ANTIOXIDANT PROPERTIES (ABTS, FRAP, AND TOTAL PHENOLIC CONTENT) OF COOKED POLLOCK ROE PREMIUM ALASKA POLLOCK ROE WITH NATURAL FERMENTED SEASONING

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ABSTRACT

The distilled water and ethanol extracts raw materials (Alaska Pollock roe), premium Gochujang Pollock roe, and premium fermented seasoning Pollock roe were evaluated for their antioxidant. The total antioxidant property was estimated by 1,1-Diphenyl-2-picrylhydrazyl (ABTS), Ferric Reducing Antioxidant Potential Assay (FRAP Assay), total phenolic content. The all values of ABTS+, FRAP, and total phenolic content scavenging activity of ethanol extract for Pollock roe were higher than those of distilled water extract for Pollock roe. The all values of fermented seasoning Pollock roe were showed the highest inhibition activity of ABTS+, FRAP, and total phenolic content among three treated groups. The 50% inhibition of fermented seasoning Pollock roe on ABTS+ showed much low value (EC_{50} = 10.02 ug/ml), followed by Gochujang Pollock roe (EC_{50} = 10.58 ug/ml) (Table 4). The EC_{50} value of raw Pollock roe was 12.39 ug/ml. The EC_{50} values of distilled water and ethanol extracts of Gochujang Pollock roe on FRAP were 11.89 ug/ml and 11.43 ug/ml, respectively. The EC_{50} values of distilled water and ethanol extracts of fermented seasoning Pollock roe on total phenolic content were 11.68 ug/ml and 11.42 ug/ml, respectively.

Keywords: Alaska Pollock roe, 1,1-Diphenyl-2-picrylhydrazyl (ABTS), FRAP, total phenolic content.

INTRODUCTION

One of the most commercially important semi-demersal fishes in the eastern Bering Sea is Walleye Pollock (*Gadus chalcogrammus*), hereafter referred to as Pollock (Petrik et al., 2016). The recent change of the scientific name of Walleye Pollock from *Theragra chalcogramma* to *Gadus chalcogrammus* has created (Carr and Marshall, 2008). The species is widely distributed in the temperate to boreal North Pacific, from Central California into the eastern Bering Sea, along the Aleutian arc, around Kamchatka, in the Okhotsk Sea and into the southern Sea of Japan. Eggs are found in the water column from December to August, but the annual peak occurs in either April or May (Bacheler *et al*., 2010).

The roe obtained from Alaska pollack is commercially important, since it occupies only 5% of the fish weight but contributes about 31% of the commercial value of pollack products (Balaban *et al.*, 2012). 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*"). Manufacturers of roe products in Japan have their own expertise in classifying raw Alaska pollack roe into *gamuko*, *mako*, and *mizuko* by maturity stage. Usually, only *mako* is accepted by

manufacturers as a standard roe material for manufacturing spicy pollack roe product which is a famous delicacy known as *karashi mentaiko* in Japanese (Chen et al., 2016).

Antioxidants play an important role in food preservation by inhibiting oxidation processes and contributing to health promotion rendered by many dietary supplements, nutraceuticals and functional food ingredients (Shahidi and Zhang, 2015). Many herbal plants contain antioxidant compounds which protects cells against degenerative effects of Reactive Oxygen Species (ROS) which is a free radical such as singlet oxygen, superoxide, peroxyl, radicals, hydroxyl radicals [Blois, 1958, Aruoma and Cuppett, 1997]. Several studies have described the antioxidant activity of protein hydrolysates from animal sources like, egg yolk (Park et al., 2001), yellowfin sole (Jun et al., 2004), shrimp processing discards (Guerard et al., 2007), and tuna liver (Je et al., 2009).

Enzyme property is an important on the release of antioxidant peptides by hydrolysis of fish protein (Laroque et al., 2008). However, antioxidants of a Pollock roe and its application to fermentation has not been reported. The aims of this investigation were to determine the antioxidant activity, 1,1-Diphenyl-2-picrylhydrazyl (ABTS), Ferric Reducing Antioxidant Potential Assay (FRAP Assay), total phenolic content.

METHODOLOGY

Sample extract

Alaska Pollock roe (raw or natural materials), premium Pollock roe with Gochujang (Gochujang is a typical Korean sauce), and fermented premium seasoning Pollock roe were obtained from Deok-Hwa Food Co., Busan-ci, Republic of Korea. The premium brand is a special processing of the company, which has made the raw material more advanced. First, 50 g of each sample and 500 ml of water heated to 95°C and cooled. Samples were divided into two groups. Second, 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 and stored at -20°C in storage vials for experimental use.

