to act as free radical scavengers or hydrogen donors and to evaluate the antioxidant activity of foods and beverages.[4]

Edible seaweed are algae that can be eaten and used in the preparation of food. They may belong to one of several groups of multicellular algae: the red algae, green algae and brown algae. Seaweeds are used extensively as food in coastal cuisines around the world. Seaweed has been a part of diets in China, Japan and Korea since prehistoric times. Today those China, Japan and the Republic of Korea are the largest consumers of seaweed as food. Seaweed is also consumed in many traditional European societies, in Iceland, Norway, the Atlantic coast of France, Ireland and England. The Māori people of New Zealand traditionally also used a few species of red and green seaweed.[5] In the Republic of Korea seaweeds are harvested or cultivated for food. For example, *Porphyra* taxa and *Undaria* taxa are produced about 400,000 ton/year, respectively. In particular, *Undaria* has been harvested from natural resources for many years in the Republic of Korea, China and Japan. The Republic of Korea has the highest consumption of the three countries. It is cultivated because natural harvest is small. A large proportion of the Republic of Korea production is exported to Japan, where there is little activity in cultivation of this species. *Porphyra* species are the largest source of food from red seaweeds. *Porphyra*, known by the more common names of nori and laver, is dried and processed into thin purplish-black sheets. One of its common uses is in Japanese sushi, where it is wrapped on the outside of a small handful of soured, boiled rice topped with a piece of raw fish. *Porphyra* has been cultivated in Japan and the Republic of Korea since the seventeenth century.

Sargassum fusiforme (Harv.) Setch. is a brown sea vegetable growing wild on rocky coastlines around Japan, Korea and China. *S. fusiforme* is rich in dietary fibre and essential minerals such as calcium, iron and magnesium. *S. fusiforme* is normally eaten with other foods such as vegetables (for example: kimchi in Korea) or fish. It may be added to foods that have been steamed, boiled, marinated in soy sauce or fish sauce, cooked in oil, or added to soup, stir fries or quiches. *Enteromorpha linza* (L.) J. Ag. is known by the common name sea lettuce, is an edible green alga in the division Chlorophyta. The species is found in bays and river mouths around Korea, and are also found in many other parts of the world, including Europe and North America. It can thrive in both salt and brackish waters and is usually found at the top of the sublittoral zone. It contains about 20 percent protein, little fat, low sodium and high iron and calcium. Its vitamin B-group content is generally higher than most vegetables and while its vitamin A is high, it is only half of that found in spinach. It was and is collected from natural sources, but careful cultivation can ensure greater uniformity and better colour (green is good, greener is better). Seaweeds are considered to be a rich source of antioxidants.[6-8] Algal biomass and algae-derived compounds have a very wide range of potential applications for human nutrition and health products.

Noodles are a staple food in many cultures made from unleavened dough which is stretched, extruded, or rolled flat and cut into one of a variety of shapes. Instant noodles are widely consumed in Asian countries. The Korean population consumed the largest quantity of instant noodles in the world in 2008.[9] Consuming instant noodles may lead to excessive intake of energy, fats, and sodium but may also cause increased intake of thiamine and riboflavin. Therefore, nutritional education helping adults to choose a balanced meal while consuming instant noodles should be implemented. If the noodles are made with seaweeds, it would be good for the health because the various kinds of seaweed, sea mustard and kelp are particularly rich in nutrients such as calcium, iron and zinc; all of which are often missing from the diets of modern people. The purpose of the present study is to evaluate edible seaweed extracts as sources of natural antioxidants for DPPH and OH radical to examine whether the extractions of seaweeds are not losing significant OH activity during cooking noodles.

MATERIALS AND METHODS

Sample extract

Porphyra tenera Kjellman, *Undaria pinnatifida* (Harvey) Suringar, *Sargassum fusiforme* (Harv.) Setch., *Enteromorpha linza* (L.) J. Ag. were collected from Namhae-gun and Busan district in Korea. The algae samples were washed, shade dried. The alga were ground with pestles and liquid nitrogen at -70°C and homogenized prior to beginning extraction experiments for the fine powder. With water and foodstuffs it is assumed that the inevitable solvent residue is not harmful. The ground powders were dissolved in 1000 ml ethanol and treated with ultrasound at room temperature for three hours. The ultrasound extraction was carried out using an ultrasonic bath (5510, Branson, USA) to increase the permeability of cell walls and produces cavitation. The mixture was further stirred with a magnetic bar at 65°C for 12 hours. Extracted sample was filtered with Whatman filter paper No. 1. The sample was evaporated to remove solvent under reduced pressure and controlled temperature by using rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan). To get dry powder, samples placed in a low temperature vacuum chamber. The powdered plant material was weighed and a powder sample lyophilized was used in the experiment.

