



## Antioxidant and antihypertensive activities of autolysate and enzymatic hydrolysates from yam (*Dioscorea opposita* Thunb.) *ichyoimo* tubers

Takeshi Nagai <sup>1\*</sup>, Nobutaka Suzuki <sup>2</sup>, Yasuhiro Tanoue <sup>3</sup>, Norihisa Kai <sup>3</sup> and Toshio Nagashima <sup>1</sup>

<sup>1</sup>Department of Food Science and Technology, Tokyo University of Agriculture, Hokkaido 0992493, Japan. <sup>2</sup>ITE Nagoya Research Institute, Toyoake, Aichi 4701131, Japan. <sup>3</sup>Department of Food Science and Technology, National Fisheries University, Yamaguchi 7596595, Japan. \* e-mail: t1nagai@bioindustry.nodai.ac.jp, nagatakenagatake@yahoo.co.jp

Received 5 May 2007, accepted 29 July 2007.

### Abstract

Autolysate and enzymatic hydrolysates from yam *ichyoimo* tubers were prepared by autolysis and digestion using three peptic enzymes. The potential antioxidant capacity and angiotensin I-converting enzyme (ACE) inhibitory activity were also investigated. Among these sample species, autolysate and trypsin hydrolysate exhibited extremely high antioxidant activities. In addition, high ACE inhibitory activities were detected in the same sample species similar to peptides derived from food proteins, for example fish muscle, casein, zein, sake and other food proteins. Yam *ichyoimo* tubers is an excellent source of antioxidant compounds and exhibited great efficiency in scavenging against active oxygen species. The discovery of our research is helpful to produce processed foods with high antioxidant activity and antihypertensive activity and high health benefits in food industry.

**Key words:** Yam *ichyoimo* tuber, autolysate, enzymatic hydrolysate, antioxidant activity, active oxygen species, scavenging activity, antihypertensive activity.

### Introduction

Free radicals may attack life-important molecules such as DNA and membrane lipids and play a role in the pathology of numerous chronic diseases <sup>1</sup>. Growing evidence has shown an inverse correlation between the intake of dietary antioxidants and the risk of chronic diseases such as coronary heart diseases, cancer and several other aging-associated health problems <sup>2-4</sup>. This motivates the discovery and development of novel nutraceutical ingredients and functional food products rich in natural antioxidants.

In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show the immense potential of medicinal plants used in traditional systems. Various medicinal plants have been studied using modern scientific approaches, and the results have revealed the potential of medicinal plants in the area of pharmacology <sup>5</sup>.

Generally speaking, fresh vegetables and agrifoods are the source of many pharmacologically and biologically active principles used as integrators that are nutraceuticals. The main biological activity of these nutraceuticals is associated with their action as antioxidants to prevent damage caused by reactive oxygen species (ROS) *in vivo*.

Yams are perennial trailing rhizome plants of the *Dioscorea* genus and belong to the Dioscoreaceae family. The tuber of yam (*Dioscorea* spp.) contains many nutrients such as carbohydrates, essential amino acids, vitamin C, minerals and physiologically active components (musin, polysaccharide and steroidal saponins<sup>6-15</sup>). These are consumed as a food and widely used in traditional Chinese medicines <sup>16</sup>. For example, it is also one of the popular foods in recent years because of its potential health benefits in Taiwan.

The Japanese have consumed the yam tubers as yam rice boiled with barley, grated yam soup, fried food, food in sweetened vinegar, Japanese sweets, food made from fish paste and a thickener for buckwheat flour. Yam as grated yam *tororo*, in particular, is the main art of cooking in Japan. There are few reports on yams as a functional food <sup>17, 18</sup>, although it was believed that sticky foods such as okra *Abelmoschus esculentus* (L.) Moench and taro *Colocasis esculenta* (L.) Schott as well as yam are beneficial to the health from old times.

The primary aim of the investigation was to prepare autolysate and enzymatic hydrolysates from yam *ichyoimo* tubers and to evaluate the antioxidative activities, scavenging activities against ROS and antihypertensive activities of these products.