ABTS+ free radical

1,1-Diphenyl-2-picrylhydrazyl (ABTS) is a stable free radical. The antioxidant activity of the seaweed extracts was measured on the basis of the scavenging activity of ABTS+ free radical according to the method described by Brand-Williams et al. (1995) with slight modifications. ABTS+ free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. 1 ml of 0.1 mM ABTS+ solution in ethanol was mixed with 1 ml of the previous algae extracts of various concentrations (0.1, 1.0, 2.0, 4.0, and 8.0 mg/ml). ABTS+ was added to the solutions prepared with algae extracts and standard antioxidant substances and stirred. A solution of ABTS+ was prepared by dissolving 5 mg ABTS+ in 2 ml of ethanol, and the solution was kept in the dark at 4°C. A stock solution of the compounds was prepared at 1 mg/ml in DMSO. The stock solution was diluted to varying concentrations in 96-well microplates. Then, 5 μL of ethanol ABTS+ solution (final concentration 300 μM) was added to each well. The plate was shaken to ensure thorough mixing before being wrapped with aluminum foil and placed into the dark. The radical

scavenging reaction was carried out at 37 ºC in dark for 30 min. The optical density (OD) of the solution was read using the Microplate Reader 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).

Ferric Reducing Antioxidant Potential Assay (FRAP Assay)

The antioxidant capacity by ferric ion reducing power was measured according to the method of Oyaizu (1986) with modifications. 1 ml of various concentrations of sample and 1 ml of 1% w/v potassium ferricyanide solution were added to 1 ml of the phosphate buffer solution (0.2 M, pH 6.6), and the mixture was reacted at 50 ℃ for 20 minutes, and then 1 ml of 10% w/v trichloroacetic acid was added thereto. The reaction mixture was centrifuged at 12,000 rpm for 10 minutes, 1 ml of distilled water was added to 1 ml of supernatant, and 0.2 ml of 1% ferric chloride was added. Blank samples were prepared for both ethanol and deionized water extracted samples. Vitamin C (L-ascorbic acid) were used as antioxidant standards. After 10 minutes of reaction, the absorbance was measured at 700 nm. A calibration curve of ascorbic acid was established, the antioxidant capacity of the plant extracts was then expressed as mmol ascorbic acid equivalent/g dry extract.

Determination of total phenolic content

The total phenolic content of the sample extracts was determined by using Folin-Ciocalteu reagent following a slightly modified method of Ainsworth (2007). Gallic acid was used as a reference standard for plotting calibration curve. A volume of 0.5 mL of the plant extract (100 µg/mL) was mixed with 2 mL of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 765 nm using double beam Microplate Reader. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds expressed as mg/g gallic acid equivalent (GAE) of dry extract.

Statistical analysis

All the analysis were carried out in triplicate and expressed as 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). Significant differences between means were determined by Duncan's multiple range tests. *P* values less than 0.05 were considered statistically significant.

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.

The 50% inhibition (EC_{50}) is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. A dose response curve was plotted to determine EC_{50} values. To determine the EC_{50} value of the active component, the technique using 96-well microplates was employed (Lee et al., 1998).

RESULTS

ABTS+ free radical

The inhibition of ABTS+ scavenging activity increases with increasing extract concentrations (Table 1). ABTS+ scavenging activity of distilled water extracts of natural Pollock roe was evaluated 37.9% at 0.1 mg/ml and 42.7% at 0.5 mg/ml. The distilled water extract of natural Pollock roe showed 49.0% inhibition of ABTS+ activity at 1.0 mg/ml that of ethanol extract was 50.7% at same concentration. ABTS+ scavenging activity of distilled water extract with Gochujang Pollock roe evaluated was 67.2% at 1.0 mg/ml and that of ethanol extract was 68.7% at same concentration. ABTS+ scavenging activity of distilled water extract with fermented seasoning Pollock roe evaluated was 69.1% at 1.0 mg/ml and that of ethanol extract was 71.6% at same concentration. The all values of fermented seasoning Pollock roe were showed the highest inhibition activity of ABTS+ among three treated groups. The all values of ABTS+ 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 50% inhibition of fermented seasoning Pollock roe showed much low value (EC_{50} = 10.02 ug/ml), followed by Gochujang Pollock roe (EC_{50} = 10.58 ug/ml) (Table 4). The EC_{50} value of raw Pollock roe was 12.39 ug/ml. When the L-Ascorbic acid used as a control, relative ABTS+ scavenging activities of ethanol extracts raw Pollock roe, Gochujang Pollock roe, and fermented seasoning Pollock roe extracts were 47.8%, 64.8%, and 67.5%, respectively (Fig. 1). The relative ABTS+ scavenging activities of distilled water extraction were lower than those of the ethanol extract.

