Effects of the addition of *Hizikia fusiforme*, *Capsosiphon fulvescens*, and *Undaria pinnatifida* sporophyll on antioxidant and inhibitory potential against enzymes related to type 2 diabetes of vegetable extract

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Abstract

This study was conducted to investigate the effect of the addition of *Hizikia fusiforme*, *Capsosiphon fulvescens*, and *Undaria pinnatifida* sporophyll on the antioxidant and inhibitory potentials against key enzymes related to type 2 diabetes of a commercial vegetable extract. The nutritional quality and mineral concentration of a vegetable extract with seaweeds added were also analyzed. The addition levels of seaweed did not influence the proximate composition, whereas the calcium, sodium, potassium, magnesium, and iron concentrations significantly increased at the 5% *Hizikia fusiforme* and *Undaria pinnatifida* sporophyll addition levels. The 20% *Hizikia fusiforme* addition level significantly increased the total phenolic content and reducing power by 47.08% and 16.82%. The hydroxyl radical scavenging ability of the vegetable extract was not strengthened with the addition of seaweeds. The DPPH radical scavenging activity at the 20% *Hizikia fusiforme*, *Capsosiphon fulvescens*, and *Undaria pinnatifida* sporophyll addition levels significantly increased by 27.47%, 22.25%, and 17.27%, respectively. The vegetable extract with seaweeds added showed higher-level α-glucosidase inhibition activities, accompanied by relatively weaker α-amylase inhibition activity. In particular, at the 5% *Undaria pinnatifida* sporophyll addition level, the α-glucosidase activity was significantly inhibited by 98.26%. Overall, the results showed that the incorporation of seaweeds into a vegetable extract effectively increased the mineral concentration and improved the antioxidant and inhibitory abilities of the extract on key enzymes linked to type 2 diabetes.

Key words: vegetable extract, seaweed, antioxidant, α-amylase, α-glucosidase

Introduction

The incidence of diabetes is as high as 10% in Korea and patients with type 2 diabetes account for 90% of diabetic population (1). Despite much headway in diabetes research, the management of diabetes is still a formidable challenge in clinical practice. Previous studies have proved that decreasing the postprandial hyperglycemia is a feasible strategy to treat the early stage of type 2 diabetes. Inhibition of key enzyme for carbohydrate metabolizing such as α-amylase and α-glucosidase can effectively delay the absorption of glucose, and consequently blunt the postprandial plasma glucose rise (2,3). Natural plant products or extracts such as tea and raspberry, which exhibited significant inhibitory activities on α-glucosidase, are recommended to replace the synthetic drugs for type 2 diabetes patients to improve their postprandial hyperglycemia (4-6).

Oxidative stress occurs in the cell and plasma when the generation of reactive oxygen species (ROS) including...
seaweed has potential activities in inhibition of ROS and inflammation. Antioxidants and with very less side effects have received considerable attention compared to the synthetic antioxidant components.

The commercial vegetable extract used in our study, Ya Chae Soo, is very popular in Korea. The raw material for producing Ya Chae Soo included five edible vegetables, namely white radish (Raphanus sativus L.), carrot (Daucus carota L.), burdock (Arctium lappa), shiitake (Lentinus edodes), and radish tops. Radish, carrot, burdock, shiitake, and radish tops have been extensively studied for their nutritional characteristics, compound composition and biological activities including antioxidant, anticancer, antimicrobial, antipyretic activities and detoxifying effects (10-13). Seaweed, one of the most important marine resources, has been utilized traditionally as food supplement in Korea and other Asian countries. In recent years seaweed have been proved to contain various components, which are effective antioxidants (14) and also exhibit particular biological activities. For example, Alaria, Palmaria, and Aucophyllum extract significantly inhibited proliferation of cancer cells (15) and extract from Turbinaria omata had strong anti-inflammatory activity (16). More recently, seaweed extract with large amount of phenolic compounds have been suggested to suppress the postprandial hyperglycemia through inhibition of starch digestive enzymes (17). However, the effect of seaweed on nutritional quality and physiological activities including antioxidant and antihyperglycemia potential of commercial vegetable extract like Ya Chae Soo has not been investigated.