### Materials and Methods

**Materials:** Fresh yam (*Dioscorea opposita* Thunb.) *ichyoimo* tubers were obtained from a local wholesale market, Aichi, Japan (Fig. 1) and used in this study. Pepsin from porcine stomach mucosa (EC 3.4.23.1; 1:10000, 630 units/mg), trypsin from porcine pancreas crystallized (EC 3.4.21.4; 4,500 USP units/mg), papain (EC 3.4.22.2; digestive powder; 1:350), ascorbic acid,  $\alpha$ -tocopherol, nitroblue tetrazolium salt (NBT), xanthine, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), 2-deoxy-D-ribose, linoleic acid, ACE (from bovine lung; 1U), hippuryl-L-histidyl-L-leucine as substrate peptide, and ethyl acetate for spectrochemical analysis grade were purchased from Wako Chemicals Co. Ltd. (Osaka, Japan). Xanthine oxidase from butter milk (XOD; 0.27 U/mg powder) was obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan). Other chemicals were of reagent grade.



**Figure 1.** Yam (*Dioscorea opposita* Thunb.) *ichyoimo* tuber.

**Preparation of autolysate and enzymatic hydrolysates from yam *ichyoimo* tubers:** After cleaning with water, the tubers were peeled and immediately ground using a grater.

**Autolysis:** The *tororo* were added with an equal volume of distilled water and incubated with shaking at 37°C for 1 day. After centrifugation at 50,000 x g at 20°C for 1 h, the supernatants were pooled and lyophilized.

**Pepsin digestion:** The *tororo* were added with an equal volume of distilled water, and pH of the solution was adjusted at 2.0 using HCl. The digestion was started by adding 1.0% pepsin (w/w) at 37°C for 1 day, the hydrolysis was stopped by boiling for 10 min to inactivate the enzyme. The hydrolysate was centrifuged at 50,000 x g at 20°C for 1 h to remove the residue, the supernatants were pooled, adjusted pH at 7.0, and then lyophilized.

**Trypsin digestion:** The *tororo* were added with an equal volume of distilled water, and pH of the solution was adjusted at 7.6 using NaOH. It was digested with 1.0% trypsin (w/w) at 37°C for 1 day. After digestion, the hydrolysate was boiled for 10 min and centrifuged at 50,000 x g at 20°C for 1 h. The supernatants were pooled and lyophilized.

**Papain digestion:** The *tororo* were added with an equal volume of distilled water and pH of the solution was adjusted at 7.0 using NaOH. After the digestion with 1.0% papain (w/w) at 37°C for 1 day, the hydrolysis was stopped by boiling for 10 min. The hydrolysate was centrifuged at 50,000 x g at 20°C for 1 h and the supernatants were pooled and lyophilized.

The lyophilized powders were used to prepare the sample solution (1, 10 and 100 mg/ml H<sub>2</sub>O) for the inhibition test of linoleic acid oxidation, scavenging ability tests against active oxygen species and antihypertensive activity test.

**Determination of protein content and total polyphenolics:** The protein concentration was measured as the method of Lowry *et al.*<sup>19</sup> using bovine serum albumin as standard. The contents of total polyphenolics were measured by the Folin-Ciocalteu colorimetric method using catechin as standard and the absorbance was measured at 760 nm<sup>20</sup>.

**SDS-polyacrylamide gel electrophoresis (SDS-PAGE):** SDS-PAGE was performed by the method of Laemmli<sup>21</sup> using 10% gel. Myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), bovine pancreas trypsinogen (24 kDa), soybean trypsin inhibitor (20.1 kDa) and bovine milk α-lactalbumin (14.2 kDa) were used as standards. After

electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250 (Fluka Fine Chemical Co., Ltd., Tokyo, Japan) and destained with 25% ethanol and 7.5% acetic acid.

**Inhibition of linoleic acid oxidation:** The antioxidant activity was assayed in a linoleic acid oxidation system. A 0.083 ml of sample solution and 0.208 ml of 0.2 M sodium phosphate buffer (pH 7.0) were mixed with 0.208 ml of 2.5% (w/v) linoleic acid in ethanol. The preoxidation was initiated by the addition of 20.8 μl of 0.1 M AAPH and carried out at 37°C for 200 min in the dark. The degree of oxidation was measured according to the thiocyanate method<sup>22</sup> for measuring peroxides by reading the absorbance at 500 nm after coloring with FeCl<sub>2</sub> and ammonium thiocyanate. A control was performed with linoleic acid but without sample solution. Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive control. Distilled water was used as negative control.

