

# Free Radical Scavenging Activity of DPPH and ABTS on Fish Paste Products with Black Garlic

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## ABSTRACT

**Black garlic (*Allium sativum* L.) is made when heads of garlic are aged under specialized conditions of heat and humidity. Fillets of Pacific cod (*Gadus macrocephalus*), Atlantic mackerel (*Scomber scombrus*), shrimp (*Fenneropenaeus chinensis*), and squid (Caribbean Reef Squid) were used as raw material for the preparation of fish pastes. This study was conducted to investigate the effect of DPPH and ABTS free radical scavenging activity on fish paste with black garlic. The DPPH results showed that there was a significant ( $p < 0.01$ ) increase in the degree of inhibition in different black garlic treatments. DPPH scavenging activity of the fish-pasta was evaluated 57.1% on 100% solution with 15g black garlic addition. Control extract of the six food materials was evaluated 25.1% on 100% and those of the 5g and 10g black garlic extracts were 33.9% and 39.2% at the same concentration, respectively. A significant inhibition effect was observed in ABTS in all samples according to increasing concentration. The values of IC<sub>50</sub> for the DPPH and ABTS on 15mg/ml were 152.2 and 162.4  $\mu\text{g/ml}$ , respectively. The value of lightness (L) was decreased to the increase of the content of black garlic. Excessive black color may not be good for fish cake sales.**

**Keywords:** ABTS, Black garlic, DPPH, Fish paste.

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## I. INTRODUCTION

Fish-paste products or fish cakes in Korea and Japan are mainly produced from white fish (fish meat) such as Pacific cod (*Gadus macrocephalus*) that are ground and blended into a paste with seasoning and then fried. Specially, Korean fish paste is not chemically broken down by a fermentation process until it reaches the consistency of a soft creamy purée or paste. China and Japan typically made from white fish, such as Pollock or hake that is pulverized to a thick paste and cooked until it becomes dense and firm. Fish muscle is mechanically deboned, collected meats, and blended with cryoprotectants to prepare a wet concentrate of proteins called surimi [1]. Surimi is a Japanese term that is also known as washed fish mince and Korean 'Surimi' is the terms of dried cuttlefish. Other Asian nations made from fermented various raw fish, ground shrimp, sun dried and either cut into fist-sized rectangular blocks or sold in bulk [2].

Garlic (*Allium sativum* L.) is a species of the onion genus that has been used as a culinary seasoning and cultivated medical herb for a long time ago in the world [3]. Various functions attributed to the sulfur compound of garlic include antibacterial activity, anticancer, antithrombotic, blood pressure drop, cholesterol reduction, anti-aging, and antioxidant function [4], [5]. Allicin (diallyl thiosulfinate), a major component of garlic, is a substance produced by decomposing allicin (S-allylcysteine sulfoxide) by an allylase and is a major physiologically active substance such as immunoactive substance such as glutamyl-S-allyl-L-cysteine, diallylsulfide, ajoen, etc. [6], [7]. Garlic contains glutamyl-S-allyl-L-cysteine, glutamyl-S-allyl-L-cysteine,  $\gamma$ -

glutamyl-S-(trans-1-propenyl)-L-cysteine, and S-allyl-cysteine.

In particular, the content of S-allyl-cysteine involved in immune activity is known as a physiologically active substance that increases a lot when raw garlic is processed to produce black garlic [6]. Black garlic (*Allium sativum* L.) is made when heads of garlic are aged under specialized conditions of heat and humidity. The enzymes that give fresh garlic its sharpness break down. Those conditions are thought to facilitate the Maillard reaction, the chemical process that produces new flavor compounds responsible for the deep taste of seared meat and fried onions. The cloves turn black and develop a sticky date-like texture [8]. Black garlic can be considered to possess antioxidant properties due to its beneficial health effects [8]–[10].

