International Food Research Journal 24(5): 2028-2032 (October 2017)

Journal homepage: *http://www.ifrj.upm.edu.my*

Effects of cooking time, temperature, and salt concentration on the phenolic content and antioxidant activity of selected edible mushrooms

[1*]Abacan, S.F., [1]Hurtada, W.A. and [2]Devanadera, M.A.R.

[1]*Institute of Human Nutrition and Food, University of the Philippines Los Banos, Laguna, Philipines*

[2]*Food and Nutrition Research Institute, Department of Science and Technology, Bicutan, Taguig, Philippines*

Article history

Received: 29 May 2016
Received in revised form: 15 August 2016
Accepted: 5 September 2016

Keywords

Mushrooms
Edible mushroom
Wild mushroom
Phenolics
Antioxidant activity
Cooking effects
Phytochemicals

Abstract

People nowadays recognize the beneficial effects of antioxidants to health; hence, many prefer consuming foods which contain phytochemicals known for their antioxidant properties. Mushroom is one among the popular sources of natural antioxidants. However, cooking factors such as cooking method, temperature, and other cooking ingredients, could affect the level of phytochemicals and antioxidant activity in cooked food. To determine the effect of cooking time, temperature, and salt concentration on the antioxidant activity and total phenolic content of edible mushroom, four species were used in this study namely, *Agaricus bisporus, Pleurotus florida, Pleurotus ostreatus* and *Termitomyces cartilaginous*. Methanolic extracts from the four edible mushrooms were analyzed for phenolic content and antioxidant activity after subjecting to different combinations of cooking time, temperature, and salt concentration. Results showed that *A. bisporus* contain the highest phenolic content (2.84±0.47 mg CE/100g) while *T. Cartilaginous* had the highest antioxidant activity (76.67±1.50 % DPPH scavenging activity). The length of cooking time and salt concentration did not significantly affect the levels of phenolics and antioxidant activity. On the other hand, increase in temperature led to reduced level of phenolic content but increased antioxidant activity. In conclusion, boiling mushroom for ≤5 minutes with salt cannot be said that affect phenolic content and antioxidant activity. The use of high temperature in cooking mushroom can be both beneficial and disadvantageous. Boiling mushrooms is not recommended as it decreases the amount of phenolic compounds however; it can increase the antioxidant activity.

Introduction

Mushroom is a flavorful food which is long being consumed in many Asian countries. Nowadays, it is widely consumed and considered one among the healthful food commodities. Edible mushrooms are rich in essential amino acids, fiber, are very low in fat, and also provide vitamins and minerals (Mantilla *et al*., 2001). Not only with nutrients, mushroom is also a known source of antioxidant compounds (Mujic, *et al*., 2010; Witkowska *et al*., 2011).

An antioxidant is a stable molecule which can donate an electron to an unsteady free radical and neutralize it. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property (Halliwell, 1995). Today, antioxidants are important as it play a key role in helping the human body to combat many diseases involving free radical formation such as Alzheimer's and Parkinson's disease, and chronic diseases such as cancer, cardiovascular disease, cataract and inflammation (Temple, 2000). Among the antioxidative compounds present in mushroom species, phenolic compounds are said to be the highest, contributing to the good antioxidant properties of mushroom (Barros, 2007).

There are varying findings on the effect of various cooking parameters, particularly the method of cooking and temperature, on the phenolic content and antioxidant activity in food. Thermal application led to decreased level of phenolic content and antioxidant activity of the leafy vegetables in the study of Ismail and colleagues (2004). Moreover, boiling and steaming reduce the phenolic compounds and DPPH-radical scavenging activity in red bell peppers (Hwang *et al*., 2012). Similarly, boiling significantly decreased the contents of total phenolics in four Boletus mushrooms studied by Sun and his colleagues (2012). On the contrary, Hwang *et al.* (2012) observed that stir-frying and roasting preserves the phenolic content and antioxidant activity of the bell peppers. Some investigators also

*Corresponding author.
Email: *sfabacan@up.edu.ph*

found that thermal treatment increases antioxidants in the case of tomato (Dewanto *et al*., 2002) and other vegetables (Turkmen *et al.*, 2005; Faller and Fialho, 2009).