**Measurement of radical scavenging activity:** The superoxide anion radical scavenging activity assay was performed as described by Nagai *et al.*<sup>23</sup>. This system contained 0.48 ml of 0.05 M sodium carbonate buffer (pH 10.5), 0.02 ml of 3 mM xanthine, 0.02 ml of 3 mM ethylenediaminetetraacetic acid disodium salt (EDTA), 0.02 ml of 0.15% bovine serum albumin, 0.02 ml of 0.75 mM NBT and 0.02 ml of sample solution. After preincubation at 25°C for 10 min, the reaction was started by adding 6 mU XOD and carried out at 25°C for 20 min. After 20 min the reaction was stopped by adding 0.02 ml of 6 mM CuCl. The absorbance of the reaction mixture was measured at 560 nm and the inhibition rate was calculated by measuring the amount of the formazan that was reduced from NBT by superoxide. Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive control. Distilled water was used as negative control.

The hydroxyl radical scavenging activity was measured by using the method of deoxyribose. The reaction mixture contained 0.45 ml of 0.2 M sodium phosphate buffer (pH 7.0), 0.15 ml of 10 mM 2-deoxyribose, 0.15 ml of 10 mM FeSO<sub>4</sub>-EDTA, 0.15 ml of 10 mM H<sub>2</sub>O<sub>2</sub>, 0.525 ml of H<sub>2</sub>O and 0.075 ml of sample solution in Eppendorf tube. The reaction was started by the addition of H<sub>2</sub>O<sub>2</sub>. After incubation at 37°C for 4 h, the reaction was stopped by adding 0.75 ml of 2.8% trichloroacetic acid and 0.75 ml of 1.0% of TBA in 50 mM NaOH, the solution was boiled for 10 min and then cooled in water. The absorbance of the solution was measured at 520 nm. Hydroxyl radical scavenging ability was evaluated as the inhibition rate of 2-deoxy-D-ribose oxidation by hydroxyl radical<sup>23</sup>. Ascorbic acid (1 and 5 mM) and α-tocopherol (1 mM) were used as positive control. Distilled water was used as negative control.

The effect of DPPH radical was evaluated by the method of Okada and Okada<sup>24</sup> with a slight modification. The assay mixture contained 0.3 ml of 1.0 mM DPPH radical solution, 2.4 ml of 99% ethanol and 0.3 ml of sample solution. The solution was rapidly mixed in the dark and this scavenging capacity was measured spectrophotometrically at 517 nm after incubation for 30 min. Ascorbic acid (0.1 and 1.0 mM) and α-tocopherol (1 mM) were used as positive control. Distilled water was used as negative control.

**Determination of antihypertensive activity:** The ACE inhibitory activity was measured as described by Nagai *et al.*<sup>23</sup>. Twenty five

microlitres of sample solution and 75  $\mu$ l of 0.1 M sodium borate (pH 8.3) containing 5.83 mM hippuryl-L-histidyl-L-leucine and 1.0 M NaCl were preincubated at 37°C for 5 min and then incubated with 25  $\mu$ l of 0.1 M sodium borate buffer (pH 8.3) containing 1 mU ACE and 1.0 M NaCl at 37°C for 60 min. The reaction was stopped by the addition of 125  $\mu$ l of 1.0 M HCl. The resulting hippuric acid was extracted with 750  $\mu$ l of ethyl acetate by mixing intensively for 15 s. After centrifugation at 6,000 rpm for 3 min, 500  $\mu$ l of the upper layer was transported into the tube and evaporated at 80°C for 2 h. The hippuric acid was dissolved in 500  $\mu$ l of distilled water, and the absorbance was measured at 228 nm using a Perkin-Elmer model Lambda 11 (Perkin-Elmer, Tokyo, Japan) UV/VIS spectrometer. The IC<sub>50</sub> value was defined as the protein concentration of inhibitor required to inhibit 50% of the ACE inhibitory activity.

