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Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edodes*) mushroom

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Abstract

The effect of heat treatment on the changes in the overall antioxidant activity and polyphenolic compounds of Shiitake extract was investigated. Raw Shiitake was heated at 100 and 121 °C for 15 or 30 min using an autoclave. After heat treatment, the free and bound polyphenolics and flavonoids in the mushroom extracts were analyzed. 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities were measured to evaluate antioxidant activity of the extracts. The polyphenolic contents and antioxidant activities in the extracts increased as heating temperature and time increased. For example, the free polyphenolic content in the extract heated at 121 °C for 30 min was increased by 1.9-fold compared to that in the extract from the raw sample. The ABTS and DPPH radical scavenging activities were increased by 2.0-fold and 2.2-fold compared to the raw sample, respectively. There was a good correlation between total polyphenolic contents and AEAC ($p < 0.001$). Results showed that heat treatment significantly enhanced the overall antioxidant activities of Shiitake mushroom.
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Keywords: Shiitake mushroom; Heat-treatment; Antioxidant activity; Polyphenolics

1. Introduction

Free radicals produced by radiation, chemical reactions and several redox reactions of various compounds may contribute to protein oxidation, DNA damage, lipid peroxidation in living tissues and cells (Halliwell, 1996; Morrissey & O'Brien, 1998). This oxidative stress may be related to many disorders, such as cancer, atherosclerosis, diabetes and liver cirrhosis (Halliwell & Gutteridge, 1984; Muramatsu et al., 1995; Steinberg, Parthasarathy, Carew, Khoo, & Witztum, 1989). Recent epidemiological studies have indicated that increased consumption of certain foods, such as fruits and vegetables, is associated with reduced risks of chronic diseases (Hu, 2002). This association may be attributed from the antioxidants in the foods including vitamin C, vitamin E, carotenoids, polyphenolic compounds and flavonoids, which prevent free radical damage (Diplock et al., 1998).

Mushrooms have been used for traditional foods and medicines in Asia (Chang, 1996). Generally, mushrooms are rich in dietary fiber, minerals, vitamins and low in fat (Manzi, Aguzzi, & Pizzoferrato, 2001; Mattila et al., 2001). Moreover, mushrooms contain various polyphenolic compounds recognized as an excellent antioxidant (Ishikawa, Morimoto, & Hamasaki, 1984). Especially, Shiitake (*Lentinus edodes*) is the second most popular and the third widely cultivated edible mushroom in the world (Chang, 1996). Several important compounds including bioactive polysaccharides (lentinan), dietary fiber, ergosterol, vitamin B_1, B_2 and C and minerals have been isolated from the fruiting body, mycelia, and culture medium of this mushroom. Resent numerous studies have shown its medicinal attributes including anti-tumor,

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antimicrobial, liver function improving and cholesterol lowering activity (Fukushoma, Ohashi, Fujiwara, Sonoyama, & Nakano, 2001; Mizuno, Sakai, & Chihara, 1995; Takehara, Kuida, & Mori, 1979).

Thermal processing is generally applied to extend shelf life of food products. However, it is well known that natural nutrients could be significantly lost during the thermal processing due to the fact that most of the bioactive compounds are relatively unstable to heat. Therefore, heat processed foods are considered to have a lower health promoting capacity than the corresponding fresh one. However, recent studies have shown that thermally processed foods, especially fruits and vegetables, have higher biological activities due to their various chemical changes during heat treatment (Dewanto, Wu, Adom, & Liu, 2002a; Kim et al., 2000). It is also reported that bioavailability of lycopene and β-carotene from cooked tomatoes and carrots was higher than the raw one (Stahl & Sies, 1992).

Therefore, the purpose of this study was aimed to evaluate the effect of heat treatment on the changes in the overall antioxidant activities and antioxidant compounds of Shiitake mushroom measured by ABTS radical scavenging activity, DPPH radical scavenging activity, polyphenolic and flavonoid contents in the free and bound extracts of Shiitake mushroom.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu reagent, gallic acid, (+)-catechin, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and potassium persulfate were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and all other reagents were of analytical grade.

2.2. Heat treatment of mushroom

Raw Shiitake (*Lentinus edodes*) was purchased form local market in South Korea from January to March 2004. About 500 g of raw Shiitakes was sliced and randomly divided into five groups (accurately 50 g each, weighed as it is). The samples were subjected to four different heat treatments: heated at 100 and 121 °C for 15 and 30 min using an autoclave.

