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ARTICLE

Evaluation of Antioxidant Activities of Ethanol Extracted Garlic and Onion as Affected by Pre-heating for the Application of Meat Products

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Abstract

The objective of this study was to evaluate the pre-heating treatment effects on the antioxidant properties of ethanolic garlic and onion extracts. Garlic and onion with or without heating (100°C, 30 min) were extracted with ethanol, and the total phenolic content, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability, iron chelating ability, reducing power, and antioxidant activity in a linoleic acid emulsion system were evaluated. Garlic (41%) had a higher drying yield than onion (11%). Regardless of pre-heating, ethanol extracts of onion resulted in an approximately 25-fold higher yield than those of garlic. Thermal treatment before extraction decreased the levels of ethanol-soluble phenolics for both garlic and onion. Regardless of pre-heating, the radical scavenging abilities of ethanol extracts from garlic were greater than the ethanol extracts from onion. The iron chelating abilities of ethanol extracts from fresh and heated garlic were 85 and 81% at 10 mg/mL, respectively, whereas those of onion extracts were 10 and 9% at the same concentration, respectively. However, no differences in reducing power between garlic and onion extracts were observed. Both garlic and onion inhibited the formation of hydroperoxide in linoleic acid emulsion systems when ethanol was used as a solvent. Overall, garlic extracts had greater antioxidant activity than onion extracts, and the antioxidant activity of garlic and onion extracts were not significantly affected by thermal treatment.

Key words: garlic, onion, antioxidant activity, pre-heating

Introduction

Animal fats in muscle foods are considered to be important factors affecting food quality. These fats provide nutritional values, essential fatty acids and desirable sensory characteristics. However, lipid oxidation deteriorates nutritional values and shortens shelf-life of meat and meat products, and produces undesirable flavor and color, which decreases consumer's acceptance. Therefore, lipid oxidation is a major cause of quality deterioration in flavor, color, texture, nutritional values and safety of meat and meat products. To reduce lipid oxidation, many studies related to natural or synthetic antioxidants have been widely performed (Ahn *et al*., 2007; Bozkurt, 2006a). However, synthetic antioxidants such as butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA) and *tert*-butyl hydroquinone (TBHQ) are limited to use as additive agents in food systems because they contain many factors hazardous to health (Branen, 1975). Hence, antioxidants derived from natural resources are perceived by consumers as being better and safer than synthetics. Among natural sources, garlic (*Allium sativum*) and onion (*Allium cepa*) have been used as a spice food and medicine for over 4,000 years (Ali *et al.*, 2000). Both garlic and onion are known to possess various biological functions, including antioxidant and antimicrobial activities (Corzo-Martinez *et al.,* 2007; Lanzotti, 2006). These biological functions may be due to the presence of organo-sulfur and phenolic compounds (Miean and Mohamed, 2001). However, processing of garlic and onion, especially heat treatment, may change their components, and consequently either increase or decrease the functionality of garlic and onion. Therefore, the objective of this study were to evaluate the antioxidant properties of ethanolic garlic and onion extracts as affected by pre-heating.

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Materials and Methods

Preparation of garlic and onion extracts

The garlic (*Allium sativum*) and onion (*Allium cepa*) were purchased from a local market. The extracts from the garlic bulbs and onion were obtained as follows: The garlic and onion were peeled and washed with double deionized (dd) water. One batch of garlic or onion was heated at 100ºC for 30 min to determine the effect of heating. Two batches (fresh and heated) of garlic or onion were freeze-dried. The freeze-dried samples were crushed and homogenized with 10 volumes of ethanol (94.0%) at 10,000 rpm for 5 min using a homogenizer (AM-3, Ace Homogenizer, Nissei, Tokyo, Japan), and then extracted by stirring at room temperature for 24 h. The ethanol extracts were filtered using filter paper (Whatman No. 1). The filtrates were rotary-evaporated at 40ºC to evaporate the ethanol (Rotavapor, R110, Buchi, Flawil, Switzerland), and then the extracts were freeze-dried to remove the residual moisture.

