ejpmr, 2018, 5(11), 44-50

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ANALYSIS OF ANTIOXIDANT PROPERTIES (DPPH, OH, AND NO) OF COOKED PREMIUM ALASKA POLLOCK ROE WITH NATURAL FERMENTED SEASONING

Ji-Young Hwang[1], Jong-Soo Jang[1] and Man Kyu Huh[2]*

[1]Research and Development Institute, Deok-Hwa Food, Busan, 49277, Republic of Korea.
[2]Food Science and Technology Major, Dong-eui University, Busan 47340, Republic of Korea.

***Corresponding Author: Man Kyu Huh**

Food Science and Technology Major, Dong-eui University, Busan 47340, Republic of Korea.

Article Received on 01/09/2018 **Article Revised on 22/09/2018** **Article Accepted on 13/10/2018**

ABSTRACT

The distilled water and ethanol extracts raw materials (Alaska pollock roe), Gochujang pollock roe, and fermented seasoning pollock roe were evaluated for their antioxidant activities. The total antioxidant property was estimated by 2, 2- diphenyl - 1- picrylhydroazyl (DPPH), hydroxyl radical (OH) reducing activity, and nitric oxide radical (NO). The overall values of fermented seasoning pollock roe except Gochujang pollock roe at 1.0 mg/ml was showed the highest inhibition activity of DPPH among three treated groups. The all values of DPPH scavenging activity of ethanol extract for pollock roe were higher than those of distilled water extract for pollock roe. OH scavenging activity of distilled water extract with Gochujang pollock roe evaluated was 73.3% at 1.0 mg/ml and that of ethanol extract was 77.8% at same concentration. OH scavenging activity of distilled water extract with fermented seasoning pollock roe evaluated was 74.9% at 1.0 mg/ml and that of ethanol extract was 78.6% at same concentration. NO scavenging activity of distilled water extract of Gochujang pollock roe was evaluated 58.3% at 1.0 mg/ml and that of ethanol extract was 68.3% at same concentration. NO scavenging activity of distilled water extracts of fermented seasoning pollock roe was evaluated 62.4% at 1.0 mg/ml and that of ethanol extract was 69.4% at same concentration. Higher DPPH, OH, and NO scavenging abilities were shown in Gochujang and fermented seasoning pollock roe than instant pollock roe.

KEYWORDS: Alaska pollock roe, DPPH, hydroxyl radical (OH), nitric oxide radical (NO)

INTRODUCTION

The Alaska pollock or walleye pollock (*Gadus chalcogrammus*) is a marine fish species of the cod family Gadidae.[1] It is a semipelagic schooling fish widely distributed in the North Pacific with largest concentrations found in the eastern Bering Sea**.** While belonging to the same family as the Atlantic pollock, the Alaska pollock is not a member of the same genus, *Pollachius*. Alaska pollock was long put in its own genus, *Theragra* and classified as *Gadus chalcogrammus*, but more recent research has shown it is rather closely related to the Atlantic cod and should be moved back to genus *Gadus*, in which it was originally described.[2-3] A pollock roe is eggs of *Theragra chalcogramma*. Pollock roe is a popular culinary ingredient in Korea, Japan, and Russia. In Korea, the roe is called *myeongnan* (literally "Alaska pollock's roe"), and the salted roe is called *myeongnan-jeot* (literally "pollock roe *jeotgal*").

Antioxidants are compounds that inhibit oxidation. Antioxidants are also substances that neutralize free radicals and their actions. Oxidation is a chemical reaction that can produce free radicals. The oxidation process is one of the most important routs for producing free radicals in food, drugs and even living systems.[4] Free radicals and other reactive oxygen species generated in living organisms leads to many diseases including cancer, cardiovascular diseases, cataracts, asthma, hepatitis, liver injury and immunodeficiency diseases.[5]

Plant species still serve as a rich source of many novel biologically active compounds. Fruits, vegetables, cereal grains, edible macrofungi, microalgae, and medicinal plants are known to contain number of phenolic compounds with strong antioxidant activity.[6-7] Many researches have been taken regarding antioxidative effect from fish and shellfish, as well as their by-products.[8] However, little information regarding antioxidants of a pollock roe and its application has been reported. The objective of this investigation was to characterize and determine antioxidative activities of pollock roe in food industry. Therefore, the present study were to determine the antioxidant activity, 2, 2- diphenyl - 1- picrylhydroazyl (DPPH), hydroxyl radical (OH) reducing activity, and nitric oxide radical (NO). Appropriate seasonings are expected to remove the oxides from the digestion process of the food.