## Results and Discussion

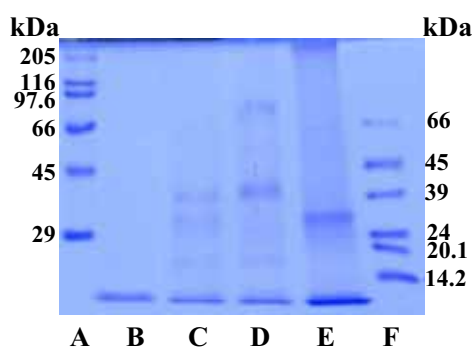
**Preparation of autolysate and enzymatic hydrolysates from ichyoimo tubers:** The autolysate was prepared from *ichyoimo* tubers by autolysis, and the hydrolysates were by digestion using three kinds of enzymes such as pepsin, trypsin and papain for only 1 day. The yields of these lyophilized powders were low and were as follows: 2.0% for autolysate, 3.0% for pepsin hydrolysate, 4.0% for trypsin hydrolysate and 3.5% for papain hydrolysate on the basis of fresh tuber, respectively. The protein contents were as follows: 61.8  $\mu$ g/mg sample powder for autolysate, 53.6  $\mu$ g/mg sample powder for pepsin hydrolysate, 66.3  $\mu$ g/mg sample powder for trypsin hydrolysate and 64.7  $\mu$ g/mg sample powder for papain hydrolysate, respectively (Table 1). The contents of total phenols were as follows: 6.5  $\mu$ g/mg sample powder for autolysate, 18.2  $\mu$ g/mg sample powder for pepsin hydrolysate, 13.0  $\mu$ g/mg sample powder for trypsin hydrolysate and 7.4  $\mu$ g/mg sample powder for papain hydrolysate, respectively (Table 1). In our previous paper<sup>18</sup>, we prepared autolysate and enzymatic hydrolysates using three kinds of proteinase such as pepsin, trypsin and papain from yam *nagaiimo* tubers. As a result, these yields were low, about 0.75-2.0% on the basis of fresh tuber. Moreover, the contents of these hydrolysates were 24-31  $\mu$ g/mg sample powder<sup>18</sup>. SDS-PAGE was performed to easily compare the digestive pattern in sample species. The autolysate was perfectly digested and the protein band was not detected in this condition (Fig. 2). Trypsin hydrolysate showed the same digestive pattern as pepsin one except for the existence of the protein band with molecular weight of about 82 kDa. Nagai *et al.*<sup>17</sup> reported that a 82 kDa protein band was not shown under non-reducing conditions in SDS-PAGE. It suggests that interactions between monomeric units of dioscorin to form oligomers seem not to occur in the extract from the *D. opposita nagaiimo* tuber mucilage. On the other hand, the only protein band with a molecular weight of about 32 kDa showed in papain hydrolysate. This suggests that the 32 kDa main band may be dioscorin, the major tuber storage protein of yam species, similar to that of the *D. batatas* Decne<sup>25, 26</sup>.

**Inhibition of linoleic acid oxidation of autolysate and enzymatic hydrolysates from ichyoimo tubers:** To evaluate the inhibition effects at the initiation stage of lipid peroxidation, the antioxidant activities of autolysate and enzymatic hydrolysates from *ichyoimo* tubers were investigated *in vitro*. As a result, each sample species possessed this activity and the activity increased with increasing

**Table 1.** The contents of protein and total phenolic components of autolysate and enzymatic hydrolysates from yam *ichyoimo* tuber.

Sample species	Protein ( $\mu$ g/mg sample powder)	Total phenols ( $\mu$ g/mg sample powder)
autolysate	61.8	6.5
pepsin hydrolysate	53.6	18.2
trypsin hydrolysate	66.3	13.0
papain hydrolysate	64.7	7.4

the concentration of sample (Table 2). For 1 mg/ml sample species the activity was lower than that of 1 mM ascorbic acid. The activities for 10 mg/ml sample species were high, although the activities of these sample species did not amount to that of 5 mM ascorbic acid. On the other hand, the sample species for 100 mg/ml were the same activity as 1 mM  $\alpha$ -tocopherol, except for pepsin and papain hydrolysates. These results indicate that the highest activities possessed to prevent linoleic acid peroxidation in autolysate and enzymatic hydrolysates from *ichyoimo* tuber. Nagai *et al.*<sup>18</sup> reported that each hydrolysate from yam *nagaiimo* tubers exhibited extremely inhibition effects at the initiation stage of lipid peroxidation.



**Figure 2.** SDS-polyacrylamide gel electrophoresis of molecular weight markers, autolysate and enzymatic hydrolysates from yam *ichyoimo* tubers. A: High molecular weight markers; B: autolysate; C: pepsin hydrolysate; D: trypsin hydrolysate; E: papain hydrolysate; F: low molecular weight markers.

