

Effect of Heating on DPPH Radical Scavenging Activity of Meat Substitute

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ABSTRACT: This study was conducted to evaluate the increase of DPPH radical scavenging activity of meat substitute by heating. The meat substitute showed higher DPPH radical scavenging activity than those of other foods rich in protein such as beef, pork, chicken, and soybean curd. The DPPH radical scavenging activity of meat substitute was dependent upon concentration, heating temperature and heating time of meat substitute. The DPPH radical scavenging activity of meat substitute was enhanced with increasing heating temperature and time. The increase of DPPH radical scavenging activity was only applied to meat substitute without showing any activation in other foods rich in protein such as beef, pork, chicken, and soybean curd.

Keywords: DPPH radical scavenging activity, meat substitute, heating, polyphenols

INTRODUCTION

Meat substitutes, also referred to as meat replacers, meat alternatives, and meat analogs, are primarily vegetable based food products that contain proteins made from mainly soybean and wheat gluten (1-4). Meat substitutes have been used as a meat replacement for a long time, but currently are not consumed widely by consumers; the main reason may be attributed to the fact that meat substitutes stayed behind in overall evaluation and in particular the sensory appreciation (1,4) compared to those of real meats. Therefore, meat substitute products are primarily aimed towards and used by vegetarians and semi-vegetarians and have a strong emphasis on health and ethnic quality aspects (3-6). However, Elzerman et al. (7) reported appropriateness seemed to be influenced by the appearance of the meat substitute, meal combination, and less by flavor and texture. Since meat substitutes have similar flavors and textures but with different shapes (piece and mince) were rated differently in four meals on product liking, appropriateness and intention-to-use, but not differently on overall liking of the meals (7).

Several reports relate meat consumption to human diseases, including cancer. The effect of meat consumption on cancer risk is a controversial issue; however, recent meta-analyses show that high consumers of cured

meats and red meat are at an increased risk for colorectal cancer, although findings of most studies have not reached statistical significance (8-11). Beef meat and cured pork meat were reported to promote colon carcinogenesis in rats, and the possible mechanism by which meat could increase the risk of cancer is N-nitrosation or fat peroxidation of heme iron (8). Red meat was reported to enhance the colonic formation of the DNA adduct O⁶-carboxymethyl guanine (12). Dietary additives were reported to suppress the toxic effects of heme iron (13).

A previous report suggested that diet affected the intestinal microflora and its metabolic activities (14). The change in the composition of gut microflora also affects the metabolism of carcinogens (15,16). Also, the diet related differences in microflora have a strong impact on the genotoxic effects of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and suggest that heterocyclic amines are less genotoxic and carcinogenetic in individuals that consume mainly plant derived food (17).

Antioxidants have been suggested to have a well-defined role as preservatives because they neutralize free radicals by donating one of their own electrons to end the electron-stealing reaction. Antioxidants have been defined by the US Food and Drug Administration (FDA) as substances used to preserve food by retarding deterioration, rancidity or discoloration caused by oxidation

Received: September 17, 2012; **Accepted:** January 31, 2013

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(18). In recent years, however, the use of some synthetic antioxidants has been restricted because of their possible toxic and carcinogenic effects (19-22). Thus, the natural antioxidants present in foods and other biological materials have attracted considerable interest because of their presumed safety and potential nutritional and therapeutic effects (23,24). Recently, phytochemicals in fruits and vegetables have attracted a great deal of attention, concentrated mainly on their role in preventing diseases caused by oxidative stress.

Antioxidant effects in fruits and vegetables can be from phenolic compounds, such as flavonoids and phenolic acids, or nitrogen compounds, such as alkaloids, chlorophyll derivatives, amino acids and amines. These flavonoids and other phenolic compounds of plant origin have been reported as scavengers and inhibitors of lipid peroxidation (25-27). Antioxidant activities of phenolic compounds are correlated to structure-activity relationships, such as redox properties and the number and arrangement of the hydroxyl groups (28).

Consumers are more conscious of nutritional value and safety of food ingredients. At the same time, consumers prefer natural foods and food ingredients that are believed to be safer, healthier, and less subject to contamination than their artificial counterparts. Therefore, the investigation and identification of natural antioxidants from edible plants is worthwhile even though they may not be comparable in efficiency to synthetic agents (29).

Meat substitute has been produced only by food ingredients derived from plants, possibly containing antioxidant activities. However, no report has been published about antioxidant activities of meat substitute. The aim of the present study is to investigate the antioxidant effect of meat substitute to gain an overall understanding of its health effects.