Jung and Sohn [11] reported anti-oxidation, antimicrobial, and anti-thrombosis activities of aged black garlic (*Allium sativum* L.). Shin *et al.* [12] studied antioxidant activity of solvent fraction from black garlic.

Koreans boil fish cakes in water and cook them. Therefore, distilled water extract from fish cake is more realistic than ethanol or methanol extract because cooking and stomach and small intestine activities in the human body are related to moisture.

Despite many studies on black garlic were food materials that help antioxidant activities such as DPPH and ABTS, few studies on fish-pasta products are being conducted. The aim of the present work was to perform a screening of the free radical scavenging activities of the fish-pasta with black garlic extracts.

## II. MATERIALS AND METHODS

### A. Preparation of Sample

Fresh garlic was purchased from the market (Fig. 1, left) and black garlic was manufactured in the laboratory (Fig. 1, right). Put 500 g of garlic in a jar so that it doesn't overlap. Temperature was stored and aged in incubate adjusted to 70 °C for 15 days. After removing the garlic skin, it was ground with a mixer and stored at -20 °C and used in an experiment.

Fillets of Pacific cod (*Gadus macrocephalus*), Atlantic mackerel (*Scomber scombrus*), shrimp (*Fenneropenaeus chinensis*), and squid (*Caribbean Reef Squid*) were used as raw material for preparation of fish pastes. The samples purchased on the market. To make fish mince, pieces of mackerel and haddock were mixed together in Pacific cod/mackerel mass ratio of 50:50 they were put in a washing machine and smashed into small pieces. Fish paste preparation was prepared according to the mixing ratio in Table I. After 3% salt added to the sample and grinded the ingredients for about 20 minutes.

After this treatment, extract was properly diluted and measured by the intensity of the optical absorption.

Fig. 2. shows the addition of various black garlic to fish cakes. The nutritional composition and chemical stability could be depended on fish species and the processing procedure according to making fish pasta.



Fig. 1. The photos of raw garlics and black garlics.

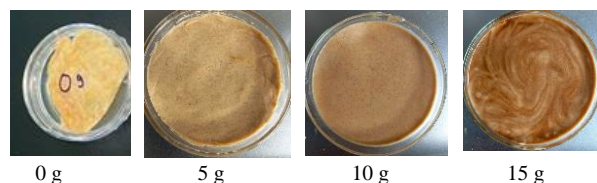


Fig. 2. The photos of fish paste with control and three added black garlic.

TABLE I: THE FORMULA FOR THE MANUFACTURING FISH PASTE WITH BLACK GARLIC

Materials	Weight (g)			
	Control	5%	10%	15%
Fish	60	60	60	60
Starch	15	15	15	15
Shrimp	2.5	2.5	2.5	2.5
Squid	2.5	2.5	2.5	2.5
Egg	2.5	2.5	2.5	2.5
Vegetable	17.5	12.5	7.5	2.5
Black garlic	0	5	10	15

### B. DPPH Radicals Scavenging Assay (DPPH Assay)

The free radical scavenging of the 1,1-diphenyl-2-picrylhydrazil (DPPH) was measured using the Microplate Reader (VersaMax, California, USA) by the method by Blois [13]

with slight modifications. Each sample stock solution (1.0 mg/ml) was diluted to final concentrations of 12.5, 25.0, 50.0, 75.0, and 100.0%. A stock solution of the compounds was prepared at 1 mg/ml in dimethyl sulfoxide (DMSO). The stock solution was diluted to varying concentrations in 96-well microplates. DPPH was added to the solutions prepared with sample extracts and standard antioxidant substances.

The plate was slowly shaken for 3 minutes and incubated for 30 minutes in darkness at 37 °C, in a water bath. The percentage of decolourisation was obtained spectrophotometrically at 515 nm using the Microplate Reader, linked to a computer equipped with (Vermax Software 7.0). Corresponding blank samples (water, DMSO, and DPPH solution) and L-Ascorbic acid (0.25, 0.50, 0.75, and 1.0 mg/ml) as positive control. The L-Ascorbic acid was used as reference standard curve (plot). Experiments were performed in triplicate.