Therefore, in this research, the influence of addition of Hizikia fusiforme, Capsosiphon fulvescens, Undaria pinnatifida sporophyll extracts at different addition levels (5, 10 and 20%) on antioxidant and inhibitory potential against key enzymes linked to hyperglycemia of commercial vegetable extract were systematically investigated. Additionally, the nutritional qualities of vegetable extracts added with seaweed were also studied.

Materials and methods

Chemicals

Ferrous sulfate, sodium salicylate, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), hydrogen peroxide, ferric chloride, α-glucosidase from saccharomyces cerevisiae (EC 3.2.1.20), soluble starch, butylated hydroxyanisole (BHA), potassium sodium tartrate tetrahydrate, gallic acid, p-nitrophenyl-α-D-glucopyranoside, potassium ferricyanide, porcine pancreatic α-amylase, type VI-B (EC 3.2.1.1), trichloroacetic acid, Folin & Ciocalteu's phenol reagent, 3,5-dimethoxyphenol, aluminum nitrate, sodium carbonate, sodium nitrite were obtained from Sigma Chemical Co., (St. Louis, MO, USA). All other chemicals and solvents used were of standard analytical grade.

Materials and sample preparation

The commercial vegetable extract was kindly provided by Hyundai Agricultural Association (Yuwol-ri, Haeje-myeon, Muan-gun, Jeollanam-do, Korea). The Hizikia fusiforme (HF), Capsosiphon fulvescens (CF), and Undaria pinnatifida sporophylls (UPS) were collected from a clean region of Wando, Korea. Fresh seaweeds were washed with tap water and dried at 50°C. These samples were then ground and sieved to obtain fine powder. Powdered seaweed materials were mixed with distilled water (1:25, w/v) and extracted at 121°C for 3 h. The filtrate was collected in bottles after vacuum filtration (Whatman No.1 filter paper). The acquired seaweed extract was formulated with vegetable extract at three different levels including 5, 10, and 20% (Table 1).

Table 1. Formulation of vegetable extract and seaweed extract (%)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>5%</th>
<th>10%</th>
<th>20%</th>
<th>50%</th>
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</thead>
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<tr>
<td>seaweed extract</td>
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<td>100</td>
</tr>
<tr>
<td>vegetable extract</td>
<td>95</td>
<td>90</td>
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<td>0</td>
</tr>
<tr>
<td>total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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</table>

5% pure vegetable extract without seaweed, 5% vegetable extract added with 5% seaweed, 10% vegetable extract added with 10% seaweed, 20% vegetable extract added with 20% seaweed, 50% pure seaweed extract.

Determination of chemical composition

The moisture, crude fat, protein and ash contents of samples were determined in accordance with the standard methods of the AOAC (18). Carbohydrate contents were calculated as the difference of 100-(crude ash+crude protein+crude fat+moisture). The concentrations of the mineral (calcium, sodium, potassium, magnesium, iron) were determined by atomic absorption spectrophotometer (spectraAA 220 Fs, Varian Co., Mulgrave, Victoria, Australia).
**Determination of total phenolic content (TPC)**

The TPC were determined according to the standard Folin-Ciocalteau method (19). 0.1 mL of seaweed extract solution were placed in a tube, to which 7.9 mL of deionized water and 0.5 mL of Folin-Ciocalteau solution were added. 15 min later, 1.50 mL of 1.85 M sodium carbonate solution was added and the tube was kept in darkness for 2 h. Absorbance were measured at 765 nm and the TPC were obtained from standard curve of gallic acid (y=0.9977x, R²=0.9915).

