

# ABTS+ Radical, Hydroxy Radical (OH), Nitric Oxide (NO), and Ferric Ion Reducing Antioxidant Power (FRAP) Effects of Ethanol Extracts from Four Seaweed Species for Noodles

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Received July 21, 2017 / Revised September 25, 2017 / Accepted October 9, 2017

The authors evaluated the scavenging activities of ABTS+ radical, hydroxy radical (OH), nitric oxide (NO), and ferric ion reducing antioxidant power (FRAP) from ethanol extracts of four edible alga, *Enteromorpha linza*, *Porphyra tenera*, *Sargassum fusiforme*, and *Undaria pinnatifida*. ABTS+ scavenging activity was analyzed according to the method of Brand-Williams et al. ABTS+ scavenging activity of *S. fusiforme* was evaluated to 61.8% at 8.0 mg/ml. ABTS+ scavenging activity of *P. tenera* was evaluated to 35.7% at 8.0 mg/ml. *P. tenera* and *U. pinnatifida* showed similar inhibitions of ABTS+ scavenging activity. According to the results of the OH assay in seaweed, inhibitory activities were in the order of *S. fusiforme* > *P. tenera* > *U. pinnatifida* > *E. linza*. The results showed scavenging activity for NO in the following order of potency: *S. fusiforme* > *P. tenera* > *U. pinnatifida* > *E. linza* with concentration values of 8.0 mg/ml. The NO scavenging activities of dough, which was instant noodles mixed with *S. fusiforme* and 3.5% salt, were 27.2% at 8.0 mg/ml. After boiling for 5 minutes, FRAP scavenging activity of instant noodles mixed with extracts of *U. pinnatifida* was evaluated to 31.5% at 8.0 mg/ml. *S. fusiforme* showed the highest inhibition activity of ABTS+, OH, NO, and FRAP among the four algae. Thus, these findings provide evidence that *P. tenera*, *U. Pinnatifida*, *S. fusiforme*, and *E. linza* extracts could become sources of natural antioxidants.

**Key words** : ABTS+ radical, ferric ion reducing antioxidant power (FRAP), hydroxy radical (OH), nitric oxide (NO)

## Introduction

Oxidation in living organisms is essential for the generation of energy during catabolism but these metabolic processes result in the continuous production of free radicals and reactive oxygen species (ROS) *in vivo*.

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 (ABTS+) 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 [19].

The hydroxyl radical (OH) is the most reactive product of ROS formed by successive 1-electron reductions of mo-

lecular oxygen in cell metabolism and is primary responsible for the cytotoxic effects observed in aerobic organisms extending from bacteria to plants and animals [9].

Nitric oxide (NO) and reactive nitrogen species (RNS) are free radicals that are derived from the interaction of NO with oxygen or reactive oxygen species [1, 22]. Nitric oxide is classified as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals such as superoxide [3].

The ferric reducing ability of plasma (FRAP) is a measure of the antioxidant power, based on the reduction of ferrous ions by the effect of the reducing power of plasma constituents, and contributed by low molecular weight antioxidants of a hydrophilic and hydrophobic character. The low molecular weight compounds are Vitamin C, Vitamin E, bilirubin and uric acid. FRAP is said to give more biologically relevant information than that provided by individual antioxidant measurements and which may describe the dynamic equilibrium between pro-oxidants and antioxidants in the plasma [8].

In nature there are a wide variety of naturally occur-

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ring antioxidants which are different in their composition, their sources, physical and chemical properties, mechanisms of action [17]. Various different mechanisms may contribute to oxidative processes in complex systems, such as foods. 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. Particularly, seaweed has been a part of diets in China, Japan, and Korea for an age. Today those China, Japan, and the Republic of Korea are the largest consumers of seaweed as food [16]. Algae are widely available aquatic plants containing natural antioxidative compounds, having biological activities that affect the pathogenesis of several diseases, with a relatively low-cost isolation/extraction process [24]. Recent studies have shown that some species of algae contain large amounts of antioxidants and phenolic compounds [20].

