

EFFECT OF COOKING ON ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT OF VARIOUS SPECIES OF EDIBLE MUSHROOMS OF INDIA

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ABSTRACT

Mushrooms are well known as medicinal foods and various health benefits are associated with dietary intake of mushrooms. Mushrooms are known for their anti-carcinogenic properties which are attributed to its antioxidant activity due to various bio-molecular components. Cooking of mushrooms has an effect on the antioxidant activity to various extents. In the present study, effect of various cooking methods on antioxidant activity by DPPH inhibition, thiobarbituric acid (TBA) reactive compounds and total phenols on common edible mushrooms of India viz., *Agaricus bisporus*, *Calocybe indica*, *Volvariella volvacea*, *Lentinula edodes* and *Pluerothus ostreatus* was done. It was found that antioxidant activity as DPPH inhibition in fresh mushrooms was found to be in the decreasing order as *A. bisporus*, *V. volvacea*, *C. indica*, *L. edodes* and *P. ostreatus*. TBA reactives were found to be in the decreasing order as *A. bisporus*, *V. volvacea*, *C. indica*, *P. ostreatus* and *L. edodes*. Total phenols as estimated by Folin ciocalteu assay was found to be in decreasing order as *P. ostreatus*, *C. indica*, *V. volvacea*, *A. bisporus* and *L. edodes*. These mushrooms were also analyzed after cooking by various methods as microwaving for 2 min, boiling in water for 5 min and stir frying in sunflower oil for 2 min. The prepared samples were analyzed for antioxidant activity, TBA reactives and total phenols. Trials were done in triplicates and results were compared statistically using t-test, it was found that antioxidant activity as DPPH inhibition in case of all the mushrooms decreased in microwave treatment as well as in boiling which can be attributed to leaching of biomolecules whereas increased slightly in stir frying of mushrooms that can be attributed to concentration of biomolecules due to frying. Similar results were observed in case of total phenols with high content measured in fried mushrooms because of higher rate of conversion to quinones during stir frying. TBA reactives decreased in all the mushrooms by all forms of cooking that indicates less of carbonyl compounds which are measured by TBA assay.

Keywords: DPPH inhibition, TBA reactives, total phenols

INTRODUCTION

Free radicals formation is a natural process associated with metabolism of cells. These free radicals can interact with macromolecules in cells like proteins, DNA and lipids, generating more reactive molecules as new radicals and lipid peroxides [1-3]. These free radicals can be damaging for the living system and are causative of many diseases such as cancer, coronary heart diseases and aging [4,5]. Antioxidants are free radical scavengers and thus limit action of free radicals on cellular biomolecules [6,7]. In food antioxidants are found as phenols, flavonoids, vitamins and certain enzymes. Their antioxidant activity is based on redox properties [8-10].

Mushrooms are known to have effective anti-cancerous, antibacterial, antiviral and immune-modulating activities [11-14]. Mushrooms, due to its inherent constituents such as phenolics, organic acids and alkaloids are used as functional foods [15-17]. Mau *et al.* [18] and Hirano *et al.* [19] have attributed the protective roles of mushroom consumption to their ability to capture metals, inhibit oxidative enzymes and scavenging free radicals.

Mushrooms are generally not consumed raw but are either cooked or processed to various culinary dishes industrially or at home. Cooking processes bring about a number of changes in physical characteristics and chemical composition of vegetables [20]. There are various studies on quantification of antioxidants in mushrooms but less work has been done on effect of cooking on antioxidant properties. The main objective of this study was to evaluate different edible mushrooms of India for antioxidant activity, TBA reactives and total phenols. *A. bisporus*, *P. ostreatus*, *C. indica*, *V. volvacea* and *L. edodes* were evaluated. Also effect of cooking by boiling, stir frying and microwaving on these properties was done to understand the carryover of antioxidants in mushrooms.

MATERIALS AND METHODS

Mushrooms

A. bisporus, *P. ostreatus*, *C. indica*, *V. volvacea* and *L. edodes* were obtained from ICAR - Directorate of Mushroom Research, Chambaghat, Solan, HP, India. The mushrooms were washed and used for analysis on the same day as harvested.

Cooking Processes

Boiling: 250 ml of water was put to boil in 500 ml beaker. 50 g of mushroom sample was added and boiled for 5 min. The sample was drained off and cooled immediately to prevent further heat damage.