### C. ABTS Radical Scavenging Activity

The assay of antioxidant activity of fish paste extracts was measured on the basis of the scavenging activity of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) free radical according to the method described by Brand-Williams *et al.* [14] with slight modifications. ABTS was dissolved in water to a 7 mM concentration, and 2.45 mM potassium persulfate was prepared. Two stock solutions were mixed and kept in the dark at room temperature for 16 h before use. After the addition of 200 µl of the diluted ABTS solution to 40 µl of the sample extracts, the decrease in absorbance was measured for 1 min after mixing the solution, and the final absorbance reading was monitored for 33 min by absorbance at 734 nm using the Microplate Reader. Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a positive control inhibiting the formation of the radical cation in a dose dependent manner.

### D. Statistical Analysis

This activity is given as the percent of DPPH and ABTS radical scavenged, which is calculated with the equation as fellow (1).

$$\text{Radical scavenging activity (\%)} = \frac{\{(\text{Abs control} - \text{Abs sample}) / \text{Abs control}\} \times 100}{(1)}$$

Where Abs control is the absorbance of DPPH or ABTS radical+methanol and Abs sample is absorbance DPPH or ABTS radical+sample extract/standard.

The IC<sub>50</sub> values were determined using Prism 5.00 software. Vitamin C (L-ascorbic acid) was used as the antioxidant standard and positive control. Results were expressed as IC<sub>50</sub> concentration where 50% inhibition of the DPPH and ABTS radical is obtained.

Differences were considered statistically significant at P<0.05 for all tests [15].

### E. Chromaticity of Fish Paste

The chromaticity of fish paste was measured using a color difference meter (Minolta CR-200, Japan). The chromometer was used to measure lightness (L), redness (a), and yellowness (b). The L value of the standard color plate was 96.21, the a value was 0.82, and the b value was 0.66.

## III. RESULTS

## A. DPPH Radicals Scavenging Assay

The DPPH inhibitory activity in black garlic was obtained by setting up the equations. The results of DPPH were shown in Table II. The degree of DPPH inhibitory activity increased during the increase of the extraction concentration. DPPH scavenging activity of the fish-pasta was evaluated 57.1% on 100% solution with 15 g black garlic addition and control (pure fish, starch, shrimp, squid, egg, and vegetable) was 28.7% at same concentration. There was significant difference among control groups without black garlic and black garlic added groups ( $t=22.553$ ,  $p<0.01$ ).

TABLE II: THE DEGREE OF INHIBITION (%) OF DPPH BY FISH PASTE WITH BLACK GARLIC

Concentration (%)	Black garlic (mg)			
	0	5	10	15
12.5	3.35±0.71	19.58±1.24	22.06±2.94	28.29±2.50
25.0	7.81±1.66	24.34±0.66	27.95±2.87	34.98±4.96
50.0	15.13±2.85	31.39±0.84	36.94±1.47	40.55±3.51
75.0	23.87±0.54	37.79±0.84	43.08±3.16	50.58±1.83
100.0	28.73±1.37	41.88±1.19	49.48±2.11	57.11±1.77
t-test	22.553, $p<0.01$			

## B. ABTS Radicals Scavenging Assay

Table III was shown the results of antioxidant activity for ABTS radical of three black garlic groups. It was observed that inhibition percentage values go on increasing with enhancements in concentration of black garlic extracts in the assay mixture. Control extract of the six food materials was evaluated 25.1% on 100% and those of the 5g and 10g black garlic extracts were 33.9% and 39.2% at same concentration, respectively. 75% black garlic extract was 43.7% at same concentration. There was significant difference among control groups without black garlic and black garlic added groups ( $t=8.688$ ,  $p<0.05$ ).