Noodles are a staple food in Chinese cuisine, with a long history and wide popularity. Its first appearance can be traced back to the East Han Dynasty, and it became very popular during Song Dynasty. It come dry or fresh in a variety of sizes, shapes and textures and are often served in soups or fried as toppings. 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 [16].

The aim of present study was to estimate the ABTS+ Radical, Hydroxy Radical (OH), Nitric Oxide (NO), and Ferric Ion Reducing Antioxidant Power (FRAP) Effects and to investigate in vitro antioxidant potential of ethanolic extracts of *Porphyra tenera*, *Undaria pinnatifida*, *Sargassum fusiforme*, *Enteromorpha linza* whether the extractions of seaweeds are losed significant OH activity during cooking noodles or not.

## Materials and Methods

### Sample extract

*Enteromorpha linza* (L.) J. Ag., *Porphyra tenera* Kjellman, *Sargassum fusiforme* (Harv.) Setch. and *Undaria pinnatifida*

(Harvey) Suringar were collected from Namhae-gun and Busan district in Korea. The algae samples were washed in water and dried in an incubator. 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 1,000 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 hr. 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

A small-scale standardized laboratory procedure was used for noodle manufacturing. One cup flour, distilled water, 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 using small scale bench top electric dough mixer machine at lowest speed until a homogenous mix was achieved. 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 laminated the strips into 0.2 cm-wide strips with roll lamination machine and cut 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.

### ABTS+ free radical

The great diversity of methods and modifications is evident from its different names. 1,1-Diphenyl-2-picrylhydrazyl (ABTS+; I) is a stable free radical. The antioxidant activity of the seaweed extracts was measured on the basis of the scavenging activity of ABTS+ free radical ac-

cording to the method described by Brand-Williams et al. with slight modifications [6]. ABTS+ free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol [7Cor---]. 1 ml of 0.1 mM ABTS+ 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). ABTS+ was added to the solutions prepared with algae extracts and standard antioxidant substances and stirred. A solution of ABTS+ was prepared by dissolving 5 mg ABTS+ 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 ABTS+ 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 37°C 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 [4].

$$\% \text{ Inhibition} = [1 - \text{OD (ABTS+ + sample)} / \text{OD (ABTS+)}] \times 100\%.$$

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

To determine the IC<sub>50</sub> value of the active component, the technique using 96-well microplates was employed [15].

#### Hydroxyl radical (OH) assay

Hydroxyl radical scavenging activity of the examined compounds was measured based on the method of Halliwell et al. [9] with a slight modification according to Baliga et al. [2]. Briefly, 200 µl deoxyribose solution (2.8 mM), 200 µl H<sub>2</sub>O<sub>2</sub> (1.4 mM) and 200 µl of the examined compound (5 mM) or oxygen free water (control), were placed in a test tube. The scavenging activity for hydroxyl radicals was measured with fenton reaction. All used solutions were oxygen free. Reaction mixture contained 60 µl of 1.0 mM FeCl<sub>2</sub>, 90 µl of 1mM 1,10-phenanthroline, 2.4

mL of 0.2 M phosphate buffer (pH 7.8), 150 µl of 0.17 M H<sub>2</sub>O<sub>2</sub>, and 1.0 mL of extract at various concentrations. Adding H<sub>2</sub>O<sub>2</sub> started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured with UV visible spectrometer (Shimadzu, UV-1800, Japan). The percent inhibition was calculated as the decolourization percentage of the test sample using the following formula:

$$\text{Inhibition \%} = (\text{IA} - \text{As}) / \text{IA} \times 100$$

Where IA is the absorbance of the 100% initial and As is the absorbance of the sample. IA and As were the values which were subtracted the average absorbance of the blank wells.