MATERIALS AND METHODS

Material

Various ingredients for meat substitute were purchased from a local market in Busan, Korea. The 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, gallic acid, ammonium thiocyanate, trichloroacetic acid (TCA), and 2,6-di-*tert*-butyl-4-methylphenol (BHT) were obtained from Sigma Chemical Co., Ltd. All other chemicals used were of analytical grade.

Production of meat substitute

Meat substitute was prepared using Kim's method with minor modifications (30). The ingredients for meat substitute were cashew nut, walnut, soybean, black sesame, sesame, beet, onion, and gluten (Table 1). The ingre-

Table 1. Ingredients for meat substitute preparation

Ingredient	Raw soy meat	
	Quantity (g)	Percentage (%)
Walnut	15.21	4.23
Sunflower seed	15.21	4.23
Dry shiitake	15.21	4.23
Peanut	15.21	4.23
Pine nut	5.07	1.41
Cashew nut	5.07	1.41
Almond	5.07	1.41
Flaxseed	5.07	1.41
Button mushroom	15.21	4.23
Onion	5.07	1.41
Beet	20.29	5.63
Soybean	5.07	1.41
Water	131.83	36.62
Gluten	101.41	28.17
Total	400.00	100

dients, except gluten, were ground into small pieces for 5 min and then mixed with gluten for 10 min. The mix was then used as meat substitute in this study. The meat substitute and other ingredients were freeze-dried and grounded. The 1.0 g of dried meat substitute and ingredients were extracted with 100 mL of water for 24 hr at room temperature. The extracts were centrifuged at 12,000×g for 20 min and the supernatants were used in the research experiments.

DPPH radical scavenging activity (RSA) of meat substitute

The DPPH radical scavenging activity (RSA) of meat substitute extract and ingredients were measured in terms of their hydrogen donation or radical scavenging activity, using the stable radical DPPH. The DPPH scavenging activity was performed as previously described (31) with some modifications. DPPH was dissolved in ethanol, and the experiments were performed on freshly prepared solutions. In this assay, reaction mixture containing 0.1 mL of meat substitute or other ingredients, was added to 2.9 mL of a DPPH solution and then shaken and left to stand for 10 min. Decolorization of DPPH-donated protons was determined by measuring the absorbance at 525 nm with a spectrophotometer (Ultrospec 3000, Pharmacia Biotech, Cambridge, England).

The scavenging activity of DPPH radical was calculated using the following equation:

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

The half maximal inhibitory concentration (IC₅₀) of meat substitute was determined as the concentration that causes 50% loss of DPPH radical scavenging activity.

The effect of heating temperature on DPPH radical scavenging activity of meat substitute was performed by heating water extract (5%) of the meat substitute ex-

tract at various temperatures from 20°C to 95°C for 10 min. The effect of heating time on DPPH radical scavenging activity was also determined by heating water extract of meat substitute for various time intervals up to 30 min at 95°C, and then each extract was examined for its DPPH scavenging activity.

Determination of total phenolic compounds

Total phenolic compounds were analyzed according to the Folin-Denis method (32), using gallic acid as the standard. The assay conditions were as follows: 0.5 mL of sample was added to 2.5 mL of Folin-Ciocalteu reagent. After 5 min, 2 mL of 7.5% aqueous sodium carbonate solution was added to the mixture and then incubated at 50°C for 5 min. Absorbance of the resulting mixture was measured at 760 nm. The content of total phenolic compound was calculated on the basis of the standard curve of gallic acid. Therefore, results are given as µg/g of gallic acid equivalents (GAE).

Statistical analysis

All analyses were performed in triplicate and the data reported as mean±standard deviation. The statistical analysis was performed by ANOVA and Duncan's multiple range test using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). A p-value of <0.05 was regarded as significant.

RESULTS AND DISCUSSION

Fig. 1 shows the comparison of DPPH radical scavenging activities and amounts of polyphenols of various foods rich in protein. Meat substitute exhibits the highest antioxidant activity among tested foods, while beef, chicken, pork, and soybean curd showed lower radical scav-