MATERIALS AND METHODS

Sample extract

Raw materials (Alaska pollock roe), Gochujang pollock roe, and fermented seasoning pollock roe were obtained from Deok-Hwa Food Co., Busan-ci, Republic of Korea. When cooking soup or stew, many cookers usual added water, pollock roe and ingredients in a pot and boiled. Therefore, in this experiment, 50 g of each sample and 500 ml of water heated to 95°C and cooled. Samples were divided into two groups. One group was added 99.9% ethanol (500 ml) and the other was added distilled water (500 ml). The samples were treated with ultrasound (5510, Branson, USA) at room temperature for one hour. The mixture was further stirred with a magnetic bar at 65°C for 6 hours. They were squeezed out with the muslin cloth and filtered through Whatman filter paper No. 1. The sample was evaporated to remove solvent or excess water under reduced pressure and controlled temperature (60°C) by using rotary vacuum evaporator (N-1001S-W, Eyela, Tokyo, Japan). To get dry powder, samples placed in a low temperature vacuum chamber (HyperCool, HC3110, Gyrozen Co., LTD, Korea). The extract was dried, weighed.

DPPH free radical

The hydrogen atoms or electrons donation ability of the corresponding extracts and some pure compounds were measured by bleaching the violet colored ethanoic solution of DPPH.[9] The effects of ethanoic extract on DPPH radicals were evaluated according to the method described by Brand-Williams et al.[10] with slight modifications. 1 ml of 0.1 mM DPPH solution in ethanol was mixed with 1 ml of the ethanol extracts of various concentrations (0.1, 0.5, and 1.0 mg/ml). DPPH was added to the solutions prepared with sample extracts and standard antioxidant substances and stirred. A solution of DPPH was prepared by dissolving 5 mg DPPH in 2 ml of ethanol, and the solution was kept in the dark at 4°C. A stock solution of the compounds was prepared at 1 mg/ml in dimethyl sulfoxide (DMSO) were plated out in triplicate in a 96-well microtiter plate. Then, 5 µl of ethanol DPPH solution (final concentration 300 µM) was added to each well and ethanol was used for control of test samples. The plate was shaken to ensure thorough mixing for 2 minutes before being wrapped with aluminum foil and placed into the dark. The radical scavenging reaction was carried out for 30 min at 37 ºC in dark. The optical density (OD) of the solution was read using the Microplate Reader (VersaMax, Califonia, USA) 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). Inhibition of free radical scavenging activity was calculated using the following equation.

Inhibition (%) = 100 × (absorbance of the control - absorbance of the sample)/ absorbance of the control. Relative inhibitor rate of treated groups and product samples for L-Ascorbic acid was calculated.

Hydroxyl radical assay

Hydroxyl radical (OH) scavenging activity was measured by the ability of the different fractions of methanol extract to scavenge the hydroxyl radicals generated by the Fe3+-ascorbate-EDTA-H2O2 system (Fenton reaction).[11] The deoxyribose method for determining the scavenging effect of the sample extracts against hydroxyl radicals was performed according to a described procedure.[11] The reaction mixture in a final volume of 1.0 ml contained 100 µl of 2-deoxy-2- ribose (28 mM in 20 mM KH2PO4 buffer, pH 7.4), 500 µl of the fractions at various concentrations (100-600 µg/ml) in buffer, 200 µl of 1.04 mM EDTA and 200 µM $FeCl_3$ (1:1, v/v), 100 µl of 1.0 mM hydrogen peroxide (H_2O_2) and 100 µl of 1.0 mM ascorbic acid.

Test samples were kept at 37°C for 1 h. The free radical damage imposed on the substrate, deoxyribose was measured using the thiobarbituric acid test. 1 ml of 1% thiobarbituric acid (TBA) (w/v) and 1 ml of 2.8% trichloroacetic acid (TCA) (w/v) were added to the test tubes and the mixture was heated in a water bath at 100 ºC for 15 min. The absorbance of the resulting solution was measured at 530 nm with Microplate Reader). This assay was also performed without ascorbic acid or EDTA, in order to check for pro-oxidant or metal chelation activities. The percent inhibition was calculated as the decolourization percentage of the test sample using the following formula.

Inhibition (%) = (IA-As)/IA×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. Relative inhibitor rate of treated groups and product samples for hydrogen peroxide was calculated.