#### Nitric oxide (-NO) scavenging assay

Nitrite oxide scavenging activity was measured by the method described by Kato et al. [12]. The reaction mixture contained 1 mM NaNO<sub>2</sub> 120 µl, 0.1 N HCl 840 µl, various concentrations of sample in a final volume of 1.2 ml. After reacting for 1 hr at 37°C, 1 ml of the reaction mixture was mixed with 3 ml of 2% acetic acid and 400 µl of Griess reagent (Sigma Co., St. Louis, MO), and the mixture was reacted at room temperature for 15 minutes. The Griess reagent was prepared by mixing an equal amount of 1% sulfanilic acid (Sigma Co.) and 1% naphthylamine (Sigma Co.), which were made with 3% acetic acid. Gallic acid was used as the positive control. All measurements were carried out in triplicates. Absorbance was measured at 520 nm using a spectrophotometer and the amount of remaining nitrite was measured.

#### Ferric ion reducing antioxidant power (FRAP) assay

Ferric ion reducing power was measured according to the method of Oyaizu [18]. 1 ml of various concentrations of sample and 1 ml of 1% w/v potassium ferricyanide solution were added to 1 ml of the phosphate buffer solution (0.2 M, pH 6.6), and the mixture was reacted at 50 °C for 20 minutes, and then 1 ml of 10% w/v trichloroacetic acid was added thereto. The reaction mixture was centrifuged at 12,000 rpm for 10 minutes, 1 ml of distilled water was added to 1 ml of supernatant, and 0.2 ml of 1% ferric chloride was added. Blank samples were prepared for both ethanol and deionized water extracted samples. After 10 minutes of reaction, the absorbance was measured at 700 nm. A calibration curve of ascorbic acid was established, the antioxidant capacity of the plant ex-

tracts was then expressed as mmol ascorbic acid equivalent/g dry extract.

### 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

As the extract concentrations of algae increase, the inhibition of ABTS<sup>+</sup> scavenging activity also increase (Table 1). ABTS<sup>+</sup> scavenging activity of extracts of *P. tenera* was evaluated 8.0 at 0.1 mg/ml and 35.7% at 8.0 mg/ml. *U. pinnatifida* and *P. tenera* were showed similar inhibition of ABTS<sup>+</sup> scavenging activity. *S. fusiforme* was showed the

highest inhibition activity of ABTS<sup>+</sup> among four alga. ABTS<sup>+</sup> scavenging activities of extracts of *S. fusiforme* were evaluated 16.2% at 0.1 mg/ml and 61.8% at 8.0 mg/ml. The overall values of ABTS<sup>+</sup> 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 ABTS<sup>+</sup> scavenging activities of *P. tenera*, *U. Pinnatifida*, *S. fusiforme*, and *E. linza* extracts were 45.6%, 48.2%, 78.8%, and 28.4%, respectively (Fig. 1). Dough usually uses about 3.5% salt for gluten formation. The ABTS<sup>+</sup> scavenging activities of dough which was instant noodles mixed with *P. tenera* and 3.5% salt were 5.8% at 0.1 mg/ml and 29.8% at 8.0 mg/ml (Table 5). *U. pinnatifida* was 29.5% at 8.0 mg/ml. *S. fusiforme* was evaluated 37.5% at 8.0 mg/ml. *E. linza* was 15.3% at 8.0 mg/ml. Antioxidant was measured after boiling for 5.0 minutes. The overall values of ABTS<sup>+</sup> activity of mixed dough were lower than those of pure algae extracts. The 50% inhibition of *S. fusiforme* showed much low value ( $IC_{50} = 112 \text{ ug/ml}$ ), followed by *P. tenera* activity ( $IC_{50} = 175 \text{ ug/ml}$ ) (Fig. 2). The  $IC_{50}$  values of *U.*

Table 1. ABTS<sup>+</sup> radical scavenging activity of four algae species at different concentrations (Mean of three replications  $\pm$  standard deviation)