**Radical scavenging activities of autolysate and enzymatic hydrolysates:** Superoxide anion radical scavenging activities of autolysate and enzymatic hydrolysates from *ichyoimo* tubers were measured using xanthine/xanthine oxidase system; namely NBT method. Pepsin and trypsin hydrolysates for 1 mg/ml had the same or slightly higher activities as 1 mM ascorbic acid (Table 3). On the contrary, the activities of autolysate and papain hydrolysate were not detected in 1 mg/ml sample species. Moreover, papain hydrolysate for 10 mg/ml also did not show the scavenging activity. For 10 mg/ml sample species autolysate and trypsin hydrolysate were higher activities than that of 1 mM ascorbic acid, although these activities did not amount to that of 1 mM  $\alpha$ -tocopherol. For 100 mg/ml sample species autolysate and papain hydrolysate showed remarkably high scavenging activities and possessed the same or higher activity than that of 1 mM  $\alpha$ -tocopherol. Among these samples, trypsin hydrolysate strongly scavenged the superoxide anion radical with the same activity as 5mM ascorbic acid (Table 3). These activities tended to increase with an increasing degree of the content of the sample.

**Table 2.** Antioxidant activities of autolysate and enzymatic hydrolysates from yam *ichyoimo* tuber.

Time (min)	Absorbance at 500 nm															
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	CN
0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
50	0.089	0.030	0.000	0.077	0.032	0.000	0.062	0.039	0.000	0.094	0.066	0.033	0.022	0.016	0.006	0.379
100	0.253	0.134	0.053	0.256	0.096	0.027	0.186	0.067	0.018	0.448	0.129	0.049	0.135	0.032	0.025	0.715
200	0.650	0.211	0.037	0.611	0.184	0.060	0.516	0.121	0.041	0.922	0.276	0.102	0.469	0.090	0.028	1.406

(A) 1 mg/ml autolysate; (B) 10 mg/ml autolysate; (C) 100 mg/ml autolysate; (D) 1 mg/ml pepsin hydrolysate; (E) 10 mg/ml pepsin hydrolysate; (F) 100 mg/ml pepsin hydrolysate; (G) 1 mg/ml trypsin hydrolysate; (H) 10 mg/ml trypsin hydrolysate; (I) 100 mg/ml trypsin hydrolysate; (J) 1 mg/ml papain hydrolysate; (K) 10 mg/ml papain hydrolysate; (L) 100 mg/ml papain hydrolysate; (M) 1 mM ascorbic acid; (N) 5 mM ascorbic acid; (O) 1 mM  $\alpha$ -tocopherol; (CN) control.

Hydroxyl radical scavenging activities of autolysate and enzymatic hydrolysates were measured using the Fenton reaction system. As a result, each sample species exhibited high scavenging activity and the activity increased with increasing concentration of the sample (Table 3). For only 1mg/ml concentration the sample species showed extremely high activities in comparison to 1 mM  $\alpha$ -tocopherol. On the other hand, the sample species of 100 mg/ml had an activity of about 87-93%, and the activities were fairly higher than that of 1 mM  $\alpha$ -tocopherol (Table 3).

DPPH radical scavenging activities of autolysate and enzymatic hydrolysates were measured and compared with those of ascorbic acid and  $\alpha$ -tocopherol. As a result, most of 1 mg/ml sample species showed no activities or low ones as well as that of 0.1 mM ascorbic acid (Table 3). With increasing the concentration of sample the activities increased. For 10 mg/ml autolysate and trypsin hydrolysate showed the same or higher activities than that of 1 mM ascorbic acid. On the contrary, sample species for 100 mg/ml exhibited higher activities and these scavenged it about 61-80%, although each activity did not amount to those of 1 mM  $\alpha$ -tocopherol (Table 3).

**Table 3.** Superoxide anion radical, hydroxyl radical and DPPH radical scavenging activities of autolysate and enzymatic hydrolysates from yam *ichyoimo* tuber.

Sample	Scavenging activity (%)		
	Superoxide anion radical	Hydroxyl radical	DPPH radical
A	0.0	82.7	0.0
B	36.5	84.8	31.1
C	173.9	91.0	74.9
D	7.3	81.4	0.0
E	7.6	86.2	14.7
F	29.3	90.4	58.9
G	19.5	77.3	1.2
H	22.6	78.4	42.7
I	90.9	92.9	80.2
J	0.0	80.5	5.3
K	0.0	85.6	22.8
L	53.8	87.2	61.4
M	14.7	13.2	3.1*
N	89.9	17.6	34.1**
O	52.6	67.6	87.6
CN	0.0	0.0	0.0

(a) See sample nomenclature in Table 2. \*0.1 mM ascorbic acid; \*\*1.0 mM ascorbic acid.