2.3. Extraction

Free and bound polyphenolic compounds of Shiitake were extracted as described by Krygier, Sosulski, and Hogge (1982a) with some modifications. Samples (50 g) were homogenized with 100 ml of 80% ethanol for 15 min using a waring blender and then homogenized further using Polytron® for 10 min at room temperature. After centrifugation (10,000 rpm, 10 min), the supernatant was separated from the residue and concentrated to approximately 10 ml with a rotary vacuum evaporator at 40 °C. The free extracts were diluted up to 50 ml with distilled water and stored at −20 °C until analysis.

The residue from free polyphenolic compound extraction was hydrolyzed with 20 ml of 4 N NaOH for 1 h under nitrogen and adjusted to pH 2 with 6 N HCl. The bound polyphenolic compounds were extracted six times with 20 ml of ethyl acetate. The organic extracts were then evaporated at 40 °C to dryness, redissolved in 25 ml of distilled water and stored at −20 °C until analysis.

2.4. Determination of free and bound polyphenolics

Polyphenolic contents in the free and bound extracts were determined using the Folin-Ciocalteu method (Dewanto, Wu, & Liu, 2002b) with some modifications and results were expressed as mg gallic acid equivalents per 100 g of Shiitake. Standard solution or mushroom extract (200 μl) was mixed with 2 ml of 2% sodium carbonate solution and 100 μl of a 50% Folin-Ciocalteu reagent. After incubation for 30 min at room temperature, the absorbance was measured at 750 nm. All extracts were analyzed in triplicate.

2.5. Determination of free and bound flavonoids

Flavonoid contents in the free and bound extracts were determined by a colorimetric method described by Jia, Tang, and Wu (1999) with some modifications and results were expressed as mg (+)-catechin equivalents per 100 g of Shiitake. Standard solution or mushroom extract (250 μl) was mixed with 1.25 ml of distilled water and 75 μl of a 5% $NaNO_2$ solution. After 5 min, 150 μl of a 10% $AlCl_3 \cdot H_2O$ was added. After 6 min, 500 μl of 1 M NaOH and 275 μl of distilled water were added to the mixture. The solution was mixed well and the intensity of pink color was measured at 510 nm. All extracts were analyzed in triplicate.

2.6. DPPH radical scavenging activity

The scavenging activity of the free and bound extracts on DPPH radical was measured according to the method of Cheung, Cheung, and Ooi (2003) with some modifications. Aliquots of 0.8 ml of 0.2 mM DPPH ethanolic solution was mixed with 0.2 ml of the extracts. The mixture was vigorously shaken and left to stand for 10 min under subdued light. The absorbance was measured at 520 nm. The DPPH radical scavenging activity (%) was calculated by the following equation:

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

where A_{sample} is the absorbance in the presence of sample and A_{control} is the absorbance in the absence of sample, respectively. All extracts were analyzed in triplicate.

2.7. ABTS radical scavenging activity

The scavenging activity of the free and bound extracts from Shiitake on ABTS radical cation was measured according to the method of Re et al. (1999) with some modifications. ABTS radical cation was generated by adding 7 mM ABTS to 2.45 mM potassium persulfate solution and the mixture was left to stand overnight in the dark at room temperature. The ABTS radical cation solution was diluted with distilled water to obtain an absorbance of 1.4–1.5 at 414 nm (molar extinction coefficient, $\varepsilon = 3.6 \times 10^4$ mol^{-1} 1 cm^{-1}) (Forni, Mora-Arellano, Packer, & Willson, 1986). Diluted ABTS radical cation solution (1 ml) was added to 50 μl of the extract or ascorbic acid standard solution or distilled water. After 90 min, the absorbance was measured at 414 nm using spectrophotometer (Berckman Instruments Inc., Fullerton, CA, USA). The ABTS radical cation scavenging activity was expressed as ascorbic acid equivalent antioxidant activity (AEAC) and defined as mg of ascorbic acid equivalents per 100 g of sample (Leong & Shui, 2002). The AEAC was calculated by the following equation:

$$\text{AEAC} = (\Delta A_{\text{sample}}/\Delta A_{\text{aa}}) \times C_{\text{aa}} \times V \times (100/W_{\text{sample}}),$$

where ΔA_{sample} is the change of absorbance in the presence of extract, ΔA_{aa} is the change of absorbance after addition of ascorbic acid standard solution, C_{aa} is the concentration of ascorbic acid standard solution (mg/ml), V is the volume of extract (ml) and W_{sample} is the weight of sample used for extraction (g). All extracts were analyzed in triplicate.