Concentration (mg/ml)	<i>P. tenera</i>	<i>U. pinnatifida</i>	<i>S. fusiforme</i>	<i>E. linza</i>
0.1	8.04 $\pm$ 1.22	10.17 $\pm$ 1.65	16.15 $\pm$ 1.42	6.81 $\pm$ 1.08
0.5	12.46 $\pm$ 1.36	14.20 $\pm$ 1.34	25.04 $\pm$ 1.72	9.51 $\pm$ 0.61
1.0	16.96 $\pm$ 1.40	18.65 $\pm$ 2.20	34.83 $\pm$ 1.65	13.65 $\pm$ 1.10
2.0	23.11 $\pm$ 2.93	27.33 $\pm$ 1.85	40.70 $\pm$ 2.71	16.60 $\pm$ 1.34
4.0	30.08 $\pm$ 1.21	31.27 $\pm$ 2.82	49.75 $\pm$ 4.10	19.62 $\pm$ 1.97
8.0	35.74 $\pm$ 1.57	37.87 $\pm$ 2.02	61.80 $\pm$ 4.66	22.24 $\pm$ 2.27

F-test

F = 3.279,  $p>0.05$

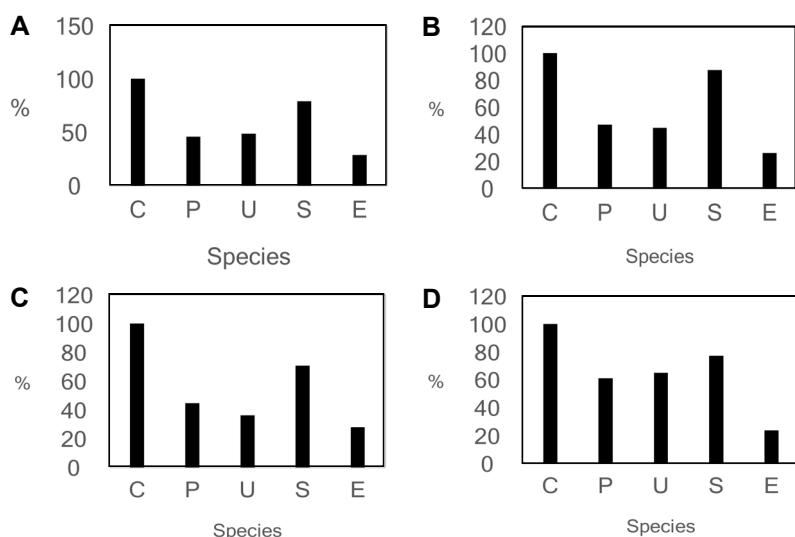


Fig. 1. Relative antioxidant values of the *Porphyrira tenera* (A), *Undaria pinnatifida* (B), *Sargassum fusiforme* (C), and *Enteromorpha linza* (D) extracts for control group: L-Ascorbic acid, H<sub>2</sub>O<sub>2</sub>, Gallic acid, and L-Ascorbic acid, respectively. C: control, P: *Porphyrira tenera*, U: *Undaria pinnatifida*, S: *Sargassum fusiforme*, E: *Enteromorpha linza*.

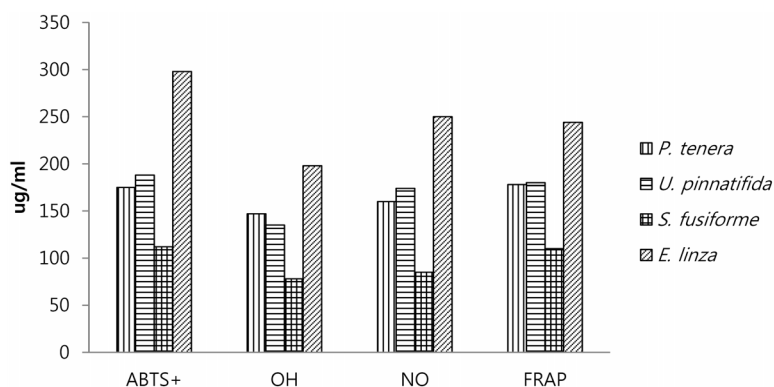


Fig. 2. The 50% inhibitory effects {IC<sub>50</sub> (ug/ml)} on ABTS+, OH-, NO-, and FRAP radical activity of 1.0 M four algae species.