Determination of total phenolic content

The total phenolic contents of the garlic and onion extracts were determined using the method described by Lin and Tang (2007). Briefly, 0.1 g of extract was dissolved in dd-water (10 mL). Then, 0.1 mL of this mixture was added to dd-water (2.8 mL), 2% sodium carbonate (2 mL, Na_2CO_3) and 50% Folin-Ciocalteau reagent (0.1 mL), and vortexed. After the reaction mixture was then incubated at room temperature for 30 min, the absorbance of mixture was measured at 750 nm against a blank on a spectrophotometer (UV-1601, UV-Visible Spectrophotometer, Shimadzu, Australia). Gallic acid was used as a standard (0-200 ug/mL). All data were expressed as g gallic acid equivalents (GAE)/100 g of dried extracts.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The radical scavenging activities of garlic and onion extracts were measured based on their ability to scavenge DPPH-radicals, which was determined according to the method described by Huang *et al.* (2006). Approximately 4 mL of each extract (1-20 mg/mL in dd-water) was mixed with 1 mL of 0.2 mM methanolic DPPH-radical solution. The mixture was then vortexed, and it was incubated at room temperature for 30 min in dark room. Then, the absorbance was measured at 517 nm against a blank (UV-1601, UV-Visible Spectrophotometer, Shimadzu, Australia). Ascorbic acid was used for com- parison with garlic and onion extracts. The scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = \frac{\Delta A_{517}\text{ of control} - \Delta A_{517}\text{ of sample}}{\Delta A_{517}\text{ of control}} \times 100$$

Ferrous iron chelating ability

The ferrous ion chelating abilities of the garlic and onion extracts were determined by measuring the inhibition of the formation of a Fe^{2+}-ferrozine complex using the method described by Le *et al*. (2007). The extracts (0.5 mL of 1-20 mg/mL in dd-water), ferrous chloride (0.1 mL, 0.6 mM in dd-water) and methanol (0.9 mL) were combined, shaken well and then allowed to react at room temperature for 5 min. After the reaction, ferrozine (0.1 mL, 5 mM in methanol) was added and the sample was then allowed to react at room temperature for 10 min. The absorbance of the Fe^{2+}-ferrozine complex was then measured at 562 nm against a blank (UV-1601, UV-Visible Spectrophotometer, Shimadzu, Australia). 2Na·EDTA ·$2H_2O$ (Ethylene diaminetetraacetic acid disodium salt dihydrate) was used for comparison with garlic and onion extracts. The chelating effect was then calculated as a percentage using the following equation:

$$\text{Chelating effect (\%)} = \frac{\Delta A_{562}\text{ of control} - \Delta A_{562}\text{ of sample}}{\Delta A_{562}\text{ of control}} \times 100$$

Reducing power

The reducing power was measured using the method described by Huang *et al.* (2006). Briefly, each extract (2.5 mL of 1-20 mg/mL in dd-water) was mixed with sodium phosphate buffer (2.5 mL, 200 mM, pH 6.6) and potassium ferricyanide (2.5 mL, 10 mg/mL), after which the mixture was incubated at 50ºC for 20 min. Then, trichloroacetic acid (2.5 mL, 100 mg/mL) was added and the mixture was centrifuged at 200×*g* for 10 min. After the upper layer (5 mL) was then mixed with dd-water (5 mL) and ferric chloride (1 mL, 1 mg/mL), the absorbance at 700 nm was measured against a blank in a spectrophotometer (UV-1601, UV-Visible Spectrophotometer, Shimadzu, Australia). The higher absorbance indicates the higher reducing power.

Antioxidant activity in linoleic acid emulsion

The antioxidant activity of garlic and onion extracts in linoleic acid emulsion system was determined using the thiocyanate method described by Yen and Hsieh (1998).

Briefly, extracts (500 ug in 0.5 mL of dd-water) of garlic or onion were mixed with phosphate buffer (2 mL, 0.2 M, pH 7.0) and linoleic acid emulsion (2.5 mL, 0.02 M, pH 7.0). The absorbance of mixture was measured during incubation at 37°C. Ethanol (4.7 mL, 75%), ammonium thiocyanate (0.1 mL, 30%), sample mixture (0.1 mL) and ferrous chloride (0.1 mL, 0.02 M in 3.5% HCl) were combined, vortexed and then allowed to react at room temperature for 3 min. Then, the absorbance was measured at 500 nm against a blank in a spectrophotometer (UV-1601, UV-Visible Spectrophotometer, Shimadzu, Australia). The higher absorbance indicates the lower antioxidant activity.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) with SPSS 14.0. Means were separated using Duncan's multiple range test. EC50 values were obtained by interpolation from linear regression analysis.