**Determination of DPPH radical scavenging activity**

The DPPH radical scavenging activity was determined by the method of Yang et al. (20). Four milliliters of reaction mixture contained 2.0 mL of 0.1 mM DPPH solution and 2 mL sample solution at various concentrations. The mixtures were shaken vigorously and placed in darkness. Absorbance was measured at 517 nm after 30 min and the inhibition ability was obtained from the formula (1):

\[
\% \text{ inhibition} = \frac{[(A_0 - A_1)/A_0] \times 100}{(1)}
\]

A₀: absorbance without samples
A₁: absorbance in the presence of the samples.

**Determination of hydroxyl radical scavenging activity**

Hydroxyl radical scavenging activity was measured according to the method of Smirnoff and Cumbes (21). 0.3 mL of 20 mM sodium salicylate solution was mixed with 1.0 mL of 1.5 mM ferrous sulfate solution and 1.0 mL of seaweed extract solutions in a tube, to which 0.7 mL of 6 mM H₂O₂ were added to initiate reaction. The reaction mixture was incubated at 37°C for 1 h. The absorbance was read at 562 nm and the inhibition ability was obtained using formula (1).

**Measurement of reducing power**

The reducing power was determined by the previously described method (22). Seaweed extract solution (0.5 mL) was placed in a tube, to which 1.25 mL of phosphate buffer solution (0.2 M, pH 6.6), as well as 1.25 mL of 1% potassium ferricyanide solution were added. After incubation at 50°C for 20 min, 1.25 mL of trichloroacetic acid solution were added to the tube. 1.25 mL of supernatant obtained by centrifugation at 3,000 rpm for 10 min was diluted with 1.25 mL of deionized water. Then, 0.25 mL of 0.1% ferric chloride solution was added to complete the assay. The absorbance was determined at 700 nm and represented the reducing power.

**α-Amylase Inhibition assay**

The inhibitory potential on α-amylase was measured according to the method of Ranilla et al. (4). 0.5 mL of seaweed extract solution was placed in a tube, to which 0.5 mL of 0.5 mg/mL α-amylase solution was added. After preincubation at 25°C for 10 min, 0.5 mL of 1% soluble starch solution were added to the tube. The tubes were then kept at 25°C for 10 min. 1.0 mL of dinitrosalicylic acid color reagents was added to each tube to terminate the reaction. The tubes were allowed to keep at 100°C for 5 min. Finally the reaction mixture in the tube was diluted with 10 mL of deionized water as it cooled to room temperature. The absorbance was determined at 540 nm and the inhibition ability was obtained using formula (1).

**α-Glucosidase Inhibition assay**

Inhibition effect on α-glucosidase was evaluated by the previously described method (17) with some modification. 0.5 mL of seaweed extract solution prepared by phosphate buffer (100 mM, pH 6.9) was mixed with 0.5 mL of 5 mM p-nitrophenyl-α-D-glucopyranoside (in phosphate buffer, pH 6.9). After incubation at 37°C for 5 min, 1 mL of phosphate buffer solution containing α-glucosidase (0.1 U/mL) was added into test tube at time interval. Finally the absorbance was measured at 405 nm after incubation at 37°C for 10 min and the inhibition ability was obtained using formula (1).

**Statistical analysis**

Data were reported as mean±SD. ANOVA followed by Duncan's multiple range test was conducted using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA) to compare the data.

**Results and discussion**

**Change in proximate composition and mineral content**

As shown in Table 2, there were no significant difference between vegetable extract supplemented with seaweeds and pure vegetable extract for moisture, crude protein, crude fat, crude ash, and carbohydrate content. In contrast, mineral content was meaningfully increased in a seaweed addition level dependent manner. Potassium (K) was the predominant element present in the pure vegetable extract, followed by sodium (Na), calcium (Ca), and magnesium (Mg). The iron
(Fe) content was scarce in the pure vegetable extract (Table 3). Expect for K content of *Capsosiphon fulvescens* extract, the mineral content of *Hizikia fusiforme*, *Capsosiphon fulvescens*, and *Undaria pinnatifida* sporophyll extracts was much higher than that of pure vegetable extract. When *Hizikia fusiforme* extract was added into vegetable extract at 5% level, the content of Ca, Na, K, Mg, and Fe were significantly