Nitric oxide (-NO) scavenging assay

Nitric oxide radical ($NO^{\cdot}$)-scavenging activity was generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitric ions that can be estimated by using Greiss reagent.[12] Nitrite oxide scavenging activity was measured by the method described by Kato et al.[13] The extracts were prepared from a 100.0 mg/mL ethanol crude extract. The reaction mixture contained 1 mM $NaNO_2$ 120 µl, 0.1 N HCl 840 µl, and various concentrations of samples, making final volume 1.2 ml. After reacting for 1 hour at 37℃, 1 ml of the reaction mixture was mixed with 3 ml of 2 % acetic acid and 400 µl of Griess reagent, and the mixture was reacted at room temperature for 15 minutes. Absorbance was measured at 550 nm using the Microplate Reader and the amount of remaining nitrite was also measured. Gallic acid and L-Ascorbic acid solutions were used as the positive control and used to prepare a standard curve. The percentage inhibition of the extract and standard was calculated as DPPH formula. Relative inhibitor rate of treated groups and product samples for gallic acid was

calculated.

Statistical analysis

The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The results were expressed as the mean ±SD. 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). The percent inhibition was calculated as the decolourization percentage of the test sample using the following formula.

Inhibition % = (IA-As)/IA×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.

RESULTS

DPPH scavenging assay

Table 1 was shown the antioxidant activities for DPPH radical of the pollock roe. It was observed that inhibition percentage values go on increasing with enhancements in concentration of research plant extracts in the assay mixture. DPPH scavenging activity of distilled water extracts of raw pollock roe was evaluated 43.0% at 0.1 mg/ml and 49.0% at 0.5 mg/ml. The distilled water extract of raw pollock roe showed 56.7% inhibition of DPPH activity at 1.0 mg/ml that of ethanol extract was 57.3% at same concentration. DPPH scavenging activity of distilled water extract with Gochujang pollock roe evaluated was 71.0% at 1.0 mg/ml and that of ethanol extract was 72.2% at same concentration. DPPH scavenging activity of distilled water extract with fermented seasoning pollock roe evaluated was 70.6% at 1.0 mg/ml and that of ethanol extract was 71.2% at same concentration. The overall values of fermented seasoning pollock roe except Gochujang pollock roe at 1.0 mg/ml were showed the highest inhibition activity of DPPH among three treated groups. The all values of DPPH scavenging activity of ethanol extract for pollock roe were higher than those of distilled water extract for pollock roe. However, the all did not show a statistically significant difference ($p < 0.05$). The fermented seasoning pollock roe was showed higher inhibition activity of DPPH than other treated groups. There was shown a statistically significant difference ($p < 0.001$).

When the L-Ascorbic acid used as a control, relative DPPH scavenging activities of ethanol extracts raw pollock roe, Gochujang pollock roe, and fermented seasoning pollock roe were 58.3%, 73.5%, and 72.4%, respectively (Fig. 1). The relative DPPH scavenging activities of distilled water extraction were 0.8 to 6.1% lower than those of the ethanol extract.

OH scavenging assay

The effect of extracts on hydroxyl radicals was assayed by using the deoxyribose method (Table 2). The highest OH activity was recorded in ethanol extract with fermented seasoning pollock roe among six groups (three samples x two solvants). The overall values of OH activity between pollock roe with Gochujang and pollock roe with fermented seasoning were show a statistically non-significant difference ($p < 0.05$). The distilled water extract of raw pollock roe showed 65.3% inhibition of OH activity at 1.0 mg/ml that of ethanol extract was 68.0% at same concentration. OH scavenging activity of distilled water extract with Gochujang pollock roe evaluated was 73.3% at 1.0 mg/ml and that of ethanol extract was 77.8% at same concentration. OH scavenging activity of distilled water extract with fermented seasoning pollock roe evaluated was 74.9% at 1.0 mg/ml and that of ethanol extract was 78.6% at same concentration. The fermented seasoning pollock roe was showed higher inhibition activity of OH than other treated groups. There was shown a statistically significant difference ($p < 0.05$ for water extracts, $p < 0.001$ for ethanol extracts). When the hydrogen peroxide (H_2O_2) used as a control, relative OH scavenging activities of raw pollock roe, Gochujang pollock roe, and fermented seasoning pollock roe extracts were 60.7%, 69.5, and 70.2%, respectively (Fig. 2). The relative OH scavenging activities of distilled water extraction were 4.3 to 9.2% lower than those of the ethanol extract.