Ferric ion reducing antioxidant power (FRAP) assay

The results (Table 2) showed that FRAP values were higher in fermented seasoning Pollock roe samples compared to other samples. However, there was a significant difference at $p < 0.05$. Raw Pollock roe was showed the lowest inhibition activity of FRAP among three groups. The distilled water extract of raw Pollock roe showed 51.0% inhibition of FRAP activity at 1.0 mg/ml that of ethanol extract was 57.9% at same concentration. FRAP scavenging activity of distilled water extract with Gochujang Pollock roe evaluated was 56.3% at 1.0 mg/ml and that of ethanol extract was 59.9% at same concentration. FRAP scavenging activity of distilled water extract with fermented seasoning Pollock roe evaluated was 59.4% at 1.0 mg/ml and that of ethanol extract was 65.1% at same concentration. The EC_{50} values of distilled water and ethanol extracts of raw Pollock roe were 12.28 ug/ml and 11.71 ug/ml, respectively (Table 4). The EC_{50} values of distilled water and ethanol extracts of Gochujang Pollock roe on FRAP were 11.89 ug/ml and 11.43 ug/ml, respectively. When the L-Ascorbic acid used as a control, relative FRAP scavenging activities of ethanol extracts raw Pollock roe, Gochujang Pollock roe, and fermented seasoning Pollock roe extracts were 53.6%, 55.4%, and 60.3%, respectively (Fig. 2). The relative FRAP scavenging activities of distilled water extraction were lower than those of the ethanol extract.

Total phenolic content

The inhibition effects (%) of total phenolic content on 1.0 mg/ml distilled water and ethanol extracts of raw Pollock roe were found to be 48.3% and 49.2%, respectively. The total phenolic content of distilled water extract with Gochujang Pollock roe evaluated was 56.3% at 1.0 mg/ml and that of ethanol extract was 58.1% at same concentration. The total phenolic content scavenging activity of distilled water extract with fermented seasoning Pollock roe evaluated was 59.8% at 1.0 mg/ml and that of ethanol extract was 62.8% at same concentration. The EC_{50} values of distilled water and ethanol extracts of raw Pollock roe on ABTS+ were 11.81 ug/ml and 12.65 ug/ml, respectively (Table 4). The EC_{50} values of distilled water and ethanol extracts of Gochujang Pollock roe were 11.88 ug/ml and 11.76

ug/ml, respectively. The EC_{50} values of distilled water and ethanol extracts of fermented seasoning Pollock roe on total phenolic content were 11.68 ug/ml and 11.42 ug/ml, respectively.

When the Gallic acid used as a control, relative total phenolic content scavenging activities of ethanol extracts raw Pollock roe, Gochujang Pollock roe, and fermented seasoning Pollock roe extracts were 48.3%, 57.0%, and 61.6%, respectively (Fig. 3). The relative total phenolic content scavenging activities of distilled water extraction were lower than those of the ethanol extract.

Table 1. The inhibition effects (%) of 1,1-Diphenyl-2-picrylhydrazyl (ABTS) scavenging activity of Alaska Pollock roe at different concentrations

Sample	Concentration (mg/ml)	Solvent		*t*-test
		Water	Ethanol	
Raw Pollock roe	0.1	37.89±0.55	39.59±0.87	-0.488
	0.5	42.74±0.29	45.86±0.99	
	1.0	49.03±0.80	50.65±0.76	
Pollock roe with Gochujang seasoning	0.1	54.24±0.85	56.13±0.96	-0.372
	0.5	58.69±0.41	61.10±2.58	
	1.0	67.15±0.72	68.72±2.22	
Pollock roe with fermented seasoning	0.1	60.45±2.05	62.78±1.39	-0.611
	0.5	65.29±0.24	67.05±1.11	
	1.0	69.13±1.07	71.58±1.85	
t-test		-0.411		