Results and Discussion

Extraction yield and total phenolic content

As shown in Table 1, the weights of garlic and onion decreased approximately 59 and 89% after freeze-drying, respectively, regardless of heating. Therefore, the garlic had a higher drying yield than onion ($p<0.05$). These results are similar to those of previous study (Abhayawick *et al*., 2002; Bae and Chun, 2003; Dini *et al*., 2008; Gorinstein *et al*., 2005; Shin *et al*., 1999). However, onion showed an approximately 25-fold higher extraction yield than garlic ($p<0.05$), regardless of thermal treatment. The levels of total phenolics ranged from 1.11 to 2.49% (Table 1), and ethanolic extracts from garlic contained more phenolic compounds than those from onion ($p<0.05$). However, thermal treatment (100°C, 30 min) significantly decreased phenolic compounds in both garlic and onion. Several previous studies have found that boiling garlic and onion decreased the level of total phenolics (Gorinstein *et al*., 2006; Gorinstein *et al*., 2008). Specifically, Roy *et al*. (2007) and Makris and Rossiter (2001) found that the amount of total phenolics extracted from heated (100°C, 60 min) garlic samples was approximately 35% lower than that of raw garlic, and that boiling (100°C, 60 min) onion resulted in 20.6% decreases in total quercetins. The results of several studies have suggested that a decrease in total phenolic content by thermal treatment may be partially due to the degradation of a portion of the phenolic compounds by thermal processing (Randhir *et al.*, 2008) or the breakdown of phenolics (Crozier *et al*., 1997). In actual meat products, types and amounts of phenolic compounds are very important in inhibition of lipid peroxidation because their radical scavenging ability, iron chelating ability and reducing power depend on position and amount of hydroxyl group in phenolic compounds (Kristinova *et al*., 2009; Medina *et al.,* 2007). Onion contains many flavonols (2720 mg/kg). Especially, quercetin (1497 mg/kg) belonging to flavonols is the most typical phenolic compounds in onion (Lanzotti, 2006). According to results of Tang and Cronin (2007) who researched effects of onion on lipid oxidation of ground turkey, quercetin in onion was the main antioxidant. Antioxidant effect of quercetin was also found in ground beef patties; 110 and 550 quercetin umol/kg of meat reduced 42.4 and 62.7% of TBARS value during storage at 2°C for 6 d, respectively (Bekhit *et al*., 2003). Garlic not only possesses phenolic compounds such as myricetin and apigenin, but also contains many organosulfur compounds that have various functions (Corzo-Martinez *et al*., 2007; Lanzotti, 2006). Yin and Cheng (2003) studied antioxidant activities of four garlic-derived organo-sulfur compounds in ground beef found that both lipophilic-(diallyl sulfide and diallyl disulfide) and hydrophilic-(*s*-ethyl cysteine and *n*-acetyl cysteine) compounds dose-dependently delayed lipid oxidation, and showed significantly greater antioxidant activity than α-tocopherol.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

Free radicals act as initiator in the process of lipid

Table 1. Dry and extraction yields, and total phenolic contents of ethanolic garlic and onion extracts as affected by pre-heating treatment

	Garlic		Onion	
	Unheated	Heated	Unheated	Heated
Dry yield (g/100 g of fresh weight)	41.35[a]	41.52[a]	10.52[b]	10.49[b]
Extraction yield (g/100 g of dry weight)	2.46[c]	2.15[c]	52.38[b]	55.18[a]
Total phenolic (g GAE/100 g of extracts)	2.49[a]	1.56[b]	1.31[c]	1.11[d]

[a-d]Means with same superscripts within same row are not different ($p>0.05$).