NO scavenging assay

NO scavenging activity of distilled water extracts of raw pollock roe was evaluated 35.6% at 0.1 mg/ml, 42.6% at 0.5 mg/ml, and 48.1% at1.0 mg/ml (Table 3). NO scavenging activity of ethanol extracts of raw pollock roe was evaluated 37.2% at 0.1 mg/ml, 45.7% at 0.5 mg/ml, and 53.0% at1.0 mg/ml. It is also observed that inhibition percentage values go on increasing with enhancements in concentration of research plant extracts in the assay mixture. However, the degree of NO inhibition activity of product samples was not high. There was no significant difference among the three experimental groups ($p > 0.05$).

NO scavenging activity of distilled water extract of Gochujang pollock roe was evaluated 58.3% at 1.0 mg/ml and that of ethanol extract was 68.3% at same concentration. NO scavenging activity of distilled water extracts of fermented seasoning pollock roe was evaluated 62.4% at 1.0 mg/ml and that of ethanol extract was 69.4% at same concentration. The high antioxidant activity for NO-scavenging found on ethanol extracts of fermented seasoning pollock roe. Although the all groups on ethanol extracts were higher than those of distilled water extract, there were not shown a statistically significant difference ($p > 0.05$). NO scavenging activity of Gochujang and fermented seasoning pollock roe were higher than those of raw materials, there was significant difference at ethanol extracts ($p < 0.05$). When the gallic acid used as a control, relative NO scavenging activities of raw pollock roe, Gochujang pollock roe, and fermented seasoning

pollock roe extracts were 50.4%, 65.1, and 66.1%, respectively (Fig. 3). The relative NO scavenging activities of distilled water extraction were 4.2 to 14.7% lower than those of the ethanol extract.

Table 1: The inhibition effects (%) of DPPH properties of Alaska pollock roe at different concentrations

Sample	Concentration (mg/ml)	Solvent		*t*-test
		Water	Ethanol	
Raw pollock roe	0.1	42.95±0.77	44.06±1.09	-0.294
	0.5	49.01±2.05	52.21±0.15	
	1.0	56.73±1.06	57.32±0.87	
Pollock roe with Gochujang seasoning	0.1	59.91±0.75	61.73±3.00	-0.324
	0.5	66.52±0.81	67.73±3.06	
	1.0	70.96±1.83	72.22±4.85	
Pollock roe with fermented seasoning	0.1	62.08±2.09	64.40±1.54	-0.550
	0.5	66.04±2.94	68.38±3.82	
	1.0	70.61±3.40	71.19±3.04	
F-test		13.977***	16.051***	-0.358

Data represent the mean ± SD from three replicates. *** = $p < 0.001$.

Table 2: The inhibition effects (%) of hydroxyl radical (OH) scavenging activity of Alaska pollock roe at different concentrations.

Sample	Concentration (mg/ml)	Solvent		*t*-test
		Water	Ethanol	
Raw pollock roe	0.1	56.79±1.38	60.81±1.48	-0.977
	0.5	62.28±1.71	65.11±1.23	
	1.0	65.31±2.92	67.98±1.69	
Pollock roe with Gochujang seasoning	0.1	65.24±1.93	69.08±0.37	-1.346
	0.5	67.93±1.14	74.81±0.26	
	1.0	73.27±2.88	77.81±0.91	
Pollock roe with fermented seasoning	0.1	68.47±2.69	74.19±1.47	-2.080
	0.5	71.96±2.83	76.64±1.15	
	1.0	74.92±2.49	78.58±0.13	
F-test		9.254*	19.045***	-1.460

* = $p < 0.05$, *** = $p < 0.001$.

Table 3: The inhibition effects (%) of nitric oxide radical (NO) scavenging activity of Alaska pollock roe at different concentrations.

Sample	Concentration (mg/ml)	Solvent		*t*-test
		Water	Ethanol	
Raw pollock roe	0.1	35.64±2.07	37.19±1.72	-0.543
	0.5	42.65±2.08	45.68±1.37	
	1.0	48.08±1.44	52.96±3.57	
Pollock roe with Gochujang seasoning	0.1	45.06±2.83	50.11±0.47	-1.234
	0.5	53.29±2.65	61.68±1.90	
	1.0	58.25±1.82	68.32±1.55	
Pollock roe with fermented seasoning	0.1	46.18±2.08	53.66±4.83	-1.110
	0.5	54.08±0.77	61.16±2.65	
	1.0	62.35±3.07	69.37±1.57	
F-test		3.428	5.737*	-1.410

* = $p < 0.05$.