Table 2. The inhibition effects (%) of ferric reducing antioxidant potential (FRAP) of Alaska Pollock roe at different concentrations

Sample	Concentration (mg/ml)	Solvent		*t*-test
		Water	Ethanol	
Raw Pollock roe	0.1	43.09±0.91	46.14±3.30	-1.120
	0.5	47.28±0.73	51.03±2.08	
	1.0	50.95±0.61	57.90±0.54	
Pollock roe with Gochujang seasoning	0.1	45.34±1.45	50.95±1.41	-1.016
	0.5	50.59±0.94	53.97±1.18	
	1.0	56.33±0.45	59.87±0.72	
Pollock roe with fermented seasoning	0.1	51.84±0.45	54.15±1.79	-1.199
	0.5	54.08±2.30	60.27±2.99	
	1.0	59.54±1.36	65.08±0.68	
t-test		-1.780		

Table 3. The inhibition effects (%) of total phenolic content of Alaska Pollock roe at different concentrations

Sample	Concentration (mg/ml)	Solvent		*t*-test
		Water	Ethanol	
Raw Pollock roe	0.1	31.38±0.51	32.38±0.68	-0.195
	0.5	41.53±1.51	43.96±1.88	
	1.0	48.53±0.95	49.22±0.74	
Pollock roe with Gochujang seasoning	0.1	37.63±2.70	38.26±1.56	-0.127
	0.5	51.45±1.57	52.11±0.08	
	1.0	56.31±1.44	58.11±1.93	
Pollock roe with fermented seasoning	0.1	38.07±2.11	40.53±1.07	-0.248
	0.5	53.11±2.84	54.43±0.49	
	1.0	59.79±4.14	62.81±2.45	
t-test		-0.336		

Table 4. The 50% inhibition (EC_{50}) of ABTS+, FRAP, and total phenolic content of Alaska Pollock roe at different solvents

Sample	ABTS+		FRAP		Phenolic content	
	Water	Ethanol	Water	Ethanol	Water	Ethanol
Raw Pollock roe	12.65	12.40	12.28	11.71	11.81	12.65
Pollock roe with Gochujang seasoning	10.78	10.58	11.89	11.42	11.88	11.76
Pollock roe with fermented seasoning	10.25	10.02	11.40	10.92	11.68	11.42

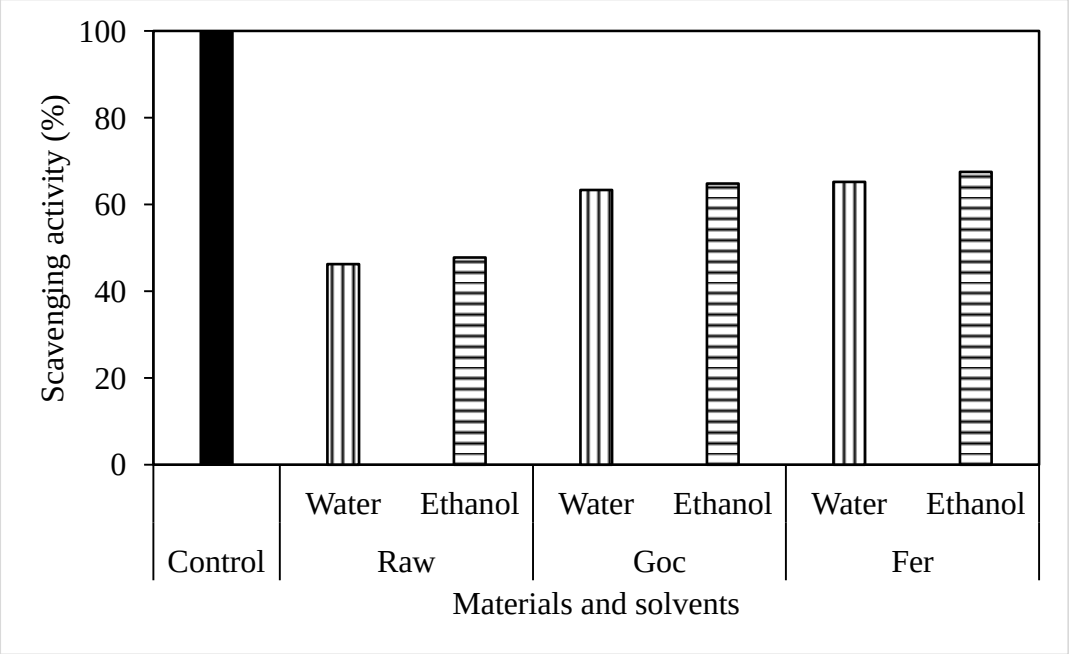

Figure 1. Relative inhibitory effects on ABTS+ by ethanol extracts from various Pollock roe is L-Ascorbic acid. Raw: raw Pollock roe, Goc: Gochujang Pollock roe, Fer: fermented seasoning Pollock roe.