2.8. Statistical analysis

The results were reported as means ± standard deviation (SD). The significance of differences among treatment means was determined by one-way analysis of variance (ANOVA) using SAS version 8.1 (SAS Institute, Cary, NC, USA) with a significant level of 0.05. Correlations from regression analysis between the parameters were also investigated.

3. Results and discussion

3.1. Effects of heat treatment on antioxidant compounds

It has been found that the major contribution on the antioxidant activities of plant foods was the amount of polyphenolic compounds in the foods (Velioglu, Mazza, Gao, & Oomah, 1998). Therefore, it is important to consider the effect of heat treatment on the total polyphenolic concentration of Shiitake extracts. Effects of heat treatments on the total polyphenolics of Shiitake extracts are shown in Fig. 1. The free polyphenolics of raw Shiitake, expressed as mg of gallic acid equivalents per 100 g of sample (weighed as it is), was 29.0 mg/100 g. After heat treatment at 100 °C for 15 and 30 min, the free polyphenolics were increased to 36.1 and 37.5 mg/100 g Shiitake, respectively. After heat treatment at 121 °C for 15 and 30 min, the free polyphenolics were increased to 38.3 and 54.6 mg/100 g (wet weight basis) Shiitake, respectively. The free polyphenolics of the heat treated samples were significantly increased ($p < 0.05$) relative to that of raw Shiitake. Among the heat treated samples, the free polyphenolics of the heat treated Shiitake at 121 °C for 30 min was significantly increased ($p < 0.05$) compared to those of the rest heat treated samples, while there were no significant differences among the rest of the heat treated samples.

The bound polyphenolics of raw Shiitakes were 1.6 mg/100 g Shiitake. After heat treatment (100 °C for 15 and 30 min), the bound polyphenolics were increased to 1.9 and

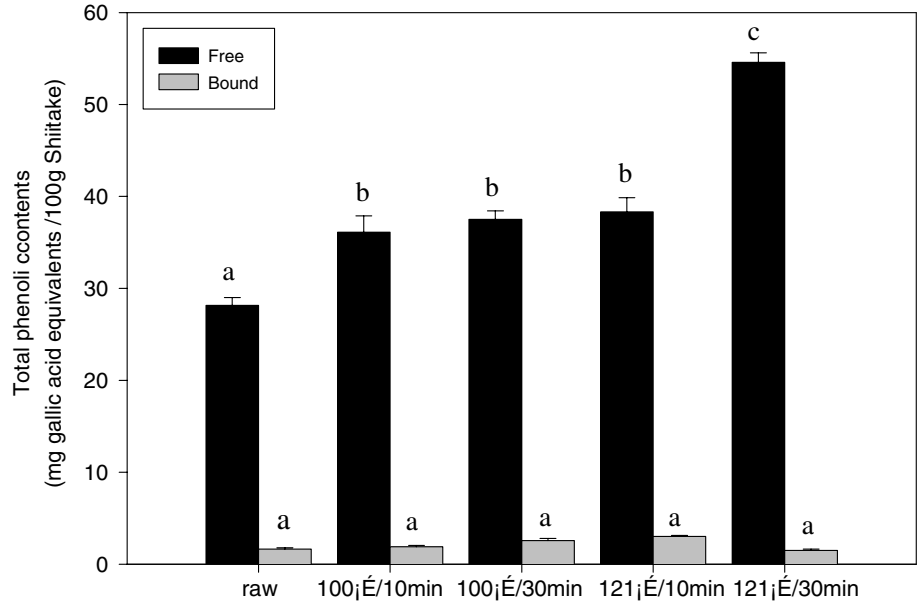

Fig. 1. Effect of heat treatment at 100 °C for 15 and 30 min and at 121 °C for 15 and 30 min on total polyphenolics content in Shiitake extract ($n = 3$). The different letters in the same bar are significantly different ($p < 0.05$).

2.6 mg/100 g Shiitake, respectively. After heat treatment at 121 °C for 15 and 30 min, the bound polyphenolics were 3.0 and 1.5 mg/100 g Shiitake, respectively. Although heat treated Shiitakes at 100 °C for 30 min and 121 °C for 15 min showed slightly higher contents of bound polyphenolics, there were no significant differences among all samples ($p > 0.05$).