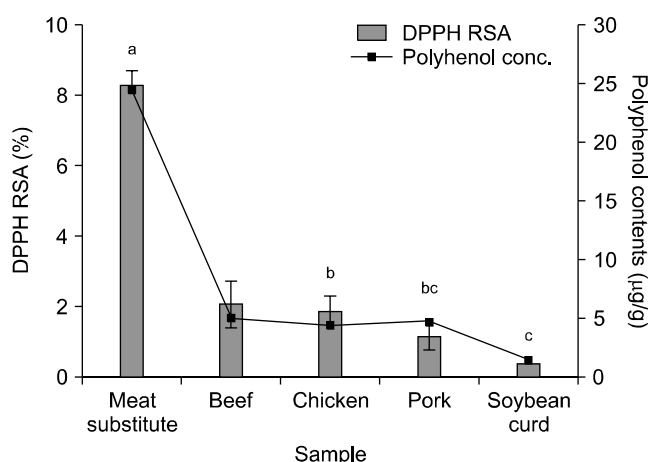


Fig. 1. Comparison of DPPH radical scavenging activity and amount of polyphenols of various foods. The foods were freeze-dried, and dissolved in water (1%). ^{a-c}Values with different alphabets on bars [DPPH RSA (%)] are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

enging activities. Previous research has shown that polyphenols may be the active compounds for antioxidant activities in plants (25-27). Therefore, the amounts of polyphenols in the samples were also determined. Total phenol contents of meat substitute, beef, chicken, pork, and soybean curd were determined by the Folin-Denis method, and expressed as 24.5 ± 1.5 , 5.1 ± 0.7 , 4.5 ± 0.9 , 4.8 ± 1.2 , and 1.6 ± 0.3 µg/g of gallic acid equivalent as shown in Fig. 1. As expected, the meat substitute possessed the highest polyphenols among tested samples, while the soybean curd showed the lowest polyphenol content. Since water-soluble compounds, including antioxidant activity, was extracted out during soybean curd preparation, soybean curd seems to have the lowest antioxidant activity even though it is derived from soybean. Since meat substitute was prepared from various foods derived from plant origin, the antioxidant activity of meat substitute seems to be due to the ingredients of meat substitute (Table 1). Table 2 shows the DPPH radical scavenging activities of meat substitute ingredients. Among the ingredients, walnut, sunflower seed, and onion possessed high DPPH radical scavenging activities.

The radical scavenging activity (RSA) with varying meat substitute concentration was plotted to calculate the IC_{50} values, which is the concentration of the extract that causes 50% loss of DPPH activity. The DPPH radical scavenging activity of meat substitute linearly increased with increasing meat substitute concentration up to 7% and the IC_{50} of the meat substitute extract was 6.2% (w/v) as shown in Fig. 2. Therefore, 1.0 g of meat substitute possesses the DPPH radical scavenging activity corresponding to that of 14.0 mg of BHT. Since meat substitute contains 39.0% moisture (data not shown), DPPH radical scavenging activity of 1.0 g of dried meat substitute corresponds to that of 22.9 mg of BHT.

Taken together, the results of DPPH radical scavenging activity and polyphenolic compounds indicate strong association between antioxidant activity and contents of phenolic compounds, suggesting that phenolic com-

Table 2. DPPH radical scavenging activity of meat substitute ingredients

Ingredient	DPPH radical scavenging activity (%)
Walnut	23.88±0.16
Sunflower seed	23.19±0.14
Dry shiitake	10.53±0.98
Peanut	9.31±0.61
Pine nut	2.21±1.10
Cashew nut	1.13±0.22
Almond	0.01±0.01
Flaxseed	5.19±1.54
Button mushroom	3.70±1.15
Onion	22.47±0.63
Beet	2.79±0.30
Soybean	0.83±0.83
Gluten	1.18±0.18

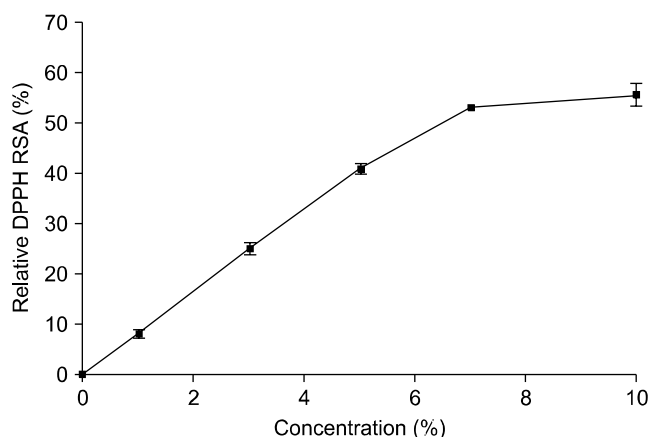


Fig. 2. Effect of meat substitute concentration on DPPH radical scavenging activity.