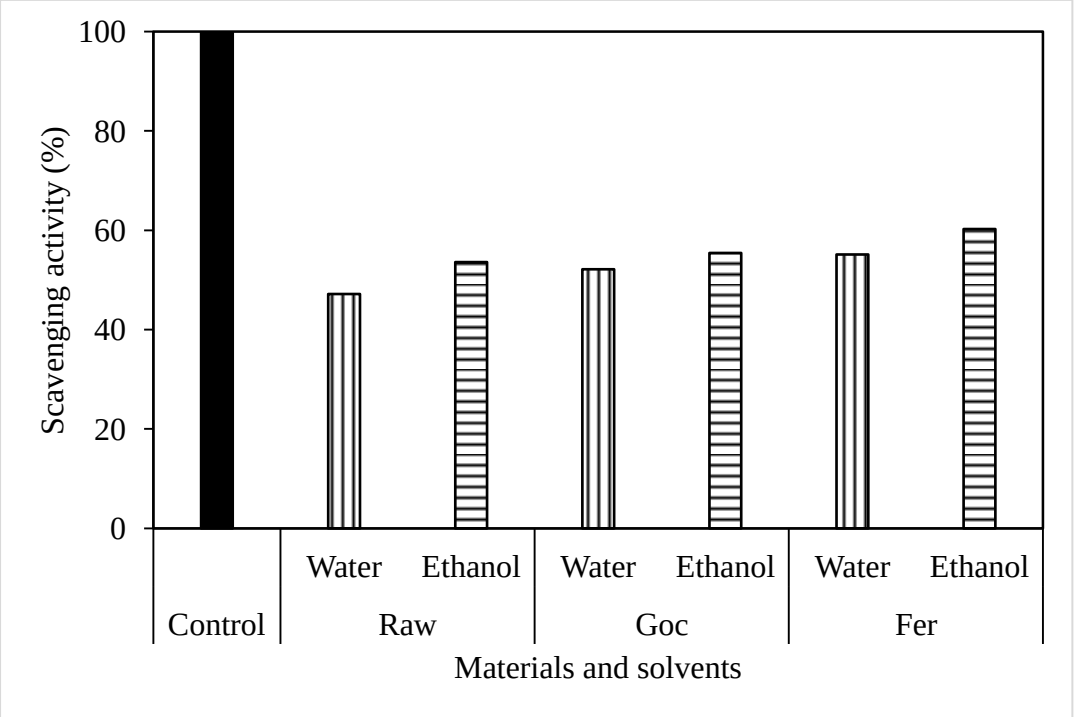

Figure 2. Relative inhibitory effects on FRAP by ethanol extracts from various Pollock roe is L-Ascorbic acid. Abbreviations of Raw, Goc, and Fer are same as Fig. 1.

