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Evaluation of antioxidant properties of button mushroom in different harvest and morphological stages

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ABSTRACT

This study was conducted to test the impact of flush number, mushroom size and cap openness on phenolic and flavonoid contents and antioxidant properties of button mushroom (*Agaricus bisporus*). Results showed that all tested facrors had a significant effect on dry matter and antioxidant properties of mushroom. The first flush had the highest dry matter in comparison with second and third flushs. Antioxiant activity and flavonoid content of mushrooms in second flush was significantly more than others but for phenol content, the first flush was the best. Surprisingly, the lowest antioxidant activity, phenol, and flavonoid contents were obseved in third flush. The highest antioxidant activity, phenol, and flavonoid content were recorded in large size, medium size, and small size of mushrooms, respectively. Cap of the mushroom showed significantly more antioxidant properties and flavanoid content, however, the phenol in stipe part was more than the cap part. Closed-cap mushrooms had significantly more dry matter and total phenol content, while no significant difference was seen in antioxidant activity and flavonoid compounds. In summary, mushrooms produced in third flush have lower dietary quality than first and second flushes, cap part of button mushroom was better than stipe and total antioxidant capacity was not affected by cap opening.

1. Introduction

Population increasing throughout the world will lead to more need of food including proteins. Because of this, edible mushrooms that grow on the agricultural waste, can be an appropriate option for providing human needs of protein (1). Besides of protein, mushrooms contain different medicinal properties including antioxidant, antihypertensive, and anticancer compounds (2). As a powerful antioxidant, mushrooms are so effective on scavenging free radicals due to the high polyphenolic contents (3).
Many types of phenolic compounds that prevent oxidative reactions and lipid oxidations were detected in natural sources (4). Antioxidant substances include different compounds like tocopherols, carotenoids, phenolic acids, flavonoids and diterpenes that all prevent lipid peroxidation (5-6).

DPPH (2,2-diphenyl-1-picrylhydrazyl) test is an indirect method for evaluation of antioxidant properties based on free radical of DPPH reaction with hydrogen donators like phenols. It is demonstrated that there is a significant relationship between antioxidant activity and phenolic compounds (7). The natural polyphenol compounds can decrease the risk of Parkinson, Alzheimer and cardiovasc ular diseases (8-9).

A great deal of efforts has been made to increase the shelf life of perishable crops and products especially by biodegradable packaging (10). Also, some composite films which has antibacterial and antioxidant impacts were developed for preserving agricultural products (11 - 12-13-14). Using biodegradable nanocomposite films based on variety of materials like pectin and gluten, is promising technique for post-harvest industry (15-16 - 17).

Nowadays, High Performance Liquid Chromatography (HPLC) is using for chemical analysis vastly (18-19).

A lot of novel methods for characterization and separation of different substances by HPLC has been developed (20 - 21). But HPLC has some limitations that

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make it inappropriate in some cases. High expense, complexity and unavailability of HPLC contributes popularity of simple alternative techniques like spectrophotometric methods. It is true especially when total amount of a compound like phenol or flavonoid and no the constituents of them is targeted.

Among 1400 species of mushrooms in the nature, button mushroom (*Agaricus bisporus*) is the most widely produced all over the world (22). Button mushroom as a commercial edible mushroom is considered a rich source of protein and antioxidants. The flush number, cap size and cap openness can potentially affect the antioxidant properties of mushrooms, but their significance is not well outlined. However, the changes of antioxidants during the harvest process are not deeply studied. The aims of this research was to test the phenolic and flavonoid contents and antioxidant properties of Button mushroom as affected by flush number, mushroom size, fruit body parts and cap development stages.

2. Results and Discussion

According to the table 1, the effect of different flushs, mushroom size and mushroom parts on the dry matter, antioxidant activity, phenol and flavonoids content were significant. Cap closeness significantly affect the dry matter and antioxidant activity but not on phenol and flavonoids content (Table 1).

Table 1. Analysis of variance of parameters related to the nutritional quality of button mushroom

SOV	df	Mean Square			
		Total flavonoids	Total phenol	Antioxidant activity	Dry matter
Flush no.	2	0.0005*	2707.7**	0.1419**	5.3461**
error	6	0.00007*	0.9906**	0.0005**	0.1448**
cv		1.61	41.05	5.78	3.33
Mushroom size	2	0.0001**	663.55**	0.1188**	3.4498**
error	6	0.000007**	0.2167**	0.0069**	0.0649**
cv		0.73	22.88	17.33	2.2
Section of the fruit body	1	170.98**	0.0093**	0.00001**	2.0666*
error	4	3.342**	0.0001**	0.000000**	0.1666*
cv		2.46	2.84	2.08	3.82
Cap closeness	1	6.18ns	0.0004ns	0.00006**	19.92*
error	4	7.86ns	0.0001ns	0.000001**	1.501*

ns, * and **: non significant, significant at 5% and 1% probablity level respectively.