autoxidation, and the DPPH radical has been widely used to evaluate the antioxidant activity of various natural plants. Radical scavenging abilities of the four extracts increased as the concentration increased. As a reference, ascorbic acid showed an excellent scavenging ability of greater than 95.0% at a concentration of 0.05 mg/mL. The scavenging abilities of ethanol extracts from fresh and heated garlic were 73.33 and 73.29% at 5 mg/mL, respectively (Fig. 1). Ethanol extracts from onion showed lower radical scavenging ability than those from garlic, regardless of pre-heating. These results were supported by the EC_{50} values of the extracts (Table 2). DPPH radical scavenging abilities in both garlic and onion were not affected by thermal treatment (100°C, 30 min). The results of the present study are supported by the results of a several previous studies. According to Pedraza-Chaverri *et al*. (2006), extracts from garlic with or without heating had similar hydroxyl radical scavenging activities of approximately 40% at a concentration of 2.61 mg/mL. In addition, aqueous extracts from garlic powder showed similar peroxynitrite scavenging capacity, regardless of boiling (Pedraza-Chaverri *et al*., 2007). Racchi *et al.* (2002) reported that boiling (100°C, 30 min) had no effect on the hydroxyl radical scavenging ability of onion. However, according to Gorinstein *et al*. (2008), although blanching (100°C, 90 sec) and frying (100°C, 10 min) white and red onion did not affect the level of antiradical activities against DPPH radicals, boiling (100°C, 10 min) significantly decreased radical scavenging ability. Lipid oxidation in meat and meat products primarily occurs through autoxidation induced by free radicals since it mainly generates hydroperoxides and carbonyl compounds through a three-phase process like initiation, propagation and termination steps (Laguerre *et al*., 2007). Therefore, strategies to control the free radicals are necessary for protection of meat and meat products from lipid peroxidation. Free radical scavenging abilities of additive agents as an antioxidant are important factor in the oxidative degradation. According to Naveena *et al*. (2008), chicken patties with the pomegranate rind powder extract that had a relatively high radical scavenging ability and reducing power had lower TBARS values than control or chicken patties with pomegranate juice. In our previous studies (Park and Chin, 2010a, b), methanolic extracts from garlic and onion, both of which had a good antiradical properties, reduced significantly TBARS values of fresh pork patties during refrigerated storage at 4°C ($p<0.05$). Therefore, we suggest that ethanolic extracts used in the present study might be effective in inhibition of lipid peroxidation of meat products.

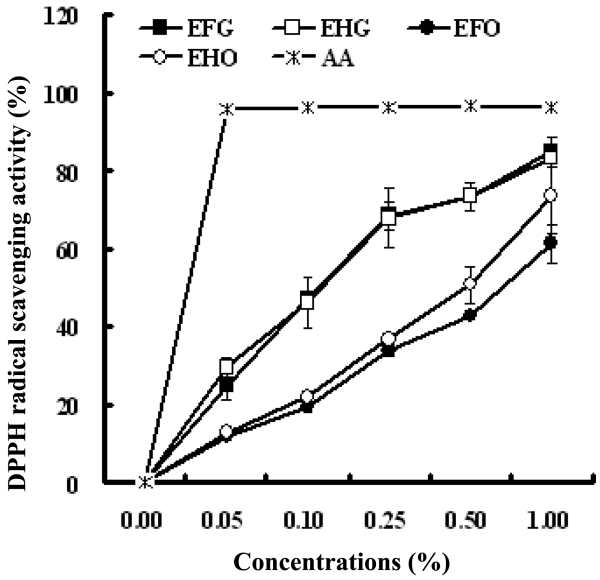

Fig. 1. DPPH radical scavenging activities of ethanolic garlic and onion extracts as affected by pre-heating treatment. EFG, ethanol extracts from fresh garlic; EHG, ethanol extracts from heated garlic; EFO, ethanol extracts from fresh onion; EHO, ethanol extracts from heated onion; AA, ascorbic acid.