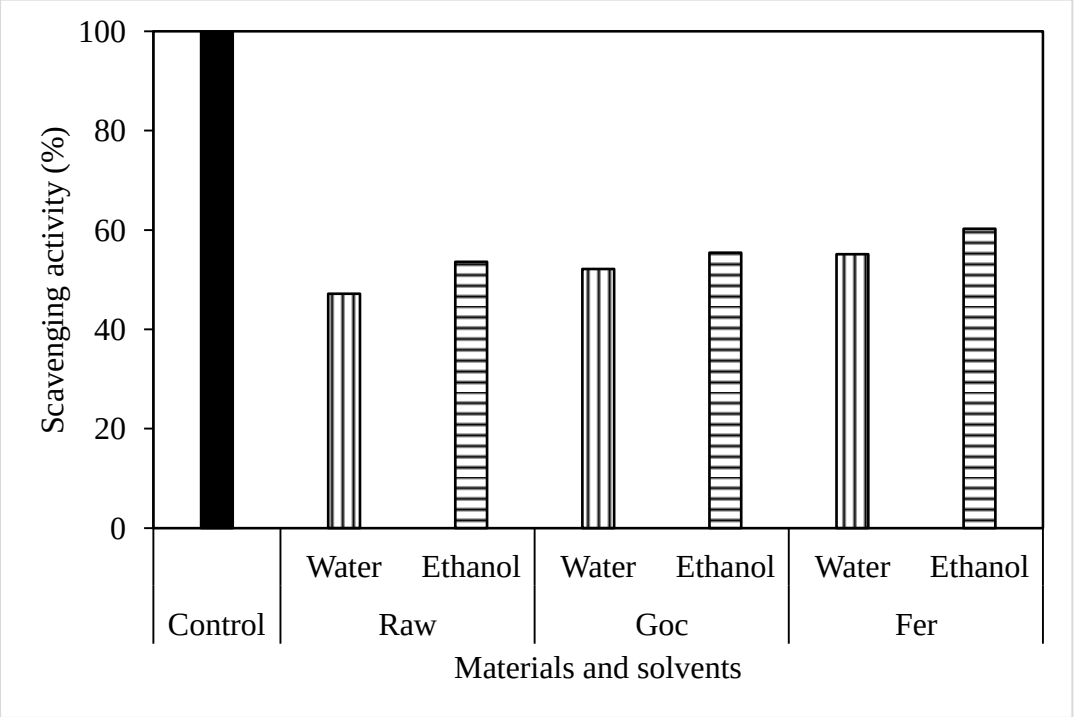

Figure 3. Relative inhibitory effects on total phenolic content by ethanol extracts from various Pollock roe is L-Ascorbic acid. Abbreviations of Raw, Goc, and Fer are same as Fig. 1.

DISCUSSION

Bioactive peptides have been identified in residual material from a range of fish species, such as Atlantic salmon (Falkenberg, 2014), Atlantic herring (Pampanin, 2012), and Alaska

Pollack (Je et al., 2005). The bone tissue of cod (*Gadus morhua*) has also shown antioxidative activity (Slizyte et al., 2009). The Atlantic salmon was found to possess higher antioxidant activity than the cod samples (Pampanin et al., 2016). In this study, Alaska Pollock roe (raw or natural materials) is not considered to be a rich source of antioxidant though it boiled to 95°C. When Pollock roes treated with Gochujang which is a typical Korean sauce and fermented seasoning were found to possess higher antioxidant activity than the raw or natural roes (Tables 1-3). Gochujang is a traditional fermented hot red pepper and soybean paste. Rosa et al. (2002) reported that capsinoids within gochujang show remarkable antioxidant activity. It is fermented with *Aspergillus* s *oryzae* and Bacillus species by mixing rice powder, salt, and dry red pepper powder, meju powder. The extract of mixed culture *doenjang* showed high antioxidant activity such as ferric reducing antioxidant power assay, 2,2-diphenyl-1-picrylhydrazyl and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activities (Kang et al., 2016). Lin et al. (2006) reported that fermented soybean meal with *Aspergillus oryzae* could enhance antioxidative and anti-mutagenic activities. Pham and Lee (2007) reported that significantly increased liver SOD activity was observed in parrot fish *Oplegnathus fasciatus* fed the diet containing 25% cheonggukjang. Kim et al. (2010) reported that total polyphenol content, flavonoids, DPPH, and superoxide anion radical scavenging activities in diets increased with increasing dietary Meju content. There have been reports on antioxidant peptides from animal proteins by enzymatic hydrolysis, which includes *myeolchi-aekjeot* (anchovy sauce) (Kim, 2003). The strong antioxidant activity was not shown in Alaska Pollock roe. From this finding it can be inferred that additives such as antioxidant seasoning such as gochujang into Alaska Pollock roe brought about an increase in the total scavenging activities of the ABTS+, FRAP, and total phenolic content.

CONCLUSIONS

The all values of ABTS+, FRAP, and total phenolic content scavenging activity of ethanol extract for Pollock roe were higher than those of distilled water extract for Pollock roe. The all values of fermented seasoning Pollock roe were showed the highest inhibition activity of ABTS+, FRAP, and total phenolic content among three treated groups. Additives such as antioxidant seasoning such as gochujang into Alaska Pollock roe brought about an increase in the total scavenging activities of the ABTS+, FRAP, and total phenolic content.

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.

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