Among the cooking parameters, temperature is said to be one of the most important factors that affect antioxidant activity and level of phenolic content. Moreover, the length of exposure to heat and the concentration of other ingredients, such as salt, might also affect the presence of phenolics and antioxidant activity in the cooked food. This study aimed to determine the effect of cooking time, temperature and salt concentration on the antioxidant activity and total phenolic content of edible mushrooms. Four species were used in the study namely, *Pleurotus florida, Pleurotus ostreatus, Termitomyces cartilagineus,* and *Agaricus bisporus.* The *Agaricus* and *Pleurotus* are the ones among the top varieties of musroom being cultivated in the Philippines. Whereas, Termitomyces cartilagineus is a wild mushroom variety which is known for its tasty flavor and is commonly available during rainy season

Materials and Methods

Materials

Four species of edible mushrooms were utilized in the study namely, *Pleurotus florida, Pleurotus ostreatus, Termitomyces cartilagineus* (locally called "Mamarang"), and *Agaricus bisporus* (button mushroom).

Sample preparation

Each sample of mushroom (45 g) was placed on a 1-L beaker with 300 mL of salt solution (0, 2, 4, 6 g/L) and cooked in hot water bath using different temperature (50, 75, and 100^{o}C) and time (1,3, and 5 minutes). Five grams (5 g) of the cooked mushroom was macerated, mixed, and stirred with 20 mL methanol then placed on a vial. Afterwards, the fresh and cooked mushroom-methanolic extracts were analyzed for total phenolic content and DPPH scavenging activity.

Total phenolic content determination

Total phenolic content was measured using the Folin–Ciocalteu method by Oyaizu (1986), with some modifications. The absorbance was measured using a UV–vis spectrophotometer at 710 nm against a reagent blank. The total phenolic content was expressed as milligrams of catechin equivalents per 100 gram of dry sample weight (mg of CE/100 g) using the calibration curve of (±)-catechin.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

The total free radical scavenging capacity of the mushroom-methanolic extract was estimated by the DPPH using the modified method of Shimada *et al.* (1992). One (1) mL of the extract was adjusted to 5 mL volume with the addition of distilled water. Freshly prepared, 1 mL DPPH solution (0.1 mM in absolute methanol) was mixed with the extract. The reaction mixture was shaken well and held for 30 minutes at room temperature, and the absorbance of the resulting solution measured at 517 nm against a reagent blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH, and expressed as percent radical quenching compared to that without the extracts.

Statistical Analysis

Experiments were carried out in triplicate. All results were analyzed using SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA). The significant difference in antioxidant activity and total phenolic content of edible mushrooms at different temperature, time, and salt concentration were analyzed using one-way ANOVA. The statistical probability was considered to be significantly different at $P< 0.05$.

Results and Discussion

Total phenolic content of raw edible mushrooms

Phenolic compounds are one of the major sources of antioxidant in vegetables, so as in mushrooms. In the study of Gan *et al*. (2013), presence of phenolic compounds contributed to high antioxidant activity in mushrooms. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In this study, the phenolic content significantly varies in each mushroom. *A. bisporus* has the highest phenolic content followed by *T. cartilagineus, P. ostreatus* and the least is *P. florida* (Table 1). *A. bisporus* or commonly known as button white mushroom is one of the most widely cultivated varieties of mushroom worldwide. According to Rajaramthnam *et al.* (2003), the phenolic content of *A. bisporus* is concentrated on its skin. Next to *A. bisporus* in terms of phenolic content is the *T. cartilaginous*, a wild mushroom commonly eaten in the provinces of Philippines. It is locally known as "Mamarang" and characterized by its brown to dark brown-colored cap. On the other hand, the *P. florida* and *P. ostreatus* varieties used are light-colored mushrooms.