Ferrous iron chelating ability

Ferrous iron is recognized as a pro-oxidant because its potent oxidizing activity induces lipid oxidation, which

Table 2. EC_{50} values of garlic and onion extracts in various antioxidant measurements

	EC_{50} values (g extract/100 mL)			
	Garlic		Onion	
	Unheated	Heated	Unheated	Heated
DPPH radical scavenging activity	0.12[c]	0.12[c]	0.70[a]	0.52[b]
Ferrous iron chelating ability	0.21[b]	0.33[b]	4.53[a]	4.60[a]
Reducing power	0.66	1.07	0.99	0.89

[a-c]Means with same superscripts within same row are not different ($p>0.05$).

EC_{50} value: the effective concentration at which DPPH radicals were scavenged by 50%, ferrous ions were chelated by 50%, and the absorbance was 0.5 for reducing power. EC_{50} value was obtained by interpolation from linear regression analysis.

results in the development of an off-flavor (Wong and Kitts, 2001). Ferrous iron-binding properties of garlic and onion extracts were measured using Fe^{2+}-ferrozine complex method. The results revealed that the iron chelating abilities of ethanol extracts from garlic were greater than those of ethanol extracts from onion, regardless of thermal treatments (Fig. 2). Specifically, the ethanol extracts from fresh and heated garlic showed high chelating abilities of 75.6-84.5% and 62.3-81.1% at 5.0-10.0 mg/mL, respectively, whereas the onion extracts showed chelating abilities of 3.3-9.6% and 2.9-8.7% at the same concentration, respectively. These trends were also found in the concentrations of extract required to chelate 50% (EC_{50}) of the ferrous ions in a chemical mixture (Table 2). Specifically, the EC_{50} values of the iron chelating abilities of the garlic extracts were lower than those of the onion extracts ($p<0.05$). As a comparison, EDTA showed a chelating ability of approximately 100% at 0.05 mg/mL. Free iron acts as an oxidation promoter in muscle system because it produces the hydroxyl radical via the chemical Fenton reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH^-$ (Erickson, 2002). In initiation of storage of beef, pork and chicken, levels of non-heme iron are low (Hazell, 1982; Rhee *et al*., 1996). However, in muscles that are ground, cooked and stored during long period, increase of free iron has been observed (Estevez and Cava, 2004; Kristensen and Purslow, 2001; Schricker and Miller, 1983). These results suggested that grinding, cooking or storage induced oxidative cleavage of porphyrin ring of heme, and hence caused the release of free ionic iron from heme iron. Therefore, free ionic iron released from heme pigments may be considered as the major catalyst for lipid peroxidation in raw meat stored for long period, and in cooked or ground meats. In our previous studies (Park and Chin, 2010a, b), we found that water extracts from garlic and onion had an excellent iron chelating abilities and significantly inhibited lipid peroxidation of fresh pork patties, as compared to counterparts, although their radical scavenging abilities were very low. Therefore, ethanolic extracts from garlic should be used as free iron chelator in muscle system, regardless of heating (Fig. 2).

Reducing power

Reducing powers of ethanolic garlic and onion extracts were estimated by measuring the ability of the extracts to reduce ferric iron to ferrous iron. In both garlic and onion, the reducing powers of the extracts increased ($p<0.05$) with increased concentrations, but were not affected by thermal treatment (Fig. 3). Specifically, reducing powers of the ethanol extracts from fresh and heated garlic ranged from 0.11-0.71 and 0.11-0.47 at 0.5-10.0 mg/mL, respectively, whereas the onion extracts ranged from 0.11-0.52 and 0.12-0.55 at the same concentration, respec-