Microwave cooking: 50 g mushroom sample was placed in 250 ml beaker and 100 ml water was added to it. The material was cooked in commercial microwave (Samsung) of 1400 W for 2 min. Samples were drained off and cooled immediately.

Stir frying: Mushroom sample (50 g) was fried in 10 ml of sunflower oil in a non-stick frying pan on a commercial induction cook top (Prestige) set at 180 °C for 2 min. Sample were then put on chilled stainless steel plate for rapid cooling.

Analytical methods

Raw and cooked mushroom samples were crushed in a blender (maxTUFF) for 1 min and analyzed for antioxidant activity (as % DPPH inhibition), TBA reactivities and total phenols.

Determination of antioxidant activity

Determination of antioxidant activity of sample was done by 2,2-diphenyl-2-picryl-hydrazyl (DPPH) inhibition method [21]. Sample (1 g) was taken in 10 ml ethanol and was kept overnight for extraction. This eluted extract was taken (0.2 ml) and to it 1 ml of DPPH solution (80 µg/ml ethanol) was added. A control was set up with 0.2 ml distilled water as blank and left at room temperature for 30 min. The sample sets were centrifuged at 3000 rpm for 15 min (Sigma laboratory centrifuge 3 K 18, Germany). In cuvette 0.5 ml of centrifuged solution was taken and to it 1 ml of ethanol was added. Absorbance was taken at 517 nm separately for blank and samples with pure ethanol as reference using PerkinElmer UV/VIS spectrometer Lambda 25, Germany.

$$\% \text{ DPPH inhibition} = (A_B - A_S / A_B) \times 100$$

Where A_B = OD for blank

A_S = OD for sample

Determination of total phenols

The total phenolic content was determined using Folin-Ciocalteu (FC) reagent, as given by Singleton and Rossi [22] with some modifications. 1 g sample was kept overnight for extraction with 10 ml of 50% aqueous methanol. The mixture was centrifuged at 10000 rpm for 15 min. 0.5 ml of centrifuged supernatant was added to test tube containing 5 ml FC reagent (10% aqueous solution) and 4 ml aq. sodium carbonate. The tubes were held for 15 min and were then analyzed by spectrometry for absorbance at 765 nm. Results were expressed as mg gallic acid equivalents/ L extract.

Determination of TBAreactivities

The method of Ottolenghi [23] was referred. 2 ml of 20% trichloroacetic acid and 2 ml of .67% 2-thiobarbituric acid was added to 1 ml of ethanolic extract of sample solution, as prepared for DPPH method. The mixture was placed in boiling water bath for 30 min and was then cooled and centrifuged at 3000 rpm for 20 min. Absorbance of supernatant was measured at 552 nm.

Statistical analysis

The results are presented as average values and standard error of triplicate readings. The data was analyzed for significance differences between raw and cooked samples by *t* test for means ($p < 0.05$).

RESULTS AND DISCUSSION

Five cultivated mushroom species (*A. bisporus*, *P. ostreatus*, *C. indica*, *V. volvacea* and *L. edodes*) were evaluated for their antioxidant activity, TBA reactives and total phenolic content. The analysis was carried out using entire mushroom fruit body. The data is presented in Table 1. Figure 1 shows the effect of cooking on antioxidant activity, TBA reactives and total phenols in mushrooms.

Effect of cooking methods on antioxidant activity of mushrooms

Antioxidant activity as % DPPH inhibition in mushroom samples was found to be in decreasing order as *A. bisporus* > *V. volvacea* > *C. indica* > *L. edodes* > *P. ostreatus*. The corresponding values are mentioned in Table 1. Amongst these *A. bisporus* displayed maximum radical scavenging activity with DPPH inhibition at 59.33% whereas least activity was found to be associated with *P. ostreatus* at 21.36%. The antioxidant activity in different varieties of mushrooms before and after cooking is shown in Fig. 1a. Though antioxidant activity declined in microwaved and boiled samples, difference was not statistically significant ($p > 0.05$). It was only found to be significant decrease in boiled and microwaved sample of *C. indica*, where the retention was 71.11 and 73% respectively. This indicates leaching of antioxidant constituents of mushrooms during boiling and microwave cooking treatments. Also cell structure damage during heating might cause release of potent radical scavengers. Zhang and Hamauzu [20] also demonstrated decrease in antioxidant activity of broccoli after aqua-thermal treatment. Puupponen-Pimia *et al.*, [24] also demonstrated that DPPH inhibition by cauliflower decreased by 23% during blanching. Conversely Turkmen *et al.*, [25] reported that boiling, microwave heating and steaming increases antioxidant activity in pepper, spinach and green beans. It was interesting to note that antioxidant activity increased in case of fried samples though not significantly except in case of *P. ostreatus* and *L. edodes* where increase was found to be 157.25 and 144.03%, respectively. This increase can be attributed to moisture loss during frying leading to concentration on biomolecules. Also heating inactivates oxidative enzymes thereby increasing antioxidant activity in fried samples. It was also observed that either no change or slight improvement in antioxidant activity was found in stir fried samples of colored pepper and paprika [26].