2.1. Flush number

Obtained results showed that the first flush has the maximum dry matter in comparison with second and third flushs (Figur 1). Higher dry matter in edible mushrooms indicates tissue hardiness and more shelflife. Therfore, the mushrooms harvested in first flush may be more appropriate for long distance market in comparison with latest flushes.

As the flush number increased, the weight loss of harvested mushroom incraesed during postharvest period at 18 °C. The first flush of mushrooms contains less water leading to less weight loss (25).

Antioxiant activity of mushrooms in second flush was significantly more than others (Figur 2). The same results obtained for flavonoids which was highest in second flush but for phenol content, the first flush was the best (Table 3 and 4). Surprisingly, the lowest antioxidant activity, phenol and flavonoids contents were obseved in third flush (Figur 2-4). It seems that weakening of the compost during the production prieod contributes less mushroom dietry value including antioxidant capacity. Liu et al. (26) reported 90.13% DPPH inhibition percentage at the concentration of 2 mg/mL, but we observed at most 80% at the concentration of 3.2 mg/ml. The cultivation conditions and extraction method may be responsible for this difference. As our extract was methanolic, it can be attributed to high etalonic antioxidant activity than methanolic one. Li et al. (27) demonstrated that the first and second flush in the *Pholiota nameko*, an edible mushroom, had high enrichment of nutrition components compared with other tide periods.