Preparation of noodles

One cup flour, each extraction solution of algae with or without 3.5% teaspoon salt were mixed and dissolve everything together well. Dough usually uses about 3.5% salt for gluten formation. And then they are gently mixing and kneading. The liquid was covered with cloth and let rest for 30 minutes. To get homemade noodles to the desired thickness was by passing the dough through a pasta machine. The dough cut the strips into 0.5 cm-wide strips and 20- to 30-cm lengths. The antioxidant activity of the seaweed extracts was measured on the basis of the scavenging activity before and after to a boil to cook noodles.

DPPH free radical

The great diversity of methods and modifications is evident from its different names. 1,1-Diphenyl-2-picrylhydrazyl (DPPH; I) is a stable free radical. The antioxidant activity of the seaweed extracts was measured on the basis of the scavenging activity of DPPH free radical according to the method described by Brand-Williams et al.[10] with slight modifications. DPPH free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol.[11] 1 ml of 0.1 mM DPPH solution in ethanol was mixed with 1 ml of the previous algae extracts of various concentrations (0.1, 1.0, 2.0, 4.0 and 8.0 mg/ml). DPPH was added to the solutions prepared with algae extracts and standard antioxidant substances and stirred. A solution of DPPH was prepared by dissolving 5 mg DPPH in 2 ml of ethanol and the solution was kept in the dark at 4°C. A stock solution of the compounds was prepared at 1 mg/ml in DMSO. The stock solution was diluted to varying concentrations in 96-well microplates. Then, 5 µL of ethanol DPPH solution (final concentration 300 µM) was added to each well. The plate was shaken to ensure thorough mixing before being wrapped with aluminum foil and placed into the dark. The radical scavenging reaction was carried out at 37ºC in dark for 30 min. The optical density (OD) of the solution was read using the UVmini-1240 Reader (Shimadzu, Kyoto, Japan) at the wavelength 515 nm. Corresponding blank sample was prepared and L-Ascorbic acid (1.0 µg/ml) was used as reference standard (positive control). The inhibition % was calculated using the following formula.

% Inhibition =[1 -OD (DPPH + sample)/OD (DPPH)] x100%.

The 50% inhibition (IC50) is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. A dose response curve was plotted to determine IC50 values.

To determine the IC50 value of the active component, the technique using 96-well microplates was employed.[12]

Statistical analysis

All measurements of free radical scavenging activity were performed in triplicate and standard deviation was calculated. Differences were tested with analysis of variance (ANOVA) followed by multiple comparison test. Correlation co-efficient (R) to determine the relationship between two or more variables among Radical Scavenging activity tests were calculated using the SPSS software (Release 21.0).

RESULTS AND DISCUSSION

As the extract concentrations of algae increase, the inhibition of DPPH scavenging activity also increase. DPPH scavenging activity of extracts of *P. tenera* was evaluated 8.2 at 0.1 mg/ml and 27.0% at 8.0 mg/ml (Table 1). *U. pinnatifida* and *E. linza* were showed similar inhibition of DPPH scavenging activity. *S. fusiforme* was showed the highest inhibition activity of DPPH among four alga. DPPH scavenging activities of extracts of *S. fusiforme* were evaluated 19.2% at 0.1 mg/ml and 69.3% at 8.0 mg/ml. The overall values of DPPH activity of *S. fusiforme* were higher than those of three other algae species and there were show a statistically significant difference ($p > 0.05$). When the L-Ascorbic acid used as a control, relative DPPH scavenging activities of *P. tenera*, *U. Pinnatifida*, *S. fusiforme* and *E. linza* extracts were 30.3%, 16.5%, 95.6% and 20.1%, respectively (Fig. 2).

Dough usually uses about 3.5% salt for gluten formation. The DPPH scavenging activities of dough which was instant noodles mixed with *P. tenera* and 3.5% salt were 7.2% at 0.1 mg/ml and 18.6% at 8.0 mg/ml (Table 2). DPPH scavenging activities of mixed dough with extracts of *S. fusiforme* were evaluated 17.9% at 0.1 mg/ml and 56.8% at 8.0 mg/ml. The overall values of DPPH activity of mixed dough were lower than those of pure algae extracts. Antioxidant was measured after boiling for 5.0 minutes. The DPPH scavenging activity of instant noodles with *P. tenera* was 6.0% (Table 3). After boiling for 5.0 minutes, DPPH scavenging activities of mixed instant noodles with extracts of *S. fusiforme* were evaluated 16.0% at 0.1 mg/ml and 54.0% at 8.0 mg/ml. The DPPH inhibitory activity of *E. linza* (IC_{50} = 153.1 ug/ml) was at the same levels as that of L-ascorbic acid (IC_{50} 1.0 ug/ml). The 50% inhibition of *S. fusiforme* showed much low value (IC_{50} = 66.5 ug/ml), followed by *P. tenera* activity (IC_{50} = 124.4 ug/ml) (Fig. 3).