*Pinnatifida* and *E. linza* were 188 ug/ml and 298 ug/ml, respectively.

### OH scavenging assay

Table 2 was shown the activity of hydroxyl radicals on various concentration of four alga extracts. The highest OH activity was recorded in *S. fusiforme* extract among four algae. The overall values of OH activity for four algae were show a statistically non-significant difference ( $p < 0.05$ ). OH activity of *P. tenera* was 35.4% at 8.0 mg/ml and *U. Pinnatifida*, *U. pinnatifida* and *S. fusiforme*, and *E. linza* were 33.5%, 65.9%, and 19.7% at same concentration, respectively. After boiling for 5.0 minutes, OH scavenging activities of mixed instant noodles with extracts of *S. fusiforme* were evaluated 6.0% at 0.1 mg/ml and 32.5% at 8.0

mg/ml (Table 6). When the H<sub>2</sub>O<sub>2</sub> used as a control, extract for stems of *S. fusiforme* was 87.2% effects on the activation of OH and those of *P. tenera*, *U. Pinnatifida*, and *E. linza* were 46.9%, 44.4%, and 26.1% (Fig. 1). *S. tuberosum* showed maximum inhibition of OH activity (IC<sub>50</sub> = 78 ug/ml) (Fig. 2). The IC<sub>50</sub> values of *P. tenera*, *U. Pinnatifida*, and *E. linza* were 147 ug/ml, 135 ug/ml, and 198 ug/ml, respectively.

### NO scavenging assay

The nitrite radical scavenging assay was carried out on the water extracts from a concentration range of 0.1 to 8.0 mg/ml (Table 3). After boiling for 5.0 minutes, NO scavenging activities of mixed instant noodles with extracts of *S. fusiforme* were evaluated 7.5% at 0.1 mg/ml and 27.2%

Table 2. OH- radical scavenging activity of four algae species at different concentrations (Mean of three replications  $\pm$  standard deviation)

Concentration (mg/ml)	<i>P. tenera</i>	<i>U. pinnatifida</i>	<i>S. fusiforme</i>	<i>E. linza</i>
0.1	8.13 $\pm$ 1.79	8.08 $\pm$ 1.59	9.25 $\pm$ 1.98	4.73 $\pm$ 0.72
0.5	13.23 $\pm$ 2.50	13.14 $\pm$ 2.85	20.34 $\pm$ 1.74	7.49 $\pm$ 1.23
1.0	19.81 $\pm$ 3.04	18.02 $\pm$ 3.64	28.44 $\pm$ 2.95	12.50 $\pm$ 1.84
2.0	25.60 $\pm$ 2.22	23.12 $\pm$ 2.63	35.24 $\pm$ 1.19	15.49 $\pm$ 1.90
4.0	30.52 $\pm$ 2.42	29.37 $\pm$ 2.78	52.53 $\pm$ 2.74	17.06 $\pm$ 1.84
8.0	35.42 $\pm$ 2.20	33.53 $\pm$ 1.32	65.87 $\pm$ 2.25	19.68 $\pm$ 1.78
F-test	F = 2.319, $p < 0.05$			

Table 3. NO- radical scavenging activity of four algae species at different concentrations (Mean of three replications  $\pm$  standard deviation)

Concentration (mg/ml)	<i>P. tenera</i>	<i>U. pinnatifida</i>	<i>S. fusiforme</i>	<i>E. linza</i>
0.1	7.04 $\pm$ 0.96	6.88 $\pm$ 1.68	13.92 $\pm$ 1.03	7.08 $\pm$ 0.78
0.5	14.45 $\pm$ 3.26	11.33 $\pm$ 0.78	21.98 $\pm$ 2.18	9.66 $\pm$ 1.99
1.0	20.58 $\pm$ 2.61	18.95 $\pm$ 3.31	30.13 $\pm$ 2.10	12.07 $\pm$ 1.66
2.0	23.30 $\pm$ 2.51	23.73 $\pm$ 1.60	39.80 $\pm$ 3.16	16.61 $\pm$ 2.02
4.0	28.46 $\pm$ 2.29	26.73 $\pm$ 1.36	46.86 $\pm$ 2.77	19.08 $\pm$ 1.74
8.0	34.97 $\pm$ 2.56	28.34 $\pm$ 2.19	55.41 $\pm$ 2.46	21.79 $\pm$ 1.79
F-test	F = 3.001, $p > 0.05$			