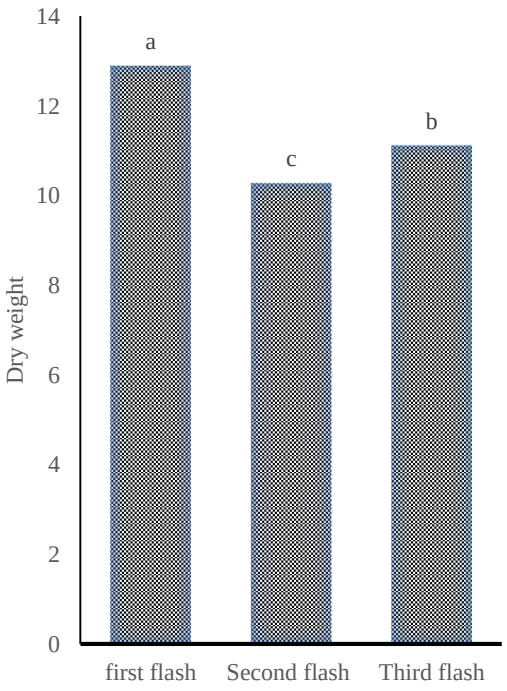

Figure 1. Effect of flush number on the dry matter of mushroom

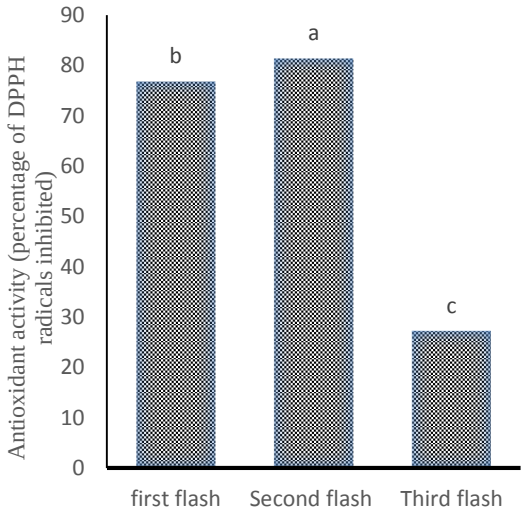

Figure 2. Effect of flush number on the antioxidant capacity of mushroom

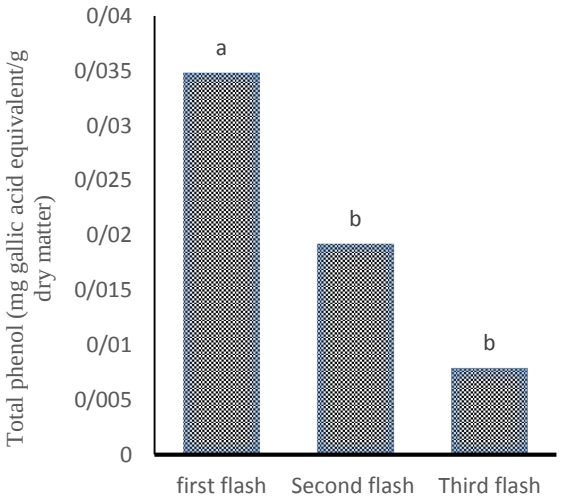

Figure 3. Effect of flush number on the total phenol of mushroom

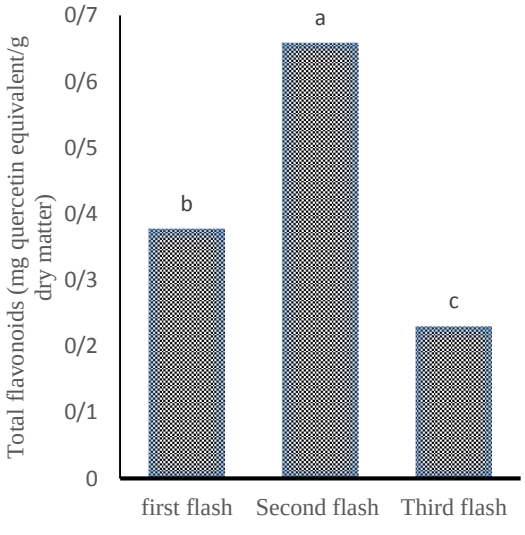

Figure 4. Effect of flush number on the flavonoid content of mushroom

2.2. Mushroom size

As shown in Figure 5, comparision of mushroom size showed that the small mushrooms has higher dry matter leading long durability in postharvest period. The highest antioxidant activity, phenol and flavonoid contents were in large size, medium size and small size of mushrooms, respectively (Figur 6-8). Based on these results, no positive correlation observed among antioxidant activity, phenol and flavonoid contents. In the other word, the main constituent of mushroom antioxidant is not phenol and the main phenolic substance is not flavonoid. This result is in consistence with findings of Czapski (28) and Savoie et al. (29) who indicated a negative correlation between the free phenolic content and antioxidant capacity. There was not relationship between phenols and antioxidant that could be due to the special characteristic of button mushroom. However, some phenolic compound plays an antiradical role (29).