Many studies have been done to determine antioxidant capacity in seaweeds and some researchers have stated high scavenging activity for algae species.[13-14] For example, *Sargassum siliquastrum* (20B17, 70B17), *Dictyota dichotoma* (70B1), *Sargassum coreanum* (70B16) and *Ecklonia cava* (70B26) among the brown seaweeds showed significantly high DPPH radical scavenging activity with 96%, 97%, 92%, 92%, 87%.[15] The percentages of DPPH free radical scavenging activity by *Kappaphycus alvarezii* extracts were ranged between 18.34 and 35.63%.[16] Yan et al.[17] studied the antioxidant activity of 27 species of seaweeds in particular: *Corallina pilulifera* (Corallinaceae, Corallinales), *Gelidium amansii* (Gelidiaceae, Gelidiales), *Ceramium boydenii* (Ceramiaceae, Ceramiales), *C. kondoi* (Ceramiaceae, Ceramiales), *Polysiphonia urceolata* (Rhodomelaceae, Ceramiales), *Rhodomela confervoides* (Rhodomelaceae, Ceramiales), *R. teres* (Rhodomelaceae, Ceramiales), *Gracilaria verucosa* (Gracilariaceae, Gracilariales) by DPPH and deoxyribose tests.

Consumption of antioxidant and addition of antioxidant in food materials protect the body as well as foods against these events.[6,18]

Noodles have been staple foods since ancient times in many countries all over the world. These cereal products are still increasingly popular worldwide for their convenience, nutritional properties, special flavor and taste. The application of some special ingredients with antioxidant or anticancer properties has received increasing attention. Natural pigments, such as anthocyanins, betalains and carotenoids, have recently gained increased research focus. They can enhance not only the sensory qualities of food products, but also contribute to functional and nutritional qualities due to the potential bioactivities such as antioxidant, anti-inflammation, cholesterol reduction and even anticarcinogenic effects.[19-20] However, most of them cannot be synthesized by human and must be consumed with the diet.[21] We have shown that 8.0 mg/ml weight of ethanol *S. fusiforme* extract has antioxidants for DPPH. Noodles produce oxidizing substances during the digestion process. The extract from *S. fusiforme* could show antioxidant properties during digestion.

Table 1: DPPH radical scavenging activity of four algae species at different concentrations (Mean of three replications ± standard deviation)				
Concentration (mg/mL)	***P. tenera***	***U. pinnatifida***	***S. fusiforme***	***E. linza***
0.1	8.16±2.34	4.46±0.77	19.17±3.96	3.32±0.92
0.5	12.41±1.02	5.63±1.54	26.17±3.81	5.26±2.77
1.0	13.94±1.54	6.80±0.91	40.73±4.53	6.39±2.30
2.0	16.24±1.78	8.21±1.11	52.59±4.51	8.21±0.74
4.0	20.91±0.68	11.62±0.74	63.97±4.74	12.18±0.95
8.0	26.97±1.63	14.29±2.33	69.28±3.08	16.19±3.14