### Table 2. Proximate composition of samples (%)

<table>
<thead>
<tr>
<th></th>
<th>$S_0$&lt;sup&gt;1&lt;/sup&gt;</th>
<th>$S$</th>
<th>$S_5$</th>
<th>$S_{10}$</th>
<th>$S_{20}$</th>
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<td>carbohydrate</td>
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<td>carbohydrate</td>
<td>0.59±0.02</td>
<td>0.64±0.02</td>
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<sup>1</sup>$S_0$: pure vegetable extract without seaweed, $S_5$: vegetable extract added with 5% seaweed, $S_{10}$: vegetable extract added with 10% seaweed, $S_{20}$: vegetable extract added with 20% seaweed, $S_{100}$: pure seaweed extracts.


<sup>3</sup>Data represent the mean±SD.

### Table 3. Mineral contents of sample (ppm)

<table>
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<th>$S$</th>
<th>$S_5$</th>
<th>$S_{10}$</th>
<th>$S_{20}$</th>
<th>$S_{100}$</th>
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<tbody>
<tr>
<td>Ca</td>
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<td>68.70±0.25&lt;sup&gt;6&lt;/sup&gt;</td>
<td>74.12±0.16&lt;sup&gt;6&lt;/sup&gt;</td>
<td>84.36±0.15&lt;sup&gt;6&lt;/sup&gt;</td>
<td>159.98±0.21&lt;sup&gt;6&lt;/sup&gt;</td>
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<tr>
<td>Na</td>
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<td>82.21±0.26&lt;sup&gt;6&lt;/sup&gt;</td>
<td>89.42±0.19&lt;sup&gt;6&lt;/sup&gt;</td>
<td>123.36±0.22&lt;sup&gt;6&lt;/sup&gt;</td>
<td>366.36±0.46&lt;sup&gt;6&lt;/sup&gt;</td>
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<tr>
<td>K</td>
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<td>880.72±0.69&lt;sup&gt;6&lt;/sup&gt;</td>
<td>907.83±0.53&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>1419.32±0.88&lt;sup&gt;6&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>33.57±0.12&lt;sup&gt;6&lt;/sup&gt;</td>
<td>45.43±0.14&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>79.82±0.14&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>Fe</td>
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<td>8.35±0.01&lt;sup&gt;6&lt;/sup&gt;</td>
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<th>$S_{10}$</th>
<th>$S_{20}$</th>
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<td>Ca</td>
<td>60.31±0.23&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>Na</td>
<td>74.50±0.21&lt;sup&gt;6&lt;/sup&gt;</td>
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<tr>
<td>Fe</td>
<td>4.51±0.01&lt;sup&gt;6&lt;/sup&gt;</td>
<td>5.92±0.01&lt;sup&gt;6&lt;/sup&gt;</td>
<td>6.44±0.01&lt;sup&gt;6&lt;/sup&gt;</td>
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</table>

<sup>1</sup>$S_0$: pure vegetable extract without seaweed, $S_5$: vegetable extract added with 5% seaweed, $S_{10}$: vegetable extract added with 10% seaweed, $S_{20}$: vegetable extract added with 20% seaweed, $S_{100}$: pure seaweed extracts.


<sup>3</sup>Data represent the mean±SD. Means with different letters in same row are significantly different at p<0.05.
(p 0.05) increased by 13.91, 10.34, 10.79, 35.33, and 31.26%, respectively. And when Undaria pinnatifida sporophyll extract was added into vegetable extract at 5% level, apart from Ca, the contents of Na, K, Mg, and Fe were significantly (p<0.05) increased by 27.28, 8.21, 27.67, and 41.91%, respectively. In the case of vegetable extract added with Capsosiphon fulvescens at 5% level, the concentration of Mg and Fe were significantly (p<0.05) increased by 30.27 and 28.82%. The increase in mineral content of vegetable extract can be attributed to the high mineral content of Hizikia fusiforme, Undaria pinnatifida sporophyll, and Capsosiphon fulvescens extracts.