Table 1. The average phenolic content and antioxidant activity of raw mushrooms.[1]

Variety	Total Phenols (mg CE/100g)	Antioxidant activity % DPPH Scavenging activity
T. cartilagineus	2.31± 0.21^{b}	76.67±1.50^{a}
P. ostreatus	1.21± 0.04^{c}	41.13±1.12^{d}
P. florida	0.72±0.46^{d}	49.79±1.27^{c}
A. bisporus	2.84±0.47^{a}	56.27±1.51^{b}

[1]Data are means ± standard deviations of three replicate determinations. Columns with different letters for each mushroom are significantly different (P < 0.05).

Antioxidant activity of raw edible mushrooms

Antioxidant is responsible for protecting the body from the damage caused by free radicals. One of the functions of antioxidant is to scavenge the unpaired electron of the free radical. To evaluate the antioxidant activity of the mushroom extracts in this study, DPPH (2,2'-diphenyl-1-picrylhydrazyl) was used. DPPH is a stable organic radical which is soluble in methanol, often used in evaluation of radical scavenging activity of antioxidant (Brand-Williams *et al*., 1995).

The four raw mushroom species showed varying levels of antioxidant activity. Table 1 shows that *T. cartilagineus* had the highest antioxidant activity (76.67 %) followed by *A. bisporus* (56.27%), *P. florida* (49.79%), and the least is *P. ostreatus* (41.13%). *T. cartilagineus* is a wild mushroom which according to Woldegiorgisa and collegues (2014), wild mushrooms have higher antioxidant properties than cultivated mushrooms. Both the *T. cartilagineus* and *A. bisporus*, which have higher phenolic content, showed higher antioxidant activity compared to the *Pleurotus* species. This follows the fact that presence of phenolic compounds contributes to total antioxidant activity. However, *T. cartilaginous* had higher %DPPH activity than *A. bisporus,* which implies that there could be other bioactive components present on it that may have contributed to the antioxidant activity, besides phenolics. On the other hand, the two *Pleureutos* species exhibited lower antioxidant activity, parallel to its low phenolic concentration.

Effect of time, temperature, and salt concentration in the total phenolic content and antioxidant activity

This study shows that temperature is the only factor which had an effect in the levels of phenolics and antioxidant activity in mushrooms. Conversely,

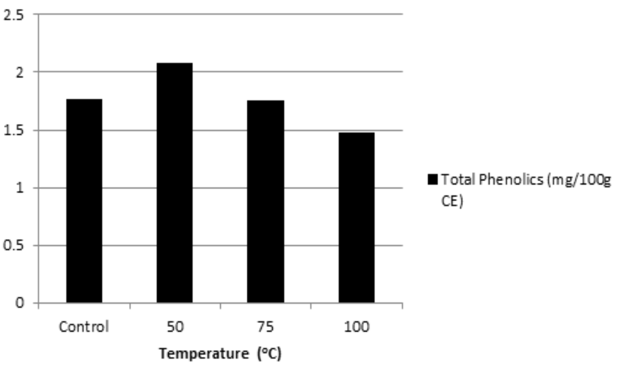

Figure 1. Effect of cooking temperature on the total phenolic content of mushrooms

length of cooking time and salt concentration did not signicantly affect the levels of phenols and antioxidant activity (data not shown). The cooking time considered (≤5 minutes) in this study might be too short to affect significantly the levels of phenolics and the antioxidant activity. This result supports the recent study of Kao *et al.* (2014) which concluded, boiling vegetables for ≤5 minutes would be better for preserving or enhancing the total phenolic content. In this study, the phenolics in mushrooms might also been preserved by cooking it for five minutes and less, which appeared also true for its antioxidant activity.