at 8.0 mg/ml (Table 7). *P. tenera* was 11.7% at 8.0 mg/ml and *U. pinnatifida* was only 9.3% at same concentration. *E. linza* was 10.0% at 8.0 mg/ml. The relative percentage radical scavenging of the nitrite radical by the ethanol extracts of algae and Gallic acid is shown in Fig. 1. The four alga extracts exhibited less free radical scavenging capacity than the Gallic acid. *S. tuberosum* showed maximum inhibition of NO activity ( $IC_{50}$  = 85 ug/ml) (Fig. 2). The  $IC_{50}$  values of *P. tenera*, *U. Pinnatifida*, and *E. linza* were 160 ug/ml, 174 ug/ml, and 250 ug/ml, respectively.

#### Ferric ion reducing antioxidant power (FRAP) assay

The results (Table 4) showed that FRAP values were higher in *S. fusiforme* samples compared to other algae. There was a significant difference at  $p < 0.05$ . *E. linza* was showed the lowest inhibition activity of FRAP among

four alga. After boiling for 5.0 minutes, FRAP scavenging activities of mixed instant noodles with extracts of *P. tenera* were evaluated 7.9% at 0.1 mg/ml and 31.2% at 8.0 mg/ml (Table 8). *U. pinnatifida* and *P. tenera* were showed similar inhibition of FRAP scavenging activity. The FRAP inhibitory activity of *E. linza* ( $IC_{50}$  = 244 ug/ml) was at the same levels as that of L-ascorbic acid ( $IC_{50}$  1.0 ug/ml) (Fig. 2). The 50% inhibition of *S. fusiforme* showed much low value ( $IC_{50}$  = 110 ug/ml), followed by *P. tenera* activity ( $IC_{50}$  = 178 ug/ml).

#### Discussion

Seaweeds are considered to be a rich source of antioxidant. Hence, many types of seaweeds have been examined to identify new and effective antioxidant com-

Table 4. FRAP radical scavenging activity of four algae species at different concentrations (Mean of three replications  $\pm$  standard deviation)

Concentration (mg/ml)	<i>P. tenera</i>	<i>U. pinnatifida</i>	<i>S. fusiforme</i>	<i>E. linza</i>
0.1	12.43 $\pm$ 2.21	14.89 $\pm$ 2.54	14.59 $\pm$ 2.72	6.88 $\pm$ 0.97
0.5	20.02 $\pm$ 1.54	20.75 $\pm$ 1.47	22.94 $\pm$ 2.25	9.76 $\pm$ 0.85
1.0	28.53 $\pm$ 2.11	27.36 $\pm$ 2.11	35.62 $\pm$ 3.45	12.23 $\pm$ 0.12
2.0	33.55 $\pm$ 2.27	33.74 $\pm$ 2.46	40.23 $\pm$ 4.07	14.43 $\pm$ 1.45
4.0	42.74 $\pm$ 2.21	41.60 $\pm$ 3.44	49.19 $\pm$ 4.43	16.55 $\pm$ 0.92
8.0	47.92 $\pm$ 2.43	50.99 $\pm$ 3.57	60.57 $\pm$ 3.02	18.58 $\pm$ 1.16
F-test	F = 2.975, $p > 0.05$			

Table 5. ABTS+ radical scavenging activity of noodles with algae and 3.5% salt after 5.0 minutes for boiling times

