

Antioxidant activity of the giant jellyfish *Nemopilema nomurai* measured by the oxygen radical absorbance capacity and hydroxyl radical averting capacity methods

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Abstract. The giant jellyfish *Nemopilema nomurai* (reaching sizes of up to 2 m diameter and 150 kg), which forms dense blooms, has caused extensive damage to fisheries by overloading trawl nets, while its toxic nematocysts cause dermatological symptoms. Giant jellyfish are currently discarded on the grounds of pest control. However, the giant jellyfish is considered to be edible and is part of Chinese cuisine. Therefore, we investigated whether any benefits for human health may be derived from consumption of the jellyfish in order to formulate medicated diets. Antioxidant activity of *Nemopilema nomurai* was measured using the oxygen radical absorbance capacity (ORAC) and hydroxyl radical averting capacity (HORAC) methods. Based on the results, the ORAC value of the giant jellyfish freeze-dried sample was 541 μmol trolox equivalent (TE)/100 g and the HORAC value was 3,687 μmol gallic acid equivalent (GAE)/100 g. On the other hand, the IC₅₀ value of hydroxyl radical scavenging activity measured by using the electron spin resonance method was 3.3%. In conclusion, the results suggest that the freeze-dried powder of the giant jellyfish *Nemopilema nomurai* is a potentially beneficial food for humans.

Introduction

The giant jellyfish, *Nemopilema nomurai* (Scyphozoa: Rhizostomeae), known as 'echizen kurage' in Japanese, is one of the largest jellyfish in the world's oceans, reaching sizes of up to 2 m diameter and 150 kg. Blooms of the jellyfish have caused serious damage to net fisheries in the Sea of Japan. Large blooms have been observed in Chinese coastal areas (1), and the cytotoxicity and hemolytic activity have been investigated (2). However, in Dalian, Qingdao and Shanghai the giant jellyfish is considered to be edible and is part of Chinese cuisine. In Japan, the giant jellyfish can be consumed in the restaurant of the Kamo Aquarium in Yamagata Prefecture, while in Tokyo Kasei University, an attempt was made to add freeze-dried giant jellyfish powder to Japanese 'Udon' noodles instead of salt (3). Okazaki performed an analysis of its general constituents and attempted to produce gelatin from *Nemopilema nomurai* (4).

Previously, we investigated the antioxidant activity of fish sauce (5) and 'Nikogori' gelatin gel food (6) using the alkaline luminol chemiluminescence method. Recently, we reported the antioxidant activity of fish sauce using the oxygen radical absorbance capacity (ORAC) method (7). The ORAC method was developed by Cao *et al* (8) and improved by the United States Department of Agriculture (USDA) (9). The latest database of ORAC values of foods was disclosed in the homepage of USDA (10).

Furthermore, findings of Schauss *et al* revealed the antioxidant activity of freeze-dried Amazonian palm berry using ORAC and hydroxyl radical averting capacity (HORAC) methods (11). In addition, Miller *et al* revealed that a proanthocyanidin-rich Amazonian medicinal plant has significant chondroprotective and antioxidant actions using ORAC, HORAC and peroxy-nitrite averting capacity (NORAC) methods. Additionally, these authors found that the plant directly inhibited matrix metalloproteinases and promoted the

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production of the cartilage repair factor, insulin-like growth factor 1 (IGF-1) (12). In this study, in addition to the ORAC and electron spin resonance (ESR) methods (6,7,13), we investigated the benefits for human health using the HORAC method (14) in order to assess the potential of formulating a medicated diet from the freeze-dried powder of *Nemopilema nomurai*.

Materials and methods

***Nemopilema nomurai* samples.** *Nemopilema nomurai* samples were extracted from the Sea of Japan off the coast of Tsuruoka, a city in Yamagata Prefecture, Japan, in November 2007. The sample was freeze-dried using vacuum freeze-drying equipment (SF-02 freeze cabin, Okawara Corporation, Shizuoka, Japan). Another raw edible commercial sample of giant jellyfish from the Bahraini waters, similar to the *Nemopilema nomurai*, which had been treated with 3% NaCl and sodium hydrosulfite as a bleaching agent, was provided to us by Kyushu Maruichi Foods Co., Ltd., Japan.