Effects of heat treatments on the flavonoids of Shiitake mushroom are shown in Fig. 2. The free flavonoids of raw Shiitake, expressed as mg of (+)-catechin equivalents per 100 g sample, were 0.8 mg/100 g Shiitake. After heat treatment at 100 °C for 15 and 30 min, the free flavonoids were 2.4 and 2.5 mg/100 g Shiitake, respectively. After heat treatment at 121 °C for 15 and 30 min, the free flavonoids were 2.3 and 2.1 mg/100 g Shiitake, respectively. The free flavonoid contents of the Shiitake heat treated at 121 and 100 °C were significantly increased ($p < 0.05$) compared to that of raw Shiitake. Although the free flavonoid contents slightly decreased with heat treatment at 121 °C, there were no significant differences among heat treated samples. The bound flavonoid contents declined with increasing both heating time and heating temperature. The bound flavonoid content of raw Shiitake was 0.4 mg/100 g Shiitake. After heat treatment at 100 °C for 15 and 30 min, the total bound flavonoids were 0.4 and 0.2 mg/100 g Shiitake, respectively. After heat treatment at 121 °C for 15 and 30 min, the total bound flavonoids were 0.1 and 0.04 mg/100 g Shiitake, respectively.

Heat treatment of Shiitake sample increased the overall content of free polyphenolic and flavonoid compounds. This suggests that heat treatment might produce changes in their extractability due to the disruption of the plant cell wall thus bound polyphenolic and flavonoid compounds may be released more easily relative to those of raw materials (Peleg, Naim, Rouseff, & Zehavi, 1991). These results are consistent with those by Dewanto et al. (2002b) who reported that significantly higher concentrations of soluble polyphenolic in commercially processed sweet corns compared to fresh one. They suggested that soluble polyphenolics in sweet corns can be liberated by heat treatment. Jeong et al. (2004) also reported the increase of total poylphenolic contents in heated citrus peels due to the breakdown of the matrix. Heat treatment could deactivate endogenous oxidative enzymes. Therefore, another reason for increased antioxidant contents could be explained by the preventing enzymatic oxidation causing loss of the antioxidant compound in the raw plant materials (Dewanto et al., 2002a; Nicoli, Anese, Parpinel, & Franceschi, 1999).

3.2. Effects of heat treatment on antioxidant activities

The antioxidant activities of the raw and heat treated Shiitakes, as determined by scavenging DPPH radical, are presented in Fig. 3. The DPPH radical scavenging activity (%) of the free extracts of the raw sample showed 45.1%. After heat treatment at 100 °C for 15 and 30 min, the DPPH radical scavenging activities were increased to 72.4% and 73.0%, respectively. After heat treatment at 121 °C for 15 and 30 min, the DPPH radical scavenging activities were increased to 78.3% and 88.6%, respectively. The DPPH radical scavenging activities of the Shiitake treated at 121 °C for 30 min was about 2.0-fold higher than that of raw Shiitake. The DPPH radical scavenging activity of bound compounds Shiitake extracts showed 22.0% in the raw sample. After heat treatment at 100 °C for 15 and 30 min, the DPPH radical scavenging activities were slightly increased to 24.9 and 31.0%, respectively. After heat treatment at 121 °C for 15 and 30 min, the DPPH rad-