Concentration (mg/ml)	<i>P. tenera</i>	<i>U. pinnatifida</i>	<i>S. fusiforme</i>	<i>E. linza</i>
0.1	5.81 $\pm$ 1.45	7.95 $\pm$ 1.12	8.56 $\pm$ 2.79	3.35 $\pm$ 0.98
0.5	9.64 $\pm$ 2.15	11.35 $\pm$ 2.08	12.84 $\pm$ 3.75	5.44 $\pm$ 1.03
1.0	13.86 $\pm$ 2.54	15.48 $\pm$ 1.70	18.97 $\pm$ 4.28	8.02 $\pm$ 1.28
2.0	18.64 $\pm$ 1.68	19.26 $\pm$ 1.63	24.64 $\pm$ 2.46	10.75 $\pm$ 0.70
4.0	25.73 $\pm$ 2.22	24.65 $\pm$ 2.43	28.88 $\pm$ 2.32	13.32 $\pm$ 1.38
8.0	29.84 $\pm$ 2.83	29.54 $\pm$ 2.80	37.51 $\pm$ 2.18	15.34 $\pm$ 1.68
F-test	F = 1.727, $p < 0.05$			

Table 6. OH- radical scavenging activity of noodles with algae and 3.5% salt after 5.0 minutes for boiling times

Concentration (mg/ml)	<i>P. tenera</i>	<i>U. pinnatifida</i>	<i>S. fusiforme</i>	<i>E. linza</i>
0.1	4.35 $\pm$ 0.44	4.13 $\pm$ 0.83	5.97 $\pm$ 1.95	2.80 $\pm$ 0.14
0.5	6.95 $\pm$ 1.16	5.81 $\pm$ 1.55	9.29 $\pm$ 2.54	3.27 $\pm$ 0.33
1.0	10.97 $\pm$ 0.92	7.71 $\pm$ 1.23	14.81 $\pm$ 2.88	4.21 $\pm$ 0.13
2.0	13.50 $\pm$ 1.64	10.67 $\pm$ 1.31	21.19 $\pm$ 1.35	6.64 $\pm$ 1.10
4.0	16.02 $\pm$ 2.42	13.53 $\pm$ 0.64	26.27 $\pm$ 2.32	9.87 $\pm$ 1.74
8.0	18.13 $\pm$ 1.40	15.56 $\pm$ 0.98	32.50 $\pm$ 3.11	11.53 $\pm$ 1.21
F-test	F = 2.788, $p > 0.05$			

Table 7. NO- radical scavenging activity of noodles with algae and 3.5% salt after 5.0 minutes for boiling times

Concentration (mg/ml)	<i>P. tenera</i>	<i>U. pinnatifida</i>	<i>S. fusiforme</i>	<i>E. linza</i>
0.1	2.99±0.59	2.36±0.35	7.45±1.58	2.87±0.72
0.5	3.33±0.35	3.06±0.89	10.98±2.27	3.75±0.65
1.0	5.22±0.61	4.53±0.72	17.55±2.74	5.60±0.29
2.0	7.63±0.79	6.40±0.78	21.02±1.95	7.48±0.17
4.0	8.69±1.23	7.53±0.64	24.94±2.23	8.67±0.33
8.0	11.73±0.46	9.31±0.58	27.17±2.47	9.97±0.92
F-test	F = 7.551, $p > 0.05$			

Table 8. FRAP radical scavenging activity of noodles with algae and 3.5% salt after 5.0 minutes for boiling times

Concentration (mg/ml)	<i>P. tenera</i>	<i>U. pinnatifida</i>	<i>S. fusiforme</i>	<i>E. linza</i>
0.1	7.85±1.32	7.07±1.75	7.90±1.21	2.59±0.78
0.5	13.07±3.06	11.73±1.66	12.46±1.65	3.54±0.95
1.0	18.73±5.34	15.07±1.99	18.13±1.19	4.61±0.89
2.0	23.38±1.49	21.07±0.83	20.95±2.48	6.34±1.07
4.0	26.68±2.58	27.91±3.04	26.15±1.30	8.11±0.34
8.0	31.16±2.07	31.53±2.15	28.62±1.28	9.14±0.62
F-test	F = 3.603, $p > 0.05$			