In general, the phenolic content of the mushrooms decreased as the temperature was increased (Figure 1). At 50^{o}C, the phenolic content of mushrooms moderately increased (2.08 mg CE/100g) but the difference is not significant (P<0.05). Conversely, when the mushrooms were cooked at 100^{o}C, its phenolic content significantly decreased (1.48 mg CE/100g). Based from the study of Romero (2006) on Centenella asiatica, locally known as "takip-kohol", phenolics are sensitive to high temperature as they are volatile. Moreover, when subjected to high temperatures, phenolics are decomposed or undergo oxidative condition which may reduce its amount that may be extracted from the sample. On the other hand, Chism and Haard (1996) stated that with increasing temperature,more bound phenolics are released due to cellular breakdown together with the oxidative and hydrolytic enzymes. These could explain the decreased in total phenolic content of mushrooms as temperature increased in this study.

On the other hand, % DPPH scavenging activity of mushrooms decreased with the application of heat up until simmering temperature (75^{o}C), however, it increased significantly (P<0.05) when boiling temperature was reached. Similar results were found in the study of Murador *et al.* (2016) in which the level of antioxidant activity of vegetables was

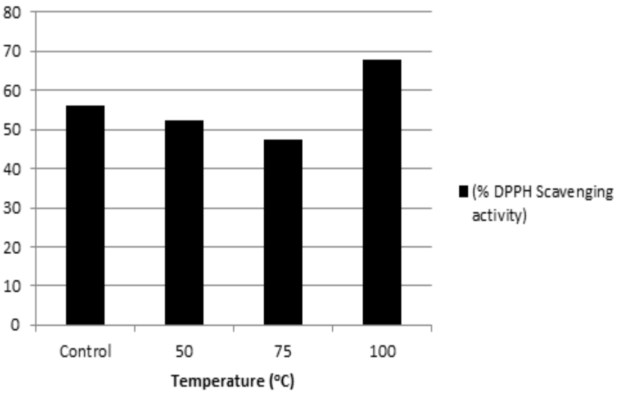

Figure 2. Effect of cooking temperature on the antioxidant activity of mushrooms

improved when steamed and stir-fried as compared to the uncooked samples. It was concluded in the said study that the cooking process can be beneficial since it softens the vegetable tissues, consequently leads to better extraction of bioactive compunds. Also, Tan and his collegues (2015) found out that pressure cooking significantly improves the scavenging abilities of three species of mushroom (*P. floridanus, P. flabellatus*, and *P. pulmonarius*) compared to the uncooked samples. The increased in antioxidant activity at high temperature could also indicate that water insoluble and heat resistant compounds might be released from the rigid tissues or have been developed in mushrooms during cooking.

Conclusion

In view of the methods used and results gathered, it cannot be said that boiling mushroom for five minutes and cooking it with salt influence the amount of phenolics and antioxidant activity. The present study can conclude that the methanolic extracts of the four species of edible mushrooms (*Pleurotus florida, Pleurotus ostreatus, Termitomyces cartilagineus,* and *Agaricus bisporus*) were affected by cooking temperature. Boiling not only reduces the amount of nutrients in food, this study proved that it can also reduce the amount of phenolics in mushrooms. Therefore, this study suggests that vegetables, including mushrooms, should be cook at lowest appropriate temperature to conserve not only nutrients but also phytochemicals, particularly phenols. On the contrary, boiling can enhance the antioxidant activity in mushroom. Consequently, further work needs to be done in order to determine the mechanism how antioxidant activity in mushroom increases with boiling temperature.

Acknowledgement

The authors would like to acknowledge the effort of Ms. Ivy M. Fernandez for analyzing the data collected in this study.

References

Barros, L., Ferreira, M.J., Queiros, B, Ferreira, I. and Baptist, P. 2007. Total phenols, ascorbic acid, b carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chemistry. 103: 413–419.

Brand-Williams, W., Cuvelier, M.E. and Berset, C. 1995. User of free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft and Technologie 28: 25-30.

Chism, G.W. and Haard, N.F. 1996. Characteristics of edible plant tissues. In Fennema, 0.R. (Ed). Food Chemistry. 3rd ed., p. 940-1011. New York: Dekker.

Dewanto, V., Wu, X., Adom, K.K. and Liu, R.H. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agriculture and Food Chemistry 50: 3010-3014.

Faller, A.L.K. and Fialho, E. 2009. The antioxidant capacity and polyphenol content of organic and conventional retail vegetables after domestic cooking. Food Research International Journal 42: 210-215.