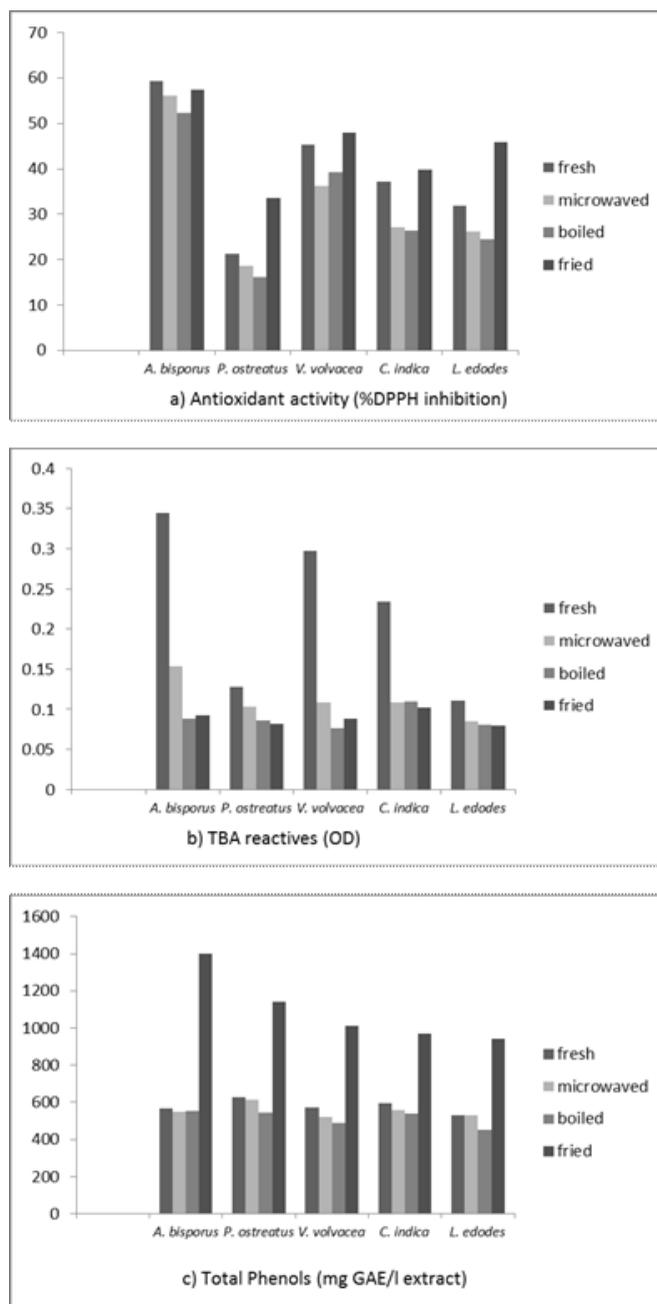


Figure 1. a) Antioxidant activity (% DPPH inhibition), b) TBA reactives, c) Total phenols (mg GAE/L extract) of *A. bisporus*, *C. indica*, *V. volvacea*, *L. edodes* and *P. ostreatus* in fresh and cooked samples