Fig. 3. was shown the rate of DPPH and ABTS inhibitory of L-Ascorbic acid (positive control) and relative inhibitory rate for four fish-pasta groups on 100% concentration. The relative DPPH values for the control, 5 mg, 10 mg, and 15 mg were 34.9%, 49.1%, 57.7% and 66.6%, respectively. The relative ABTS values for the control, 5 mg, 10 mg, and 15 mg were 30.0%, 40.4%, 46.7% and 52.1%, respectively.  $IC_{50}$  value was inversely related to the antioxidant activity of DPPH and ABTS (Fig. 4.). The values of  $IC_{50}$  for the DPPH and ABTS on 15 mg/ml were 152.2 and 162.4  $\mu\text{g/ml}$ , respectively (Table IV).

The results of chromaticity of fish paste were shown in Fig. 4. The value of lightness (L) was decreased to the increase of the content of black garlic. Redness (a) was highest on 15mg/ml black garlic (7.9). Whereas yellowness (b) was lowest on 15mg/ml black garlic (7.9).

## IV. DISCUSSIONS

Black garlic is popular after being introduced as a functional food rather than regular garlic. Black garlic has been introduced to the Korean market as a health product. It has not been long since black garlic was introduced to the Korean market and manufactured and used.

The antioxidant function of black garlic is also related to the manufacturing (aging) period. The production (aging) of black garlic varies depending on temperature and time [16]. The increase in the antioxidant activities such as DPPH, ABTS, and FRAP assays of black garlic may be due to the increase in total polyphenols, total flavonoids, and ascorbic acid contents during aging period [17].

TABLE III: THE DEGREE OF INHIBITION (%) OF ABTS BY FISH PASTE WITH BLACK GARLIC

Concentration (%)	Black garlic (mg)			
	0	5	10	15
12.5	2.44±0.72	10.76±1.19	14.06±0.73	14.10±0.54
25.0	7.99±3.17	16.46±1.74	19.30±1.88	21.13±3.07
50.0	16.52±0.32	22.08±1.83	23.43±0.83	29.06±2.79
75.0	22.71±0.81	29.84±1.37	30.13±0.93	39.16±3.74
100.0	25.13±1.63	33.92±2.10	39.19±2.97	43.70±2.26
t-test	8.688, $p<0.05$			

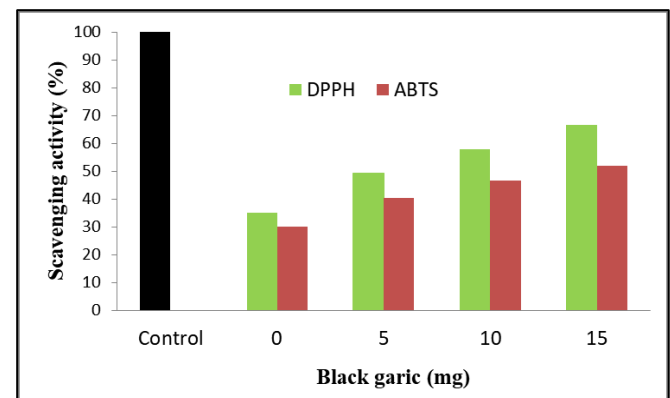


Fig. 3. The relative inhibitory rate of DPPH and ABTS for L-Ascorbic acid (positive control) on fish pastes with black garlic addition.

TABLE IV: THE 50% INHIBITION ( $IC_{50}$ ) OF DPPH AND ABTS BY FISH PASTE WITH BLACK GARLIC

Radical	Black garlic (mg)		
	0	5	10
DPPH	203.9±4.6	178.78±7.2	166.4±10.3
ABTS	225.7±9.3	200.7±11.0	190.5±4.5