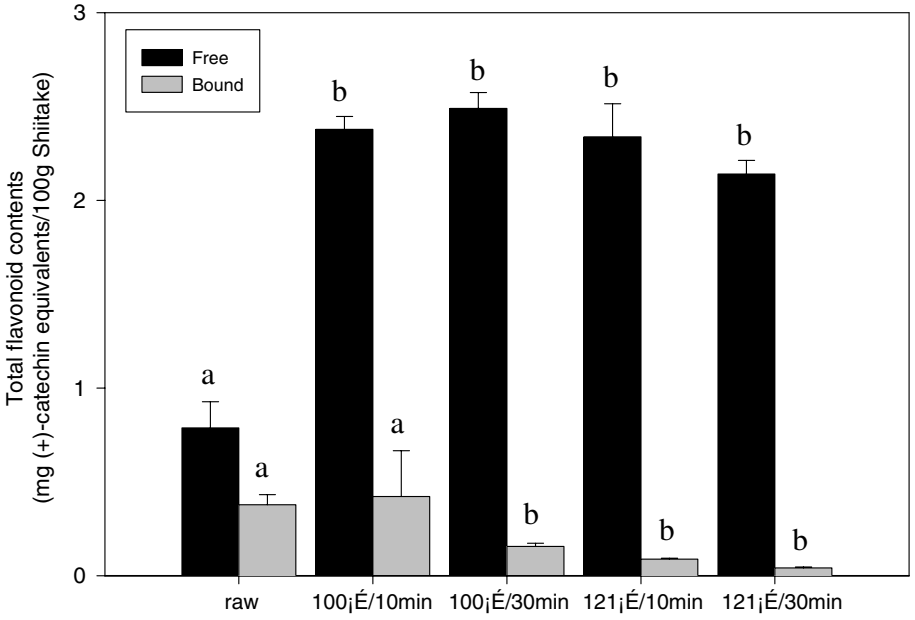

Fig. 2. Effect of heat treatment at 100 °C for 15 and 30 min and at 121 °C for 15 and 30 min on total flavonoids content in Shiitake extracts ($n = 3$). The different letters in the same bar are significantly different ($p < 0.05$).

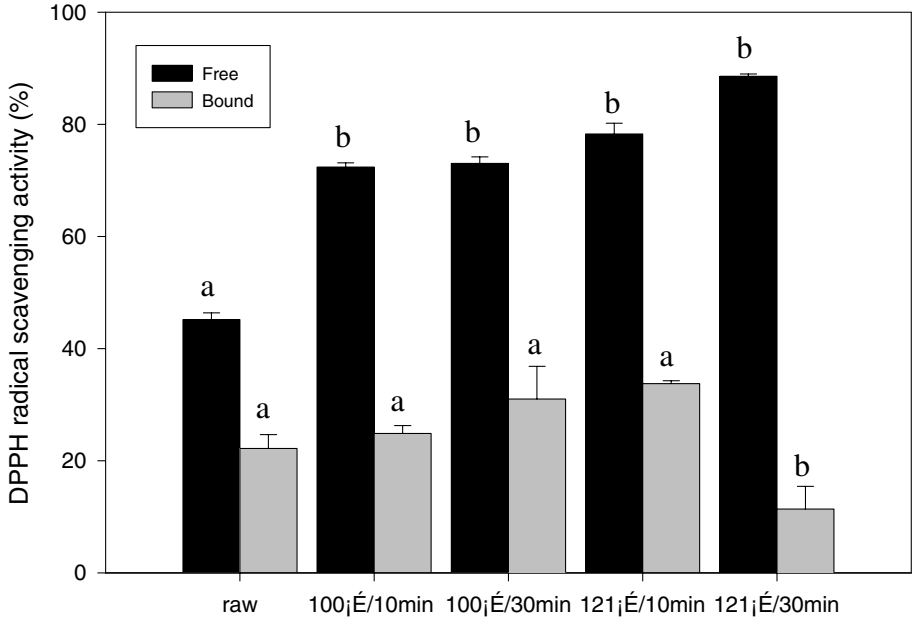

Fig. 3. Effect of heat treatment at 100 °C for 15 and 30 min and at 121 °C for 15 and 30 min on DPPH radical scavenging activity in free and bound Shiitake extracts ($n = 3$). The different letters in the same bar are significantly different ($p < 0.05$).

ical scavenging activities were 33.7% and 11.4%, respectively. The DPPH radical scavenging activity of bound compound extract heated at 121 °C for 30 min was significantly decreased ($p < 0.05$) relative to those of raw Shiitake or heat treated at 100 °C for 15 and 30 min or 121 °C for 15 min.

The total antioxidant activities of the raw and heat treated Shiitakes, as determined by scavenging ABTS radical, are presented in Fig. 4. The ABTS radical scavenging activity of the free extracts of the raw sample, expressed as ascorbic acid equivalent antioxidant activity (AEAC) and defined as the mg of ascorbic acid equivalents per 100 g Shiitake, showed 4.9 mg ascorbic acid equivalents/100 g sample. After heat treatment at 100 °C for 15 and 30 min, the AEAC values were 7.6 and 7.8 mg ascorbic acid equivalents/100 g sample, respectively. After heat treatment at 121 °C for 15 and 30 min, the ABTS radical scavenging activities were increased to 7.9 and 10.8 mg ascorbic acid equivalents/100 g sample, respectively. The ABTS radical scavenging activity of the Shiitake heat treated at 121