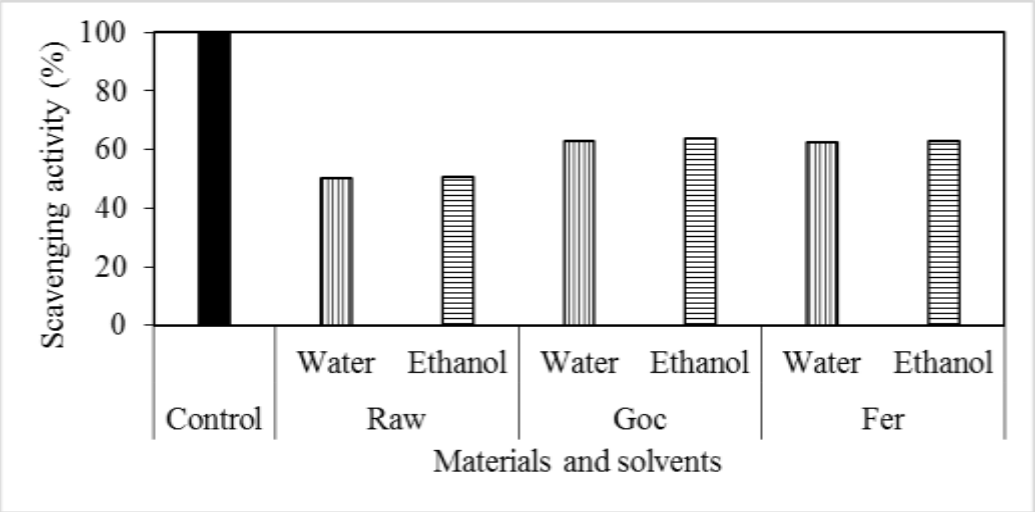

Figure 1: Relative inhibitory effects on DPPH by ethanol extracts from various pollock roe is L-Ascorbic acid. Raw: raw pollock roe, Goc: Gochujang pollock roe, Fer: fermented seasoning pollock roe.

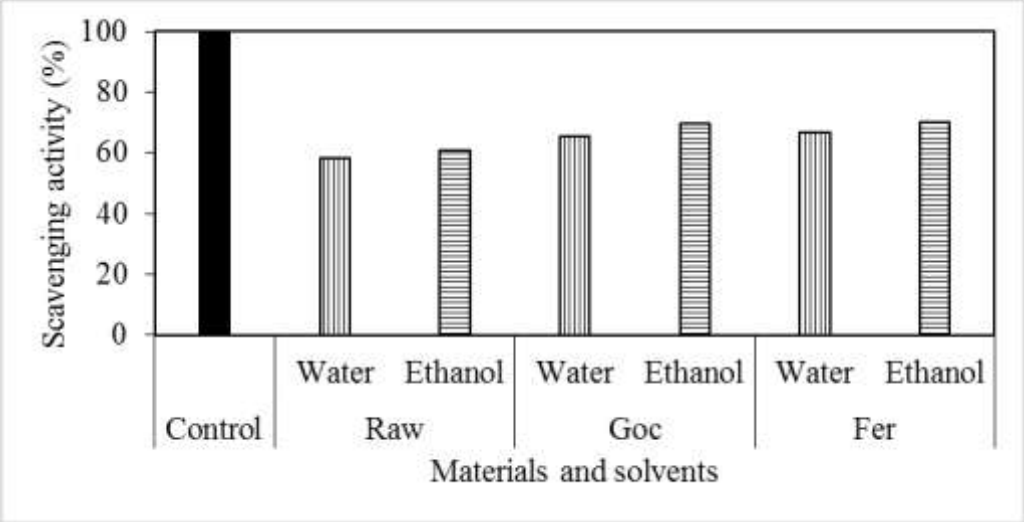

Figure 2: Relative inhibitory effects on OH by ethanol extracts from various pollock roe is L-Ascorbic acid. Abbreviations of Raw, Goc, and Fer are same as Figure 1.