**ACE inhibitory activities of autolysate and enzymatic hydrolysates:** ACE inhibitory activities of autolysate and enzymatic hydrolysates from *ichyoimo* tuber were measured and the results were indicated as IC<sub>50</sub> value. The activities of sample species were as follows: 1.33 mg protein/ml for autolysate, 2.68 mg protein/ml

for pepsin hydrolysate, 0.53 mg protein/ml for trypsin hydrolysate and 5.10 mg protein/ml for papain hydrolysate, respectively (Table 4). On the other hand, the hydrolysates from yam *nagaimo* tubers also possessed high ACE inhibitory activities as follows: 2.1 mg protein/ml for autolysate, 0.6 mg protein/ml for pepsin hydrolysate, 0.5 mg protein/ml for trypsin hydrolysate and 11.7 mg protein/ml for papain hydrolysate, respectively<sup>18</sup>.

**Table 4.** ACE inhibitory activities of autolysate and enzymatic hydrolysates from yam *ichyoimo* tuber.

Sample species	IC <sub>50</sub> (mg protein/ml)
autolysate	1.33
pepsin hydrolysate	2.68
trypsin hydrolysate	0.53
papain hydrolysate	5.10

It is well-known that yam species tuber contains main ingredients as follows: water 82.6% (*nagaimo*), 71.1% (*ichyoimo*), 68.8% (*jinenjyo*); proteins 2.2% (*nagaimo*), 4.5% (*ichyoimo*), 2.8% (*jinenjyo*); lipids 0.3% (*nagaimo*), 0.5% (*ichyoimo*), 0.7% (*jinenjyo*); carbohydrates 13.9% (*nagaimo*), 22.6% (*ichyoimo*), 26.7% (*jinenjyo*); and ash 1.0% (*nagaimo*), 1.3% (*ichyoimo*), 1.0% (*jinenjyo*), respectively<sup>27</sup>. In particular, it contains relatively high contents of proteins in *ichyoimo* tubers and its values were about twice as much as those of *nagaimo*. From this reason, it could be obtain a large amount of hydrolysates from yam *ichyoimo* tubers in comparison to *nagaimo* in this study.

It was reported that dioscorin, the storage protein of yam tuber, accounted for about 90% of extractable water-soluble proteins from different yam species such as *D. batatas*, *D. alata* and *D. pseudojaponica* using an immunostaining method<sup>28</sup>. In addition, Hoe *et al.*<sup>26</sup> reported that dioscorin obtained from these yam species exhibited high antioxidative activities. In recent paper, it was found that mucilage in yam *D. batatas* tubers also showed antioxidative activities<sup>29</sup>.

In the present study, it was found that autolysate and enzymatic hydrolysates from yam (*D. opposita*) *ichyoimo* tubers exhibited high antioxidative activities and ROS scavenging activities. Among these hydrolysates autolysate and trypsin hydrolysate possessed strongly antioxidative activities and scavenging activities against active oxygen species. Moreover, each hydrolysate from yam *ichyoimo* tubers showed high ACE inhibitory activity. Autolysate and trypsin hydrolysates, in particular, exhibited strong ACE inhibitory activities and these activities were similar to those from peptides derived from fish muscle<sup>30,31</sup>, casein<sup>32</sup> and other food proteins<sup>33-36</sup>. At present it is not clear whether dioscorin in yam *ichyoimo* tubers contributes to high antioxidative activity, active oxygen species scavenging activity and ACE inhibitory activity. Furthermore, it was still unknown whether these functional properties in yam species tubers were connected with the

intensities of viscosity of each tubers. However, it is possible to play an important role to these functional properties in order to account for about 90% of extractable water-soluble proteins in yam species tubers. Further studies on detailed identification of some compounds related to viscosity followed by studies of the yam *ichyoimo* bioactivity *in vivo* (clinical trials) and *in vitro* (anticancer and antihypertensive activities) are progress. In the future, yam *D. opposita ichyoimo* tubers will be increasingly regarded as a health-promoting food.

#### Acknowledgement

We gratefully acknowledge Mrs. Junko Nagai and Mrs. Kayo Nagai for supplying yam *ichyoimo* samples for the study.