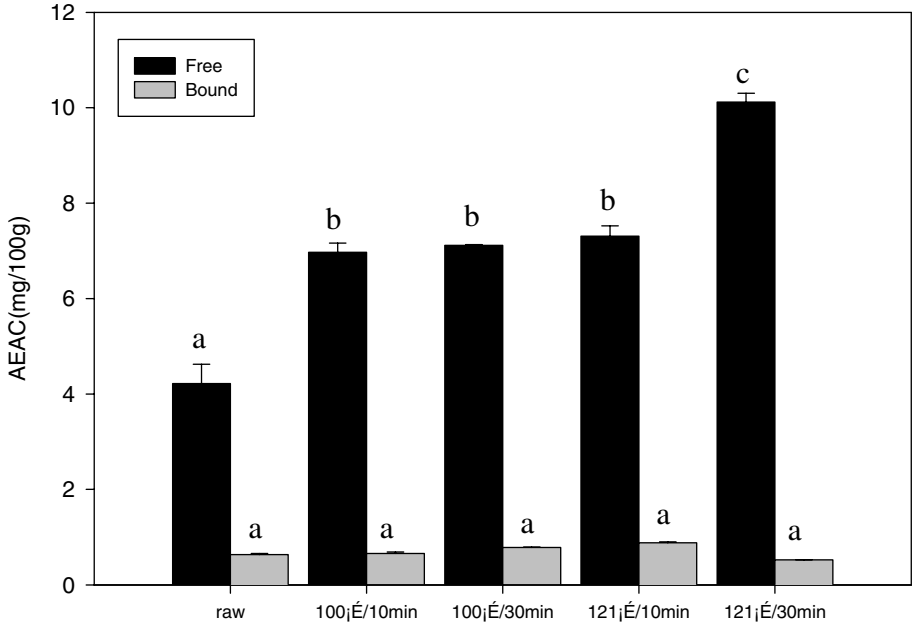

Fig. 4. Effect of heat treatment at 100 °C for 15 and 30 min and at 121 °C for 15 and 30 min on total antioxidant activity (AEAC) in free and bound Shiitake extracts ($n = 3$). The different letters in the same bar are significantly different ($p < 0.05$).

°C for 30 min was about 2.2-fold higher than that of raw sample. The ABTS radical scavenging activity of the bound extracts showed 0.7% in raw sample. After heat treatment at 100 °C for 15 and 30 min, the ABTS radical scavenging activities were 0.7% and 0.9%, respectively. After heat treatment at 121 °C for 15 and 30 min, the ABTS radical scavenging activities were 1.0% and 0.6%, respectively. Although other antioxidant compounds are probably present in Shiitake extracts, good correlation ($R^2 = 0.95$) between free polyphenolic content and AEAC was observed with a significance level ($p < 0.001$). No correlation was observed with flavonoids due to its low concentration compared to the amount of polyphenolic compounds.

Results reported in this study showed that a prolonged heating time (30 min) and higher heating temperature (121 °C) significantly enhanced the overall antioxidant activities of Shiitake mushroom. First of all, this could be explained by the increased amount of antioxidant compounds, especially free polyphenolic compounds. Many antioxidant compounds in plant materials are mainly present as a covalently bound form with insoluble polymers (Peleg et al., 1991). Therefore, it is suggested that heat treatment might disrupt the cell wall and liberate antioxidant compounds from insoluble portion of mushroom, which, in turn, increases the pool of bioaccessible antioxidant compounds. Another reason for improved antioxidant activity could be due to formation of novel compounds having antioxidant activity during heat treatment or thermal processing. In this study, non-enzymatic browning reaction products might be formed during prolonged heat treatment with the improvement of antioxidant activity. Recently, another study carried out on tomato and coffee has shown that a prolonged heat treatment enhanced the antioxidant activity of these food items (Nicoli, Anese, Parpinel, Franceschi, & Lerici, 1997). They reported that browning and antioxidant activities of the tomato and coffee samples were increased with the extension in heating and roasting time. In last decade, lots of studies have been carried on the antioxidant properties of Maillard's reaction products and have shown that Maillard's reaction products exhibit chain breaking and oxygen scavenging activities (Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2001).

4. Conclusions

It is well known that natural nutrients could be significantly lost during the thermal processing due to the fact that most of the bioactive compounds are relatively unstable to heat. In some case, however, heat treatment causes no change or improved effect on the contents and activities of naturally occurring antioxidants. Moreover, the formation of novel compounds having antioxidant property, such as Maillard's reaction products, can be formed as a result of heat treatment. Therefore, the loss of natural antioxidants or heat liable nutrients can be minimized by an enhancement of the overall antioxidant activity in plant foods due to their various chemical changes during heat treatment. Our result suggests that heat treated Shiitake mushroom may have increased health beneficial effects associated with the increase of antioxidant activities. However, this research has a limitation that only one mushroom variety was used and, therefore, further study using different varieties is needed to validate matrix effects.

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