Chemicals. 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH), dipotassium hydrogenphosphate (K_2HPO_4), cobalt (II) chloride hexahydrate, picolinic acid, gallic acid monohydrate and hydrogen peroxide (H_2O_2) were obtained from Wako Pure Chemical, Ltd. (Osaka, Japan). Potassium dihydrogenphosphate (KH_2PO_4) was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Fluorescein sodium salt and iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) were purchased from Sigma-Aldrich Japan (Tokyo, Japan). The spin trapping reagent, 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) and 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox) were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan).

Sample preparation. For the ORAC and HORAC measurements, 2 g each of the freeze-dried and commercial samples of *Nemopilema nomurai* were homogenized with 20 ml distilled water using a polytron PT2100 (Kinematica AG, Luzern, Switzerland). The solutions were centrifuged at $2,580 \times g$ for 10 min, and the supernatant was frozen as the stock sample solution. For the ESR measurement, 2 g freeze-dried and commercial samples were each homogenized using 10 ml distilled water. Treatment procedures of the sample solutions were prepared in the same manner as described above.

Preparation of hydrophilic-oxygen radical absorbance capacity reaction solution. Hydrophilic-ORAC reaction solution was prepared as previously described (7).

Measurement of hydrophilic-ORAC. The experimental procedure of the hydrophilic-ORAC method was performed as previously described (7).

Preparation of HORAC reaction solution. Phosphate buffer was utilized as the assay (control) buffer and was prepared by combining 75 mM KH_2PO_4 and 75 mM K_2HPO_4 , adjusted to pH 7.4. Fluorescein working solution was prepared at a concentration of 6.7×10^{-2} nM by dissolving fluorescein disodium in the buffer. A cobalt solution was prepared by dissolving 47.1 mg of cobalt (II) chloride hexahydrate and 60 mg picolinic acid in 30 ml of distilled water. A 1.1 M

hydrogen peroxide solution was prepared by diluting 30% H_2O_2 solution (8.8 M) with distilled water. Gallic acid standard solutions were prepared at concentrations of 800, 600, 400, 200 and 100 μ M by dissolving gallic acid in the buffer.

Measurement of hydrophilic-HORAC. The hydrophilic-HORAC value was obtained by measuring the hydroxyl radical elimination activity of hydroxyl radicals generated from Fenton's reaction [cobalt (II) chloride hexahydrate added to hydrogen peroxide reaction mixture], and measuring the time lapse degradation of fluorescein (i.e., the rate of decrease in the intensity of fluorescence). The HORAC assay was performed in a 96-well multilabel microplate reader (Mithras LB940, Berthold Technologies GmbH & Co. KG, Germany) as described by Ou *et al.* (14). Briefly, 20 μ l each of sample buffer (obtained by appropriate dilution with assay buffer), various concentrations of gallic acid standard solution (for construction of a standard curve) or blank buffer (as a control) were placed in individual wells of a 96-well transparent microplate (Sanplatec Corp., Osaka, Japan). Fluorescein working solution (270 μ l) was added and the well was agitated at 37°C for 10 min. Subsequently, 10 μ l H_2O_2 and 10 μ l cobalt (II) chloride solutions were continuously added to each of the wells to initiate the reaction. The total volume of each reaction solution was 310 μ l. The fluorescence intensity [485 nm (excitation)/535 nm (emission)] was then measured every 1 min for 65 min at 37°C. As the reaction progressed, fluorescein was consumed and the fluorescence intensity decreased. The inhibition of fluorescence decay indicated the presence of an antioxidant.

Typical HORAC assay kinetic curves in the presence of different concentrations of gallic acid were determined. HORAC values were then determined.

The area under the kinetic curve (AUC) of the standards and samples was calculated as follows:

$$AUC = (0.5 + f_{1min}/f_{0min} + f_{2min}/f_{0min} + f_{3min}/f_{0min} + \dots + 0.5 \times (f_{65min}/f_{0min}), (f_{xmin} = \text{fluorescence reading at cycle } x \text{ min}).$$

The standard regression line was obtained by plotting the gallic acid concentration against the net $AUC_{\text{gallic acid}}$ of each concentration:

$$\text{Net } AUC_{\text{gallic acid}} = AUC_{\text{gallic acid}} - AUC_{\text{control}}, \text{ Net } AUC_{\text{sample}} = AUC_{\text{sample}} - AUC_{\text{control}} \quad (AUC_{\text{gallic acid}}, AUC \text{ in the presence of gallic acid; } AUC_{\text{control}}, AUC \text{ with blank control; } AUC_{\text{sample}}, AUC \text{ with sample buffer}).$$

The horizontal axis was regarded as the net $AUC_{\text{gallic acid}}$ and the vertical axis as the concentration of gallic acid.