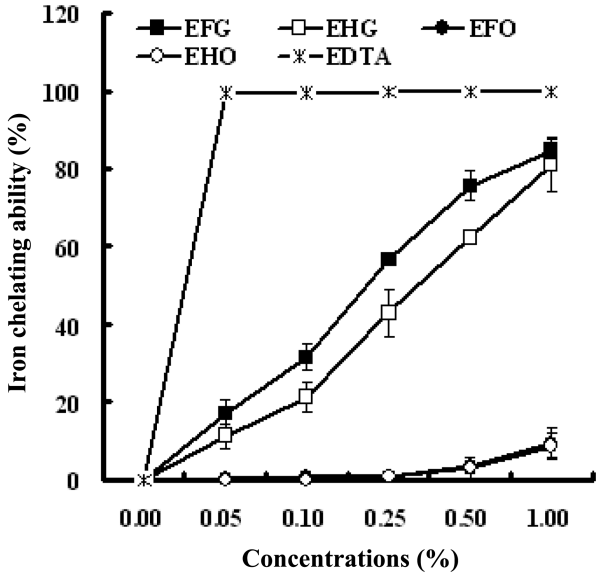

Fig. 2. Ferrous iron chelating abilities of ethanolic garlic and onion extracts as affected by pre-heating treatment. EFG, ethanol extracts from fresh garlic; EHG, ethanol extracts from heated garlic; EFO, ethanol extracts from fresh onion; EHO, ethanol extracts from heated onion; EDTA, ethylenediaminetetraacetic acid disodium salt dihydrate.

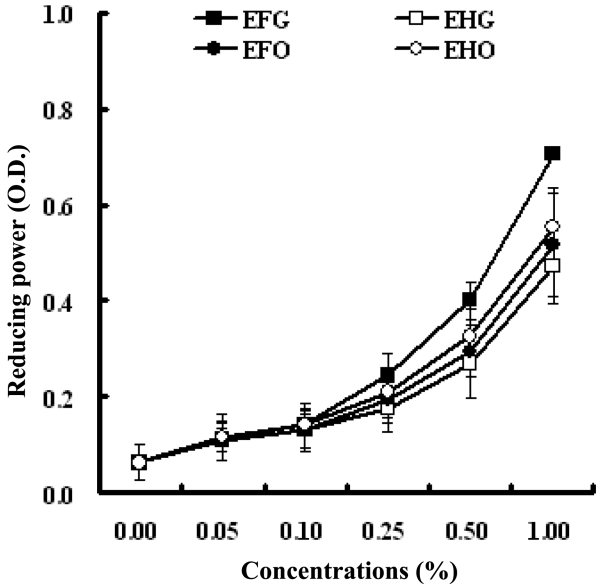

Fig. 3. Reducing powers of ethanolic garlic and onion extracts as affected by pre-heating treatment. EFG, ethanol extracts from fresh garlic; EHG, ethanol extracts from heated garlic; EFO, ethanol extracts from fresh onion; EHO, ethanol extracts from heated onion.

tively. With regard to the EC_{50} values, the ethanol extracts from garlic and onion had similar reducing power, regardless of heating (Table 2). However, several studies found that heating garlic and onion bulbs prior to extraction decreased their reducing activity (Gorinstein *et al*., 2008; Jastrzebski *et al*., 2007). According to Gorinstein *et al.* (2008), heat processing (boiling at 100°C) of garlic and onion lead to a decrease in the amounts of their bioactive compounds such as phenolic compounds, resulting in a decrease in the reducing power of extracts. But on the contrary, Dini *et al*. (2008) found that the ferric reducing antioxidant power of onion increased significantly after boiling at 100°C for 20 min. Ferric reducing power assay is developed for direct test of total antioxidant power of compounds, measuring electron-donating ability (Benzie and Strain, 1999). According to Medina *et al.* (2007), correlations between reducing power of hydroxycinnamic acids and rate of generation of TBARS, and peroxides during oxidation of minced mackerel muscle were -0.96 and -0.97, respectively. And they concluded that electron-donating ability seems to play an important role for antioxidant efficiency of phenolics. We found that using garlic and onion extracts that had EC_{50} values ranging from 0.60 to 3.30 could reduce about 50-70% of TBARS values developed in fresh pork patties stored at 4°C for 14 d (Park and Chin, 2010a,b). In this study, EC_{50} values of the ethanol extracts from garlic and onion with heating or not ranged from 0.66 to 1.07. Therefore, we think that they might have potential possibility as antioxidants when they are added to meat products.