Change in the total phenolic content

In the present study, the total phenolic content of vegetable extract which was 15.40 mg GAE/100 mL, was significantly (p<0.05) increased to 20.35 mg GAE/100 mL when supplemented by Hizikia fusiforme extract at 10% level (Fig. 1). However, significant increase of total phenolic content was not found in vegetable extract added with Undaria pinnatifida sporophyll and Capsosiphon fulvescens extracts. All of these results can be attributed to the higher total phenolic content of Hizikia fusiforme (40.29 mg GAE/100 mL) and relatively lower total phenolic contents of Capsosiphon fulvescens and Undaria pinnatifida sporophyll (12.03-17.06 mg GAE/100 mL). Son et al. (23) also reported that the total phenolic content of carrot juice was influenced by the addition level (1, 3, and 5%) of beet extract. The total phenolic content (5.49 mg GAE/100 mL) of carrot juice was increased to 11.42 mg GAE/100 mL with the addition of beet extract at 5% level.

Change in DPPH radical scavenging activity

As can be seen from Fig. 2, the vegetable extract exhibited a relatively higher DPPH scavenging ability (69.52%). This higher DPPH radical scavenging activity may be due to the higher total phenolic compound content of vegetable extract. The vegetable extract added with seaweed scavenged DPPH radical in an addition level dependent manner. With the addition of seaweed extracts, the DPPH scavenging activities were gradually increased. In particular, when Hizikia fusiforme, Capsosiphon fulvescens, and Undaria pinnatifida sporophyll were added into vegetable extract at 20% level, the DPPH radical scavenging activities were significantly (p<0.05) increased by 27.47, 22.25, and 17.27%, respectively. Previous study (24) in this area had also reported the similar addition effect (7, 14, 21, and 28%) of maca (Lepidium meyenii) on DPPH radical scavenging activity of syrup.

Change in hydroxyl scavenging activity

It can be observed that the hydroxyl radical scavenging activity of vegetable extract was good (53.64%). However, in contrast to the DPPH radical scavenging performance, when adding Hizikia fusiforme into vegetable extract, the scavenging activities on hydroxyl radical were not found to be changed at each addition level (Fig. 3). The hydroxyl radical scavenging activity of vegetable extract was increased, though not significantly, with the addition of Capsosiphon fulvescens, and Undaria pinnatifida sporophyll at 20%
addition level. In addition, Chung et al. (24) also reported that the addition of maca into syrup did not increase the hydroxyl radical scavenging activity.

Fig. 3. Hydroxyl radical scavenging activity (%).

HF, Hizikia fusiforme; CF, Capsosiphon fulvescens; UPS, Undaria pinnatifida sporophyll.

S0: pure vegetable extract without seaweed; S5: vegetable extract added with 5% seaweed; S10: vegetable extract added with 10% seaweed; S20: vegetable extract added with 20% seaweed; S100: pure seaweed extracts. Data represent the mean±SD, different letters marked above the same bar means significantly different (p<0.05).

Change in reducing power

Fig. 4 showed the reducing power, an important indicator of antioxidant ability, for vegetable extract added with seaweeds and pure vegetable extract. The reducing power of vegetable extract, in term of absorbance at 700 nm, was 1.07. The reducing power was significantly (p<0.05) increased by 16.82% when adding 20% level of Hizikia fusiforme into the vegetable extract. In contrast, addition of Capsosiphon fulvescens and Undaria pinnatifida sporophyll extracts did not significantly change the reducing power (0.91-1.01). This can be partly explained by the low or medium reducing power (0.24-0.67) of Capsosiphon fulvescens and Undaria pinnatifida sporophyll extracts and higher reducing power of Hizikia fusiforme extract (1.97).