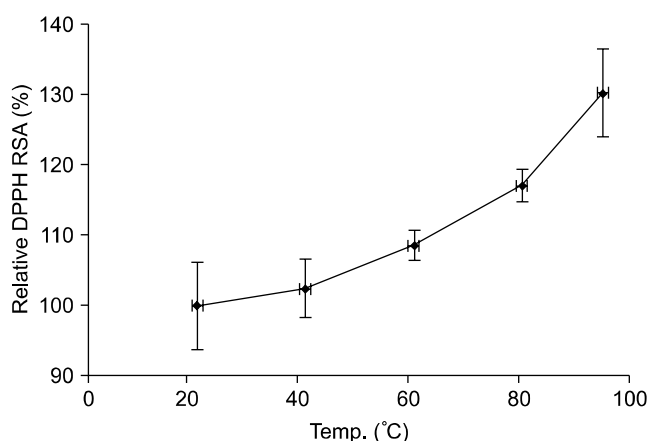


Fig. 3. Effect of heating temperature on relative DPPH radical scavenging activity of meat substitute. Meat substitute (5%, w/v) was heated for 10 min at various temperatures, and residual DPPH radical scavenging activity was expressed as relative DPPH RSA (%).

pounds are probably responsible for the antioxidant activity of meat substitute. Many previous studies conducted with vegetables or fruits have also found a positive correlation between total phenolic compounds and the antioxidant activity, concluding that high total phenol contents increase antioxidant activity (33-35).

Meat substitute is usually consumed after cooking by heating with added seasoning to improve taste. Also, heating enhances the antioxidant properties of some plants (36,37). Therefore, we investigated whether heating changes the DPPH radical scavenging activity of the meat substitute. Fig. 3 shows DPPH radical scavenging activity of meat substitute increased by heating at various temperatures for 10 min. The enhancement of DPPH radical scavenging activity was dependent on the heating temperature (Fig. 3). Fig. 4 demonstrates the increase in DPPH radical scavenging activity was also dependent on the temperature employed. As the temperature increased, the antioxidant activity of meat substitute also increased. Therefore, we investigated whether

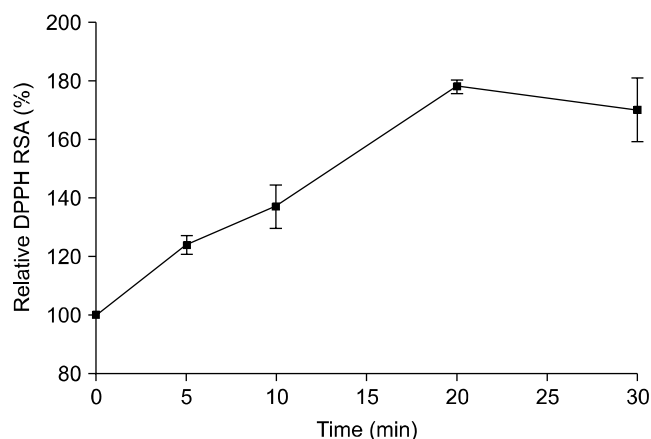


Fig. 4. Effect of heating time on relative DPPH radical scavenging activity of meat substitute. Meat substitute was heated for various time intervals at 95°C, and the residual DPPH radical scavenging activity was expressed as relative DPPH RSA (%). The concentration of meat substitute was 5% (w/v).

er heating had any effect on DPPH radical scavenging activities of meat substitute ingredients. Among the tested ingredients of meat substitute, heating at 95°C for 10 min enhanced DPPH radical scavenging activities of peanut (108.2%), dry shiitake (36.1%), and onions (10.7%). Therefore, an increase in DPPH radical scavenging activity of meat substitute may be attributed to the enhancement of DPPH radical scavenging activities of peanut, dry shiitake, and onion by heating. As previously reported, the Maillard reaction products contain antioxidant activity by scavenging oxygen radicals or chelating metals (38). Therefore, attempts were made to determine the amount of Maillard reaction products produced in meat substitute by heating. Beets were used as a coloring agent in the meat substitute and its red color decolorized during heating at 95°C. The production of Maillard reaction can be determined by measuring color change at 420 nm. However, this method was difficult to measure the amount of Maillard reaction products in meat substitute because of the color change of beet during heating. The reduct one moiety present in Maillard reaction products has been reported to exhibit both reducing and chelating properties (39). Possibly, various reduct ones produced in the Maillard reaction process may contribute to the increased DPPH radical scavenging activity of meat substitute by heating.

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