The equation of $Y = ax + b$ was derived from the above data, and the values for a and b were obtained.

The final HORAC values of the samples were calculated using the equation:

$$\text{HORAC value } [\mu\text{mol gallic acid equivalent (GAE)/100 ml}] = [a \times (\text{net } AUC_{\text{sample}})] \times 100/[\text{sample}],$$

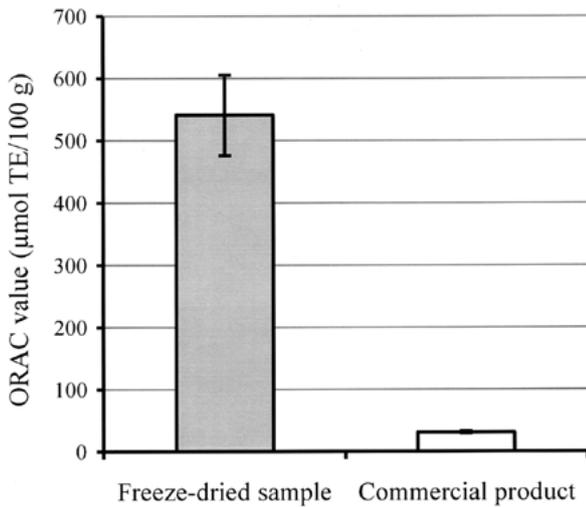


Figure 1. Hydrophilic-ORAC values [μmol trolox equivalent (TE)/100 g] representing the peroxy radical elimination capacity of freeze-dried powder and raw commercial samples of the giant jellyfish (n=3).

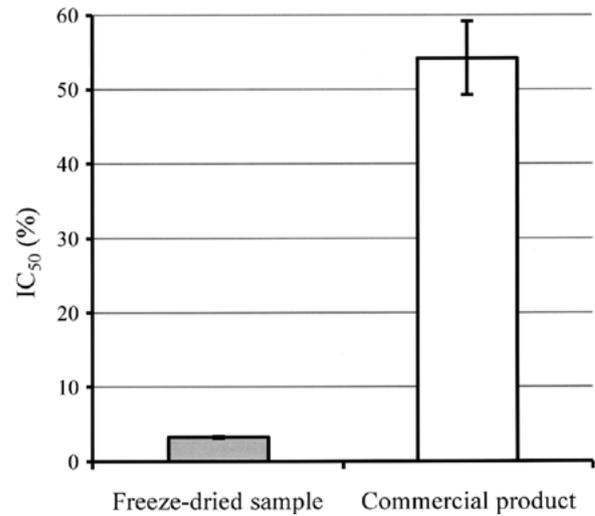


Figure 3. IC₅₀ values (%) representing the hydroxyl radical scavenging activities of freeze-dried powder and raw commercial samples of the giant jellyfish (n=3).

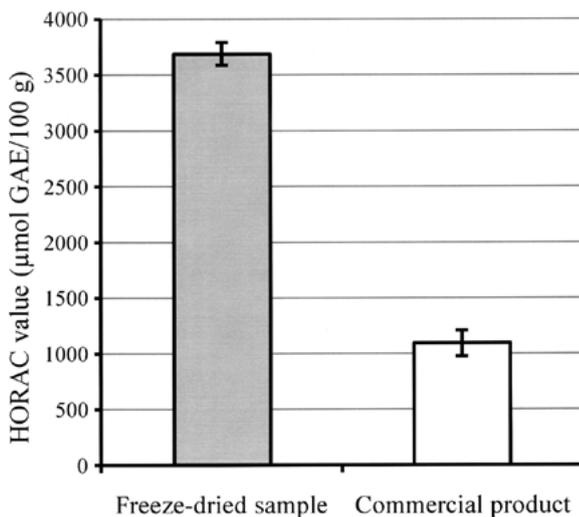


Figure 2. Hydrophilic-HORAC values [μmol gallic acid equivalent (GAE)/100 g] representing the hydroxyl radical elimination capacity of freeze-dried powder and raw commercial samples of the giant jellyfish (n=3).

where [sample] = the diluted concentration ratio of the sample.

Data were analyzed using Microsoft Excel.