Gan, C. H., Nurul-Amira, B. and Asmah, R. 2013. Antioxidant analysis of different types of edible mushrooms (*Agaricus bisporous* and *Agaricus brasiliensis*). International Food Research Journal 20(3): 1095-1102.

Halliwell B. 1995. How to characterize an antioxidant- An update. Biochemical Society Symposia 61: 73–101.

Hwang, I.G., Shin, Y.J., Lee, S., Lee, J. and Yoo, S.M. 2012. Effects of Different Cooking Methods on the Antioxidant Properties of Red Pepper (*Capsicum annuum* L.). Preventive Nutrition and Food Science 17: 286-292.

Ismail, A., Marjan, Z. M. and Foong, W. 2004. Total antioxidant activity and phenolic content in selected vegetables. Food Chemistry 87: 581-586.

Kao, F.J., Chiu, Y.S. and Chiang, W.D. 2014.Effect of water cooking on antioxidant capacity of carotenoid-rich vegetables in Taiwan. Journal of Food and Drug Analysis 22(2): 202-209.

Mattila, P., Könkö, K., Eurola, M., Pihlava, J.M., Astola, J., Vahteristo, L., Hietaniemi, V., Kumpulainen, J., Valtonen, M. and Piironen, V. 2001. Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms.Journal of Agriculture and Food Chemistry 49(5): 2343-2348.

Mujić, I., Zeković, Ž., Lepojević, Z., Vidović, S. and Živković, J. 2010. Antioxidant properties of selected edible mushroom species. Journal of Central European Agriculture 11(4): 387-392.

Murador, D.C., Mercadente, A.Z. and Vera de Rosso,

V. 2016. Cooking techniques improve the levels of bioactive compounds and antioxidant activity in kale and red cabbage. Food Chemistry 196: 1101-1107.

Oyaizu, M. 1986. Studies on products of browning reactions: Antioxidative activities of browning products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition 44: 307–315.

Rajarathnam, S., Shashirekha, M.N. and Rashmi, S. 2003. Biochemical changes associated with mushroom browning and *Agaricus bisporus* (Lange) Imbach and *Pleurotus florida* (Block and Tsao): commercial implications. Journal of the Science of Food and Agriculture 83: 1531-1537

Romero, M.D. 2006. Antioxidant potential and total phenolic content of freeze-dried and air-dried Centenella asiatica L. Laguna, Philippines: University of the Philippines Los Baños, MSc Thesis.

Shimada, K., Fijikawa, K., Yahara, K. and Nakamura, T. 1992. Antioxidative properties of xanthan on the autooxidation of soybean oil in cyclodextrin emulsion. Journal of Agriculture and Food Chemistry 40: 945-948.

Sun, L., Bai, X., and Zhuang, Y. 2012. Effect of different cooking methods on total phenolic contents and antioxidant activities of four *Boletus* mushrooms. Journal of Food Science and Technology 51(11): 3362–3368.

Tan,Y.S., Baskaran, A., Nallathamby, N., Chua, K.H., Kuppusamy, U.R. and Sabaratnam, V. 2015 . Influence of customized cooking methods on the phenolic contents and antioxidant activities of selected species of oyster mushrooms (*Pleurotus* spp.). Journal of Food Science and Technology 52(5): 3058-64.

Temple M.J.2000. Antioxidants and disease: more questions than answers. Nutrition Research 20: 449–459.

Turkmen, N., Sari, F. and Velioglu, Y.S. 2005. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chemistry 93: 713-718.

Witkowska, A.M., Zujko, M.E. and Mirończuk-Chodakowska, I. 2011. Comparative study of wild edible mushrooms as sources of antioxidants. International Journal of Medicinal Mushrooms 13(4): 335-41.

Woldegiorgisa, A.Z., Abateb, D., Hakia, G.D. and Zieglerc, G.R. 2014. Antioxidant property of edible mushrooms collected from Ethiopia. Food Chemistry 157: 30-36.