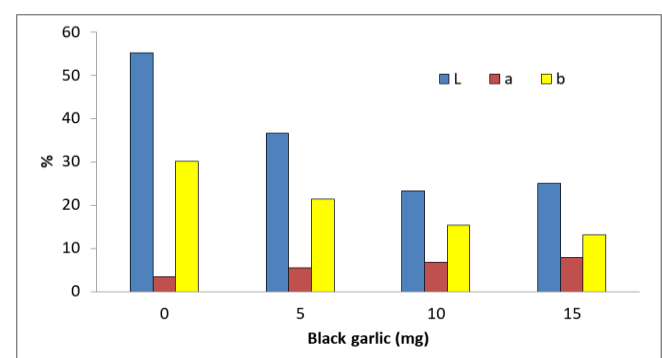


Fig. 4. The analyses of color parameters of fish pastes with black garlic addition. L: Lightness, a: Redness, b: Yellowness.

The antioxidant function of black garlic varies depending on the extraction solvent. When the antioxidant activities of hot water and ethanol extracts from fresh, steamed and black garlic were compared, the highest level was  $69.40\pm0.13$  in concentration of 10 mg/mL from black garlic ethanol extract, ethanol extracts showed  $50.55\pm1.40$  in concentration of 15 mg/mL [18]. Moreno *et al.* [19] said that the amadori compound obtained in the first step of the Maillard reaction

of the aged garlic extract obtained by aging at room temperature for more than 10 months has an antioxidant effect.

Increasing the temperature can shorten the ripening period of garlic. The total phenol and flavonoid content increased with the aging period as the aging temperature increased, and in the case of the aged 6-day sample, the total phenol was about 3.5 times higher and the flavonoid was about 9.1 times higher in the 90 °C aging sample than 60 °C [18]. Highest total polyphenol content was 18.155 mg/g at 140 °C and 2 hours, seven times higher than untreated garlics [20].

The antioxidant level varies depending on the fish material used in black garlic. For example, puffer fish sauce and fish sauces containing soybean, wheat and koji mold tended to have higher ORAC values and lower ESR IC<sub>50</sub> values [21]. Puffer fish sauce had a high oxygen radical absorbance capacity (ORAC) value (8,365 µmol TE/100 ml) and the highest hydroxyl radical scavenging activity (0.081). Many kinds of free amino acids were detected in fresh and steamed garlic, while five more free amino acids, O-phosphoethanolamine, and urea were additionally detected in black garlic [17]. This change in ingredients can be seen as an increase in antioxidant function compared to the control group when black garlic is added to fish pasta (Tables II and III).

Choi *et al.* [8] reported lightness and yellowness values of black garlic radically decreased during the aging period, whereas redness values increased significantly. The results of this study are consistent with their findings (Fig. 4.). Thermal processing induces many chemical reactions in garlic, such as enzymatic browning and the Maillard reaction, causing its color to change from white and yellow to dark brown [3]. This intensity is the most convenient measurable index of the Maillard reaction because it can be estimated visually [22]. There are reports that garlic is caused by browning at 60 °C [16]. In fish cakes, black is not the original color of fish cakes that have been recognized for a long time. Because it is fried during the manufacture of fish cakes, black fish cakes can be mistaken for burnt during the frying process. Therefore, excessive black color may not be good for fish cake sales. In Fig. 2. fish cakes containing more than 10 g are of an undesirable color.

Xiang *et al.* [23] reported both black garlic residue and black garlic had stronger capacities to scavenge DPPH than raw garlic and IC<sub>50</sub> values of 0.454, 0.514, and 4.236 mg/ml, respectively. In this study, IC<sub>50</sub> of fish-pasta with black garlic was 0.152 mg/ml (=152mg/L) (Table II).

## V. CONCLUSION

Black garlic is popular after being introduced as a functional food in Korea. The degree of DPPH and ABTS radical inhibitory activity increased during the increase of the extraction concentration. There was significant difference among control groups without black garlic and black garlic added groups. In addition, excessive black color may not be good for fish cake sales.

## CONFLICT OF INTEREST

Author declares that there do not have any conflict of interest.

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