Effect of cooking methods on TBA reactivities of mushrooms

At later stages of oxidation peroxides decomposes to carbonyl compounds that are measured by TBA method and is principally used to measure lipid oxidation. The TBA activity in mushrooms on day 1 were found to be in decreasing order as *A. bisporus*>*V. volvacea*>*C. indica*>*P. ostreatus*>*L. edodes*. The TBA reactivities in different varieties of mushrooms before and after cooking is shown in Fig. 1b. Cooking of all these mushroom species lead to decrease in TBAreactivities significantly ($p>0.05$) with maximum decrease in fried samples followed by boiled and least depreciation in microwaved samples that shows decreased level of oxidation in fried samples. This pattern can be attributed to inactivation of oxidative enzymes due to heat treatment in cooking processes and also to initial low lipid content of mushrooms. In *A. bisporus* microwave treatment, boiling and frying demonstrated 55.53, 74.42 and 73.26% decreased TBA reactivities in mushroom samples. This result is inverted from TBA activity of cooked meat samples that demonstrate increase in TBA reactivities accumulation on cooking [27]. Conversely, Du and Li [28] concluded that when cooking increases proteins can react with TBA solution, reducing TBA values. The TBAreactivities values and corresponding percentage retention on cooking by various methods is mentioned in Table 1.

Table 1. Mean values of antioxidant activity, TBA reactivities and total phenols of common cultivated mushrooms of India (*A. bisporus*, *C. indica*, *V. volvacea*, *L. edodes* and *P. ostreatus*) in fresh and cooked samples

S.No	Mushroom	Processing	Antioxidant activity		TBAreactivities (OD)	Total phenols (mg GAE/ L extract.)	% retention	
			(% DPPH inhibition)	% retention				
1	<i>A. bisporus</i>	Fresh	59.33	100	0.344	100	567.90	100
2		Microwaved	56.15	94.64	0.153	44.47	548.82	96.64
3		Boiled	52.28	88.11	0.088	25.58	554.43	97.62
4		Fried	57.33	96.62	0.092	26.74	1399.55	246.44
5	<i>P. ostreatus</i>	Fresh	21.36	100	0.128	100	626.26	100
6		Microwaved	18.6	87.07	0.103	80.46	612.99	97.88
7		Boiled	16.23	75.98	0.086	67.18	542.08	86.55
8		Fried	33.59	157.25	0.082	64.06	1140.29	182.07
9	<i>V. volvacea</i>	Fresh	45.33	100	0.297	100	572.89	100
10		Microwaved	36.31	80.10	0.109	36.70	520.67	90.88
11		Boiled	39.2	86.47	0.076	25.58	490.24	85.57
12		Fried	47.94	105.75	0.088	29.62	1011.56	176.47
13	<i>C. indica</i>	Fresh	37.08	100	0.234	100	596.07	100
14		Microwaved	27.07	73.00	0.108	46.15	560.04	93.95
15		Boiled	26.37	71.11	0.11	47.00	537.59	90.18
16		Fried	39.86	107.49	0.102	43.58	970.03	162.73
17	<i>L. edodes</i>	Fresh	31.86	100	0.111	100	531.98	100
18		Microwaved	26.19	82.20	0.085	76.57	528.6	99.36
19		Boiled	24.43	76.67	0.081	72.97	451.06	84.78
20		Fried	45.89	144.03	0.079	71.17	943.09	177.27

Effect of cooking methods on total phenols of mushrooms

Total phenolic content of mushrooms was measured as mg equivalents of gallic acid, which was found to decrease in order as *P. ostreatus*>*C. indica*>*V. volvacea*>*A. bisporus*>*L. edodes*. Total phenols in fresh mushrooms were found to be ranging from 626.26 to 531.98 mg GAE/L extract. The total phenolic content in different varieties of mushrooms before and after cooking is shown in Fig 1c. Results show that, after cooking phenolic content decreased very slightly in microwave heated and boiled samples of all the mushrooms and the decline is not statistically significant ($p>0.05$). This can be attributed to inactivation of polyphenoloxidases due to heat treatment thereby hindering phenol degradation. It has been reported that boiling, microwaving or further warm holding did not affect the level of polyphenols in green beans and onions. On the other hand frying lead to significant increase in phenolic content of all the mushroom samples. Stewart *et al.* [29] reported that heat treatment increased the level of free flavanols. Turkmen *et al.* [25] also concluded that cooking leads to increase in phenolic content in vegetables.

CONCLUSION

In general, it can be concluded that frying does not affect antioxidant activity but boiling and microwave cooking depletes radical scavenging ability of mushrooms. Thus it is also vital to consume water used for cooking mushrooms so that leaching losses can be minimized from diet. All the cooking methods demonstrated decrease in TBA reactivities thereby indicating inactivation of lipid oxidizing enzymes by heat. Phenolic content was not much affected by boiling or microwaving but showed exemplary increase in fried mushroom samples indicating stir frying as a cooking option might be useful in improving health properties of mushrooms.

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