Antioxidant activity in linoleic acid emulsion

The antioxidant activity of ethanolic garlic and onion extracts on peroxidation of linoleic acid was determined. As shown in Fig. 4, the amounts of hydroperoxide in all emulsion systems significantly increased with increased incubation time, and the oxidative activities of linoleic acid were inhibited by the addition of extracts when extracts were added to 0.05%. Specifically, extracts of 0.01% inhibited the rate of hydroperoxide formation from 2.87 to 16.71% during incubation time on average. Extracts of 0.05% had a inhibition rate greater than 24.0%, as compared to control (data not shown). In general, free radicals in a food matrix containing fats or oils act as the initiator of autoxidation, and resulted in the formation of hydroperoxide (Min and Ahn, 2005). A lot of researchers have studied various natural ingredients containing radical scavenging activity because various compounds, including alcohols, aldehydes, hydrocarbons and ketons, produced by decomposition of lipid hydroperoxide contri-

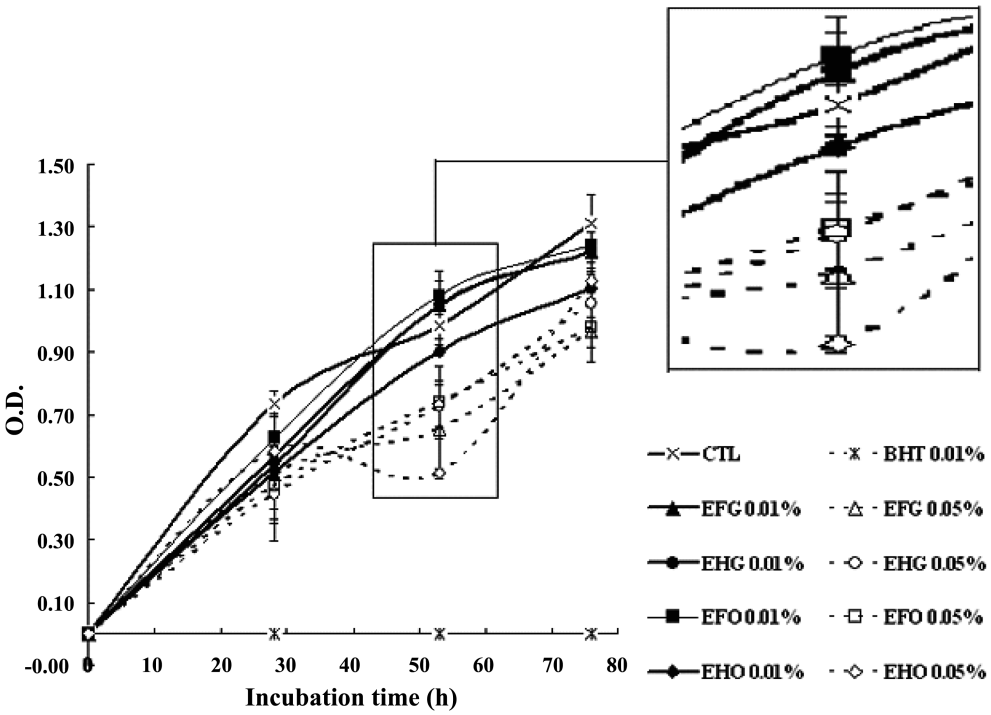

Fig. 4. Antioxidant activities of ethanolic garlic and onion extracts in linoleic acid emulsion systems as affected by preheating. CTL, control without ingredient; BHT, butylated hydroxytoluene; EFG, ethanol extracts from fresh garlic; EHG, ethanol extracts from heated garlic; EFO, ethanol extracts from fresh onion; EHO, ethanol extracts from heated onion.

bute to flavor deterioration of food (Frankel, 1991). Garlic and onion are known as natural products having radical scavenging activity (Banerjee *et al.,* 2003; Nuutila *et al*., 2003). In this study, ethanolic garlic and onion extracts showed to have excellent DPPH radical scavenging activities, regardless of heating (Fig. 1). Therefore, their antiradical properties may contribute to the inhibition of hydroperoxide formation in linoleic acid emulsion systems. Similar results were reported by Leelarungrayub *et al*. (2006) and Nuutila *et al*. (2003), who found that garlic and onion extracts inhibited the generation of lipid hydroperoxide.

In conclusion, garlic had a higher drying yield than onion, and garlic extracts had greater antioxidant activities than onion extracts. Antioxidant activities of garlic and onion extracts were not significantly affected by the thermal treatment. Both garlic and onion inhibited the formation of hydroperoxide in linoleic acid emulsion systems when ethanol was used as a solvent. Further study will be focused on their application to meat and meat products.

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