Change in α-amylase and α-glucosidase inhibition abilities

α-Amylase and α-glucosidase inhibitors, especially these, which exhibited stronger inhibition effect on α-glucosidase and weaker inhibition effect on α-amylase, can make a big difference in the prevention and improvement of hyperglycemia (25). Different from the performance of antioxidant, which were relatively strong or medium, the inhibition activity of pure vegetable extract on type 2 diabetes related enzymes, namely α-amylase and α-glucosidase, was very weak (10.82 and 13.77%). This is in contrast to the results of earlier studies (4,6), which have revealed that plant extracts rich in total phenolic content had weaker or no inhibition effect on α-amylase and stronger inhibition effect on α-glucosidase. This may be attributed to the differences of experimental methods and phenolic compounds presented in extracts. However, with the addition of Hizikia fusiforme, Capsosiphon fulvescens, and Undaria pinnatifida sporophyll extract at 20% level, the α-amylase inhibitory activity of vegetable extract was significantly (p<0.05) increased by 97.87, 103.51, and 211.37%, respectively. In the case of α-glucosidase inhibition activity, addition of Capsosiphon fulvescens extract did not change the α-glucosidase inhibition activity of vegetable extract. When Hizikia fusiforme extract

Fig. 4. Reducing power (Abs at 700 nm).

HF, Hizikia fusiforme; CF, Capsosiphon fulvescens; UPS, Undaria pinnatifida sporophyll.

S0: pure vegetable extract without seaweed; S5: vegetable extract added with 5% seaweed; S10: vegetable extract added with 10% seaweed; S20: vegetable extract added with 20% seaweed; S100: pure seaweed extracts. Data represent the mean±SD, different letters marked above the same bar means significantly different (p<0.05).

Fig. 5. α-Amylase inhibitory activity (%).

HF, Hizikia fusiforme; CF, Capsosiphon fulvescens; UPS, Undaria pinnatifida sporophyll.

S0: pure vegetable extract without seaweed; S5: vegetable extract added with 5% seaweed; S10: vegetable extract added with 10% seaweed; S20: vegetable extract added with 20% seaweed; S100: pure seaweed extracts. Data represent the mean±SD, different letters marked above the same bar means significantly different (p<0.05).
was added into vegetable extract at 10% level, the α-glucosidase inhibition activity was significantly increased (p 0.05) by 151.42%. It is worth noting that the α-glucosidase was almost completely inhibited when Undaria pinnatifida sporophyll extract was added into the vegetable extract at 5% level. In addition, vegetable extract added with Undaria pinnatifida sporophyll had a stronger α-glucosidase inhibition activity and a weaker α-amylase inhibition activity, indicating that Undaria pinnatifida sporophyll, which are underutilized and usually damped as a fishery waste, can be used as a functional food ingredient of commercial vegetable extract to improve the type 2 diabetes related postprandial hyperglycemia.

In conclusion, our results revealed that the commercial vegetable extract had relatively low mineral contents and exhibited medium or weak antioxidant and anti-hyperglycemia activities. Addition of seaweed extracts such as Undaria pinnatifida sporophyll, Hizikia fusiforme and Capsosiphon fulvescens extracts, can significantly increase the mineral contents and improve antioxidant activity and inhibitory potential against type 2 diabetes related enzymes. Thus, this study also indicated that utilization of these seaweed extracts in the development of novel vegetable beverage would be an important step toward promoting health.

**Fig. 6. α-Glucosidase inhibitory activity (%)**

HF, Hizikia fusiforme; CF, Capsosiphon fulvescens; UPS, Undaria pinnatifida sporophyll. S0: pure vegetable extract without seaweed, S1: vegetable extract added with 5% seaweed, S10: vegetable extract added with 10% seaweed, S20: vegetable extract added with 20% seaweed, S100: pure seaweed extracts. Data represent the mean±SD; different letters marked above the same bar means significantly different (p<0.05).

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References

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