pounds [10, 13, 25]. For example, seaweeds, laver, sea mustard, kelp, and fusiformis possess biological activities such as antioxidant activity and tyrosinase inhibition effect [14]. *Grateloupia filicina* 20AE (85.35%) and *Polysiphonia japonica* 20ME (94.92%) exhibited the highest scavenging activities against HO·, O<sup>2</sup>·, H<sub>2</sub>O<sub>2</sub>, and DPPH free radicals [10]. In particular, Yan et al. [23] studied the antioxidant activity of 27 seaweed species: *Corallina pilulifera* (Corallinales, 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 ABTS+ and deoxyribose tests.

Approximately 40% of the total wheat flour consumption is in the form of noodles in Asia [24]. It is widely consumed in Southeast Asia, Southern China, Korea and Japan.

Commercialized yellow alkaline noodles are rich in carbohydrate, but deficient in essential nutrients such as dietary fibre, minerals, vitamins, antioxidants and proteins. Addition of non-wheat flour containing natural antioxidants, dietary fibre and minerals in food products such as noodles, pasta, and bread products represent as the alternative technique to enhance the quality and nutritional value of food products. However, only few stud-

ies concerning the dietary fibre, minerals content and the addition of antioxidants in noodles are available. Seaweeds are good sources of dietary fibre, minerals and antioxidants to improve and enhance the nutritional content of food products. 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 [5, 21].

Quality characteristics of wet noodle with *Ecklonia cava* powder has relatively better antioxidant activity than extracts from other indigenous plants in Jeju Island [11]. This study has also shown that 8.0 mg/ml weight of ethanol *P. tenera*, *U. pinnatifida*, *S. fusiforme*, and *E. linza* extracts have few antioxidants for ABTS+, OH, NO, and FRAP. In conclusion, the extracts of four seaweeds added to noodles at a final concentration of 8.0 mg/ml can increase antioxidant properties of flour noodles even though their antioxidant function is lost during cooking.

## Acknowledgement

This Research was supported by the Tongmyong University Research Grants 2017F076.

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## 초록 : 국수에 대한 4종 해조류 에탄올 추출물에 의한 ABTS+, OH 라디칼, NO 라디칼, 철 이온 환원력

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4종의 식용 조류, 참김(*Porphyra tenera*), 미역(*Undaria pinnatifida*), 툇(*Sargassum fusiforme*), 잎파래(*Enteromorpha linza*)의 에탄올 추출물에 대해 ABTS+ 소거활성, 하이드록시 라디칼(OH), 산화질소(NO), 철 이온 환원력(FRAP)을 조사하였다. ABTS+ 소거활성은 Brand-Williams 등의 방법에 따랐다. 툇의 ABTS+ 소거활성은 8.0 mg/ml 일 때 61.8%로 가장 높은 반면 잎파래는 동일 농도에서 35.7%로 가장 낮았다. 참 김과 미역은 ABTS+에 대해 유사한 활성 저해를 나타내었다. 하이드록시 라디칼의 저해활성은 툇 > 참김 > 미역 > 잎파래 순이었다. 산화질소는 8.0 mg/ml 추출물일 때 툇 > 참김 > 미역 > 잎파래 순이었다. 3.5% 소금과 4종의 조류 추출물을 첨가한 밀가루 반죽을 국수틀에 뽑아낸 후 5분간 끓였을 때 전반적으로 4개의 산화제에 대해 활성저해가 저하되었다. 툇 8.0 mg/ml를 첨가한 국수의 경우 산화질소 활성저해는 27.2%였다. 미역 8.0 mg/ml를 첨가한 국수는 철 이온 환원력이 31.5%였다. 종합적으로 툇 추출물이 ABTS+, OH, NO, FRAP에 대해 활성저해가 가장 높았다. 본 연구 결과 자연적으로 분포하는 참김, 미역, 툇, 파래가 국수의 항산화력 증진에 도움을 준다고 사료되었다.