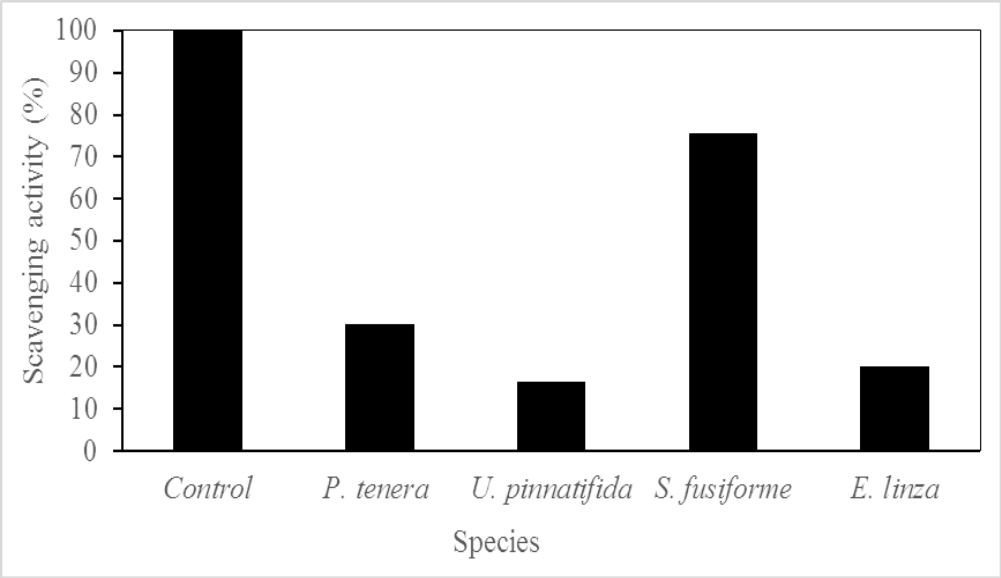

Figure 1: Relative antioxidant values of the algae extracts for control group (L-Ascorbic acid).

Table 2: DPPH radical scavenging activity of mixed noodles with four algae species at different concentrations (Mean of three replications ± standard deviation)

Concentration (mg/mL)	***P. tenera***	***U. pinnatifida***	***S. fusiforme***	***E. linza***
0.1	7.23±1.92	2.22±0.98	18.92±4.31	2.24±0.12
0.5	8.38±1.89	2.83±0.63	23.51±5.69	3.00±0.70
1.0	9.73±1.21	5.02±0.46	33.52±8.01	4.18±0.23
2.0	11.71±0.77	5.72±0.69	47.52±4.19	5.05±0.50
4.0	14.15±0.87	7.68±0.70	56.97±3.90	6.22±0.71
8.0	18.55±2.06	10.95±0.82	64.68±1.52	8.70±0.82

Table 3: DPPH radical scavenging activity of noodles with algae and 3.5% salt after 5.0 minutes for boiling times.				
Concentration (mg/mL)	***P. tenera***	***U. pinnatifida***	***S. fusiforme***	***E. linza***
0.1	7.23±1.92	2.22±0.98	16.01±3.79	2.72±0.74
0.5	8.38±1.89	2.83±0.63	22.11±3.19	3.48±0.51
1.0	9.73±1.21	5.02±0.45	34.02±3.01	4.89±0.55
2.0	11.71±0.77	5.71±0.89	39.62±0.89	6.00±1.18
4.0	14.15±0.87	7.68±0.70	47.63±4.66	7.65±1.79
8.0	18.55±2.06	10.95±0.81	53.95±4.38	10.65±1.97

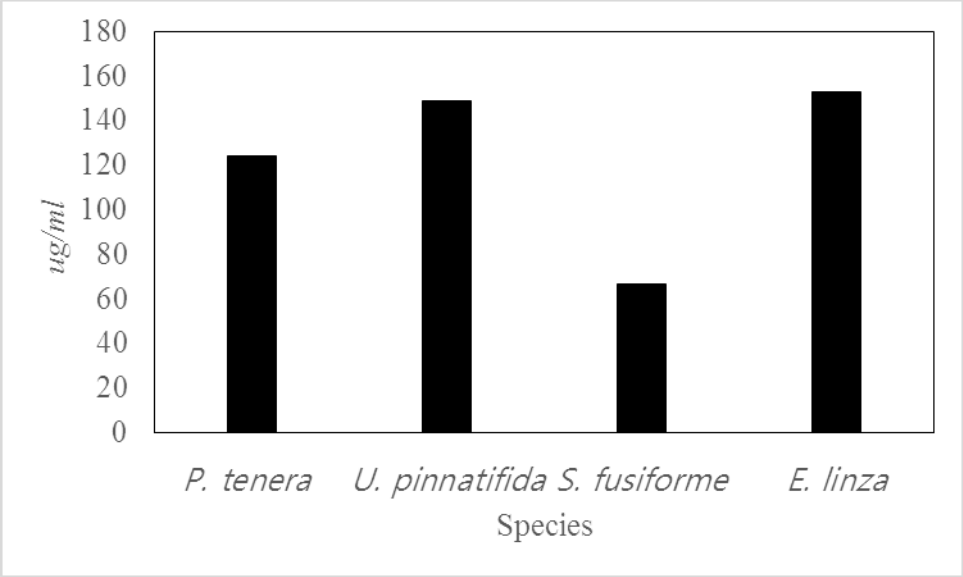

Figure 2: The 50% inhibitory effects {IC_{50} (ug/ml)} on DPPH radical activity of 1.0 M four algae species.

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