#### References

- <sup>1</sup>Young, I. S. and Woodside, J.V. 2001. Antioxidants in health and diseases. *J. Clin. Pathol.* **54**:176-186.
- <sup>2</sup>Hertog, M.G., Feskens, E. J., Hollman, P. C., Katan, M.B. and Kromhout, D. 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen elderly study. *Lancet* **342**:1007-1011.
- <sup>3</sup>Block, G., Patterson, B. and Subar, A. 1992. Fruit, vegetable and cancer prevention: A review of the epidemiological evidence. *Nutr. Cancer* **18**:1-29.
- <sup>4</sup>Steinmetz, K. A. and Potter, J. D. 1996. Vegetables, fruits, and prevention: A review. *J. Am. Diet. Assoc.* **96**:1027-1039.
- <sup>5</sup>Dahanukar, S. A., Kulkarni, R. A. and Rege, N. N. 2000. Pharmacology of medicinal plants and natural products. *Indian J. Pharmacol.* **32**:S81-S118.
- <sup>6</sup>Wanasundera, J. P. D. and Ravindran, G. 1992. Effects of cooking on the nutrient and antinutrient of yam tubers (*Dioscorea alata* and *Dioscorea esculenta*). *Food Chem.* **45**:247-250.
- <sup>7</sup>Wanasundera, J. P. D. and Ravindran, G. 1994. Nutritional assessment of yam (*Dioscorea alata*) tubers. *Plant Foods Hum. Nutr.* **46**:33-39.
- <sup>8</sup>Lape, I. M. and Treche, S. 1994. Nutritional quality of yam (*Dioscorea dumetorum* and *D. rotundata*) flours for growing rats. *J. Sci. Food Agric.* **66**:447-455.
- <sup>9</sup>Agbor-Egbe, T. and Treche, S. 1995. Evaluation of chemical composition of Cameroonian yam germplasm. *J. Food Compos. Anal.* **8**:274-283.
- <sup>10</sup>Hikino, H., Konno, C., Takahashi, M., Murakami, M., Kato, Y., Karikura, M. and Hayashi, T. 1986. Isolation and hypoglycemic activity of dioscorans A, B, C, D, E, and F; glycans of *Dioscorea japonica* rhizophors. *Planta Med.* **52**:168-171.
- <sup>11</sup>He, S., Zhan, T. and Wang, S. 1994. Study on chemistry and antioxidation activity of water soluble polysaccharides of rhizoma *Dioscoreae oppositae*. *J. Clin. Pharm. Univ.* **25**:369-372.
- <sup>12</sup>Hu, K., Dong, A., Yao, X. S., Kobayashi, H. and Iwasaki, S. 1996. Antineoplastic agents. I. Three spirostanol glycosides from rhizomes of *Dioscorea colletii* var. *hypoglauca*. *Planta Med.* **62**:573-575.
- <sup>13</sup>Hu, K., Yao, X., Kobayashi, H. and Iwasaki, S. 1997. Antineoplastic agents. II. Four furostanol glycosides from rhizomes of *Dioscorea colletii* var. *hypoglauca*. *Planta Med.* **63**:161-165.
- <sup>14</sup>Yang, D. J., Lu, T. J. and Hwang, L. S. 2003. Isolation and identification of steroidal saponins on Taiwanese yam cultivar (*Dioscorea pseudojaponica* Yamamoto). *J. Agric. Food Chem.* **51**:6438-6444.
- <sup>15</sup>Yang, D. J., Lu, T. J. and Hwang, L. S. 2003. Simultaneous determination of furostanol and spirostanol glycosides in Taiwanese yam (*Dioscorea* spp.) cultivars by high-performance liquid chromatography. *J. Food Drug Anal.* **11**:10-15.
- <sup>16</sup>Chen, Y. and Wu, Y. 1994. Progress in research and manufacturing of steroidal saponins in China. *J. Herbs, Spices Med. Plants* **2**:59-70.
- <sup>17</sup>Nagai, T., Suzuki, N. and Nagashima, T. 2006. Antioxidative activity of water extracts from the yam (*Dioscorea opposita* Thunb.) tuber mucilage *tororo*. *Eur. J. Lipid Sci. Technol.* **108**:526-531.
- <sup>18</sup>Nagai, T., Suzuki, N. and Nagashima, T. 2007. Autolysate and enzymatic hydrolysates from yam (*Dioscorea opposita* Thunb.) tuber mucilage *tororo* have antioxidant and angiotensin I-converting enzyme inhibitory activities. *J. Food Agric. Environ.* **5**(1):39-43.