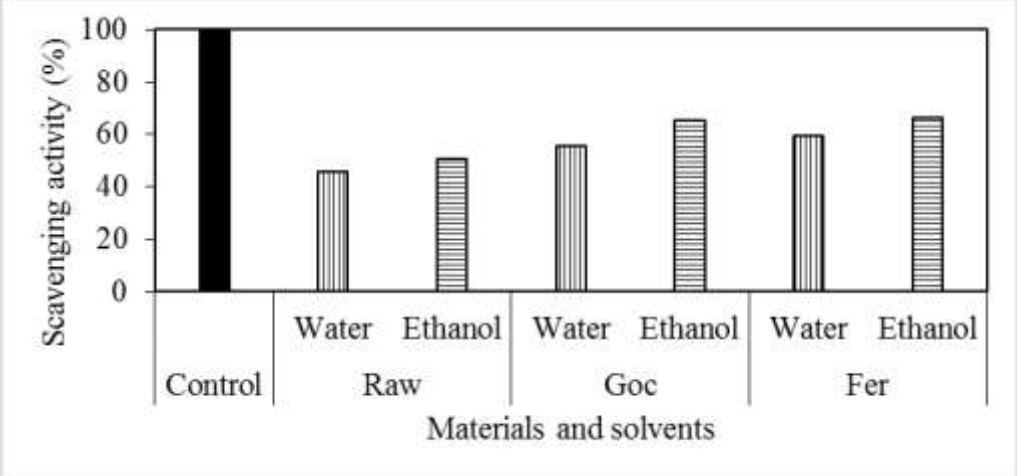

Figure 3: Relative inhibitory effects on NO by ethanol extracts from various pollock roe is L-Ascorbic acid. Abbreviations of Raw, Goc, and Fer are same as Figure 1.

DISCUSSION

Alaska Pollack is fished mainly in the Gulf of Alaska in

the U.S., the Bering Sea, the Sea of Okhotsk in Russia and in the seas off Japan. Pollock accounted for 54 percent of Alaska's total commercial seafood harvest volume in 2014/2015.[14] This is one of the most popular ways that cod roe is served in Japan and Korea. A 1696 Japanese book records the use of Alaska pollock's roe in Northern land. Fishermen in the Korean Peninsula began eating Mentaiko in the 17th to 18th century.[15] They discovered the rich flavor of fish eggs and used salt as a preservative for them. Later on, chili was added as well to add a hot and spicy touch. Mentaiko is identical to tarako in that it is a sack of salted cod roe, but differ from tarako in that the roe is marinated in different seasonings and spices to create an array of subtly different flavor profiles. Alaska pollock or walleye pollock in Korea, whether raw, dried, and/or cooked, is a common side dish and food served with alcoholic beverages. Antioxidant substances can be mostly extracted from plants including fruits and vegetables as phenolic compounds, while it is rare in animals. However, there have been reports on antioxidant peptides from animal proteins by enzymatic hydrolysis, which includes *myeolchi-aekjeot* (anchovy sauce).[16] Higher buffer capacity was observed in both roe protein concentrates in alkali medium and antioxidant activity determined by the DPPH radical scavenging activity was higher.[17] The scavenging effects of papain hydrolysate on DPPH and hydroxyl radicals were found to be about 72% and 56% at 1 mg/ml, respectively.[18]

Nashimoto et al.[19] investigated the effect of katsuo-dashi acted as an antioxidant during cooking process. Ando et al.[20] also reported the effect of instant-dashi (commercially available types of katsuo-flavored mix seasoning) on hydroxyl-radical scavenging activity during cooking process. The strong the antioxidant activity observed various seasoning and fermented foods.[21] Also, it has been suggested that the ABTS assay strongly depends on the nature of the compound being studied.[22] In the present study, higher DPPH, OH, and NO scavenging abilities were shown in Gochujang and fermented seasoning pollock roe than instant pollock roe. From the foregoing, it may be concluded that the ethanol extract of pollock roe with proper seasoning, pollock roe demonstrated good antioxidant and free radical scavenging activities.

CONCLUSION

The all values of DPPH, OH, and NO scavenging activity of ethanol extract for pollock roe were higher than those of distilled water extract for pollock roe. DPPH, OH, and NO scavenging activity of Gochujang and fermented seasoning pollock roe were higher than those of raw materials

ACKNOWLEDGEMENTS

This research was supported by the Fisheries Industry Investment Support Project (2018) of BUSAN TECHNOPARK. We would like to express our sincere gratitude to the BUSAN TECHNOPARK in Korea.

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