Electron spin resonance analysis. ESR was performed as previously described (6,7,13). Briefly, hydroxyl radical generation was first examined using the DMPO method and iron (II) sulfate with or without the *Nemopilema nomurai* samples. The addition of 8.8 mM H₂O₂ (50 μl) to the reaction mixture (320 μl) was then used to initiate Fenton's reaction as depicted in the chemical equation: $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^-$. After 1 min of hydroxyl radical generation, spin adduct DMPO-OH \cdot was measured using an ESR spectrometer (JES-FR30; JEOL Ltd., Tokyo, Japan). ESR measurement conditions were: output, 4 mW (9.4 GHz); magnetic field,

342,790 \pm 5 mT; modulation amplitude, 0,079 mT; time constant, 0.1 sec; sweeping time, 1 min; and amplification ratio, 32-125.

Calculation of IC₅₀ value of hydroxyl radical scavenging. IC₅₀ values were defined as previously described (6,7,13).

Results and Discussion

The results of hydrophilic-ORAC values of the giant jellyfish samples are shown in Fig. 1. The ORAC value of the freeze-dried sample was 540.9 \pm 64.8 μmol trolox equivalent (TE)/100 g, and the commercial sample was 30.8 \pm 1.5, respectively. The low ORAC value of the commercial sample was due to the high body fluid content of the jellyfish in the sample. According to the USDA database, the ORAC value of the freeze-dried sample was similar to that for raw sweet corn (593 μmol TE/100 g), raw cabbage (508), raw celery (512) and raw pumpkin (414).

On the other hand, for the first time, we reported the hydrophilic-HORAC value for a fishery product in this study. The results of HORAC values of the giant jellyfish samples are shown in Fig. 2. The HORAC value of the freeze-dried sample was 3,687.4 \pm 100.9 μmol GAE/100 g, and the commercial sample was 1,093.4 \pm 116.8. Since a database for HORAC values has yet to be established, we cannot currently assess whether or not the hydroxyl radical elimination activity of the freeze-dried powder sample of the giant jellyfish is high. Therefore, the hydroxyl radical scavenging activity of the freeze-dried powder sample was measured using the ESR method. From the results shown in Fig. 3, the IC₅₀ values of hydroxyl radical scavenging activity were found to be 3.27 \pm 0.12 and 54.20 \pm 4.94% for 0.2 g/ml freeze-dried powder and raw commercial sample solutions, respectively. We observed that the IC₅₀ value of sandfish sauce obtained using the ESR method was 4.16%, and the ratio of DNA protection (%) was 60.1% (15). In addition, we noted that the average of IC₅₀ values of raw and processed sea urchin foods obtained

using the ESR method was 4.14%, and that the average of IC₅₀ values of peroxy radical scavenging activity obtained via the chemiluminescence method was 0.33% (13). The IC₅₀ value of 3.27% indicated a high hydroxyl radical scavenging activity. Therefore, it can be concluded that the HORAC value of 3,687.4 $\mu\text{mol GAE}/100\text{ g}$ suggests a high hydroxyl radical elimination activity.

In their study, Yu *et al* reported that the protein from the tentacles of the jellyfish *Rhopilema esculentum* indicated the scavenging activities of superoxide anion and hydroxyl radical (16). Zhuang *et al* reported that the collagen from *Rhopilema esculentum* alleviated the UV-induced abnormal changes of antioxidative indicators, including superoxide dismutase, glutathione peroxidase and catalase activities, on mouse skin (17). Although the *Nemopilema nomurai* jellyfish stings workers harvesting the medusae and induces cutaneous symptoms in humans, there is no significant incidence of symptoms by ingestion (18). Kim *et al* observed that the venom of the giant jellyfish produced a functional cardiac depression in mice (19). Recently, Imamichi and Yokoyama reported the purification, characterization and cDNA cloning of a novel lectin, sugar-binding protein, stemming from this jellyfish (20). Lectins are highly specific for their sugar moieties and play a role in the biological recognition phenomena involving cells and proteins.

We attempted the practical usage of freeze-dried *Nemopilema nomurai* powder as a cooking ingredient in Japanese Udon noodles, including replacing salt with the powder obtained from the giant jellyfish (3). It is common practice in Japan that Udon noodles be made from wheat, salt and water, where salt is added to alter the gluten structure and increase the viscoelasticity of the noodles. Since the giant jellyfish is directly frozen with its body fluid, the freeze-dried powder was found to be relatively salty. Consequently, this freeze-dried giant jellyfish powder may be used as a functional salt for human health benefits.

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