- <sup>19</sup>Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
- <sup>20</sup>Slinkard, K. and Singleton, V. L. 1977. Total phenol analysis. *Am. J. Enol. Viticult.* **28**:49-55.
- <sup>21</sup>Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**:680-685.
- <sup>22</sup>Mitsuda, H., Yasumoto, K. and Iwai, K. 1966. Antioxidative action of indole compounds during the autoxidation of linoleic acid. *Eiyo to Shokuryo* **19**:210-214.
- <sup>23</sup>Nagai, T., Suzuki, N., Tanoue, Y., Kai, N. and Nagashima, T. 2007. Physical properties of kamaboko derived from walleye pollack (*Theragra chalcogramma*) surimi and functional properties of its enzymatic hydrolysates. *J. Food Agric. Environ.* **5**(1):76-81.
- <sup>24</sup>Okada, Y. and Okada, M. 1998. Scavenging effect of water soluble proteins in broad beans on free radicals and active oxygen species. *J. Agric. Food Chem.* **46**:401-406.
- <sup>25</sup>Hou, W-C., Liu, J-S., Chen, H-J., Chen, T-E., Chang, C-F. and Lin, Y-H. 1999. Dioscorin, the major tuber storage protein of yam (*Dioscorea batatas* Decne), with carbonic anhydrase and trypsin inhibitor activities. *J. Agric. Food Chem.* **47**:2168-2172.
- <sup>26</sup>Hou, W-C., Lee, M-H., Chen, H-J., Liang, W-L., Han, C-H., Liu, Y-W. and Lin, Y-H. 2001. Antioxidant activities of dioscorin, the storage protein of yam (*Dioscorea batatas* Decne) tuber. *J. Agric. Food Chem.* **49**:4956-4960.
- <sup>27</sup>Standard Tables of Food Composition in Japan 2003. In Kagawa, Y. (ed.). Kagawa Education Institute of Nutrition, Tokyo.
- <sup>28</sup>Hou, W-C., Chen, H-J. and Lin, Y-H. 2000. Dioscorins from different *Dioscorea* species all exhibit both carbonic anhydrase and trypsin inhibitor activities. *Bot. Bull. Acad. Sin.* **41**:191-196.
- <sup>29</sup>Hou, W-C., Hsu, F-L. and Lee, M-H. 2002. Yam (*Dioscorea batatas*) tuber mucilage exhibited antioxidant activities *in vitro*. *Planta Med.* **68**:1072-1076.
- <sup>30</sup>Kohama, Y., Matsumoto, S., Oka, H., Teramoto, T., Okabe, M. and Mimura, T. 1988. Isolation of angiotensin-converting enzyme inhibitor from tuna muscle. *Biochem. Biophys. Res. Commun.* **155**:332-337.
- <sup>31</sup>Fujita, H. and Yoshikawa, M. 1999. LKPNM: A prodrug-type ACE-inhibitory peptide derived from fish protein. *Immunopharmacology* **44**:123-127.
- <sup>32</sup>Tauzin, J., Miclo, L. and Gaillard, J. L. 2002. Angiotensin I-converting enzyme inhibitory peptides from tryptic hydrolysate of bovine  $\alpha$ S2-casein. *FEBS Lett.* **476**:369-374.
- <sup>33</sup>Kim, S. K., Byun, H. G., Park, P. J. and Shahidi, F. 2001. Angiotensin I-converting enzyme inhibitory peptide purified from bovine skin gelatin hydrolysate. *J. Agric. Food Chem.* **49**:2992-2997.
- <sup>34</sup>Wanasundara, P. K., Ross, A. R., Amarowicz, R., Ambrose, S. J., Pegg, P. B. and Shand, P. J. 2002. Peptides with angiotensin I-converting enzyme (ACE) inhibitory activity from defibrinated, hydrolyzed bovine plasma. *J. Agric. Food Chem.* **50**:6981-6988.
- <sup>35</sup>Nakagomi, K., Yamada, R., Ebisu, H., Sadakane, Y., Akizawa, T. and Tanimura, T. 2000. Isolation of acein-2, a novel angiotensin I-converting enzyme inhibitory peptide derived from a tryptic hydrolysate of human plasma. *FEBS Lett.* **467**:235-238.
- <sup>36</sup>Saiga, A., Okumura, T., Makihara, T., Katsuta, S., Shimizu, T., Yamada, R. and Nishimura, T. 2003. Angiotensin I-converting enzyme inhibitory peptides in a hydrolyzed chicken breast muscle extract. *J. Agric. Food Chem.* **51**:1741-1745.