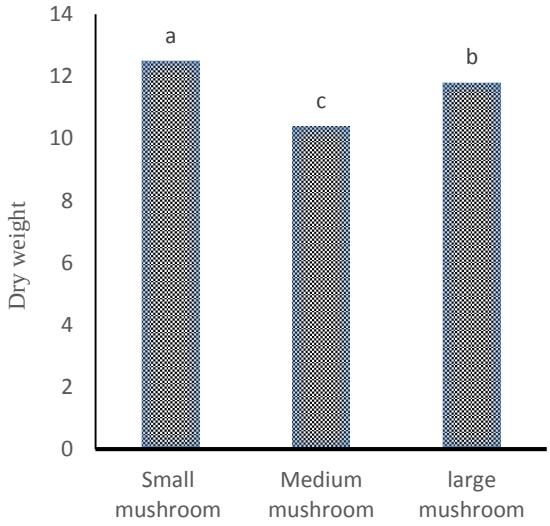

Figure 5. Effect of mushroom size on the dry matter

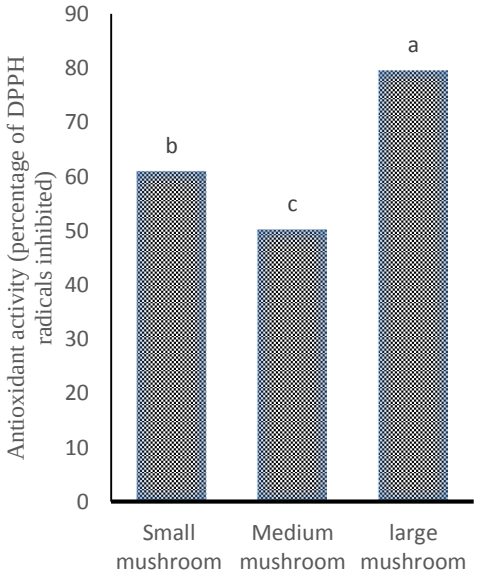

Figure 6. Effect of mushroom size on the antioxidant capacity

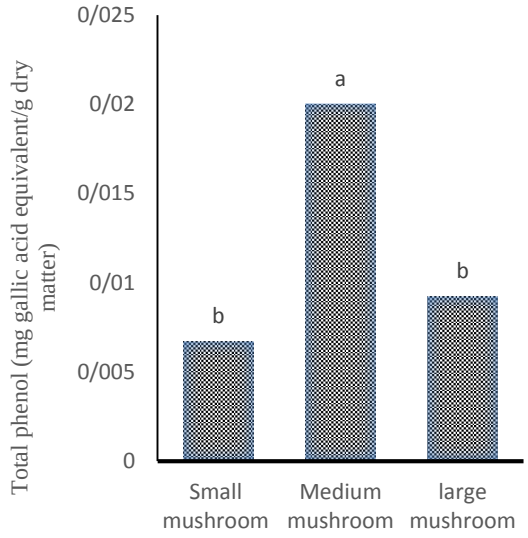

Figure 7. Effect of mushroom size on the total phenols

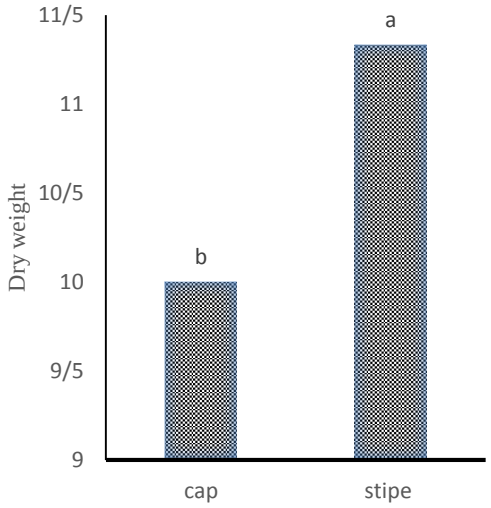

Figure 9. Effect of mushroom part on the dry matter

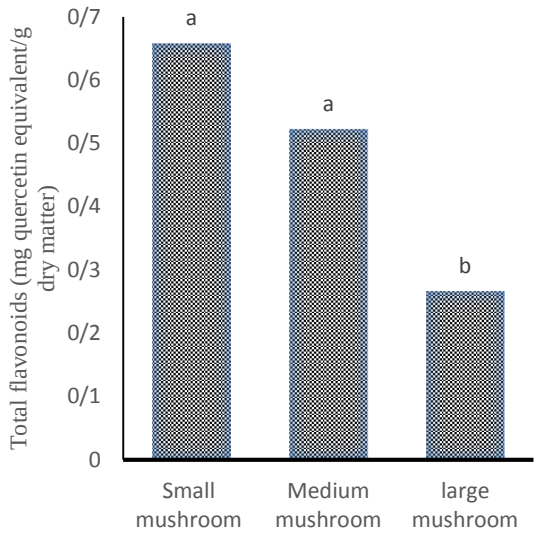

Figure 8. Effect of mushroom size on the total flavonoids

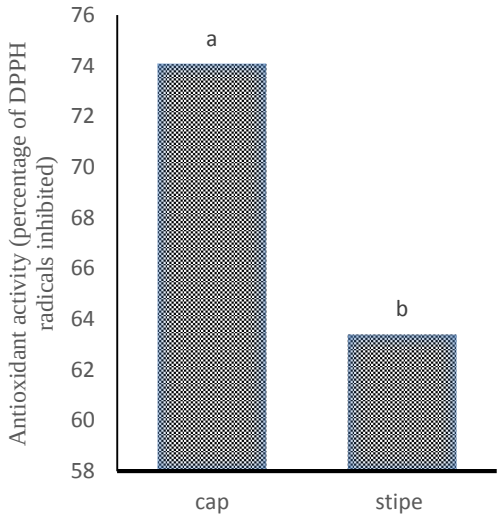

Figure 10. Effect of mushroom size on the antioxidant capacity

2.3. Section of the fruit body

Different parts of mushroom (cap and stipe) revealed the diffenet dietry quality. Stipe of botton mushroom had more dry matter indicating more shelf life and less sensitiveness for postharvest problems (Figur 9). Cap of the mushroom indicated significantly more antioxidant and flavanoid content than stipe but the phenol in stipe was more than the cap part (Figur 10-12). As antioxidant is a more comprehensive parameter in comparison with phenol, the cap part may be more valuble in dietery view. These findings are in accordance with Savoie et al. (29) who found that antioxidant power of button mushroom (determined by both scavenging DPPH and Reducing power methods) was higher in cap comparing with stipe. Both the mushroom strain and the cultivation conditions can affect cap to stipe ratio, therefore breeding plans and cultivation management should target to a nutraceutical food by focus on cap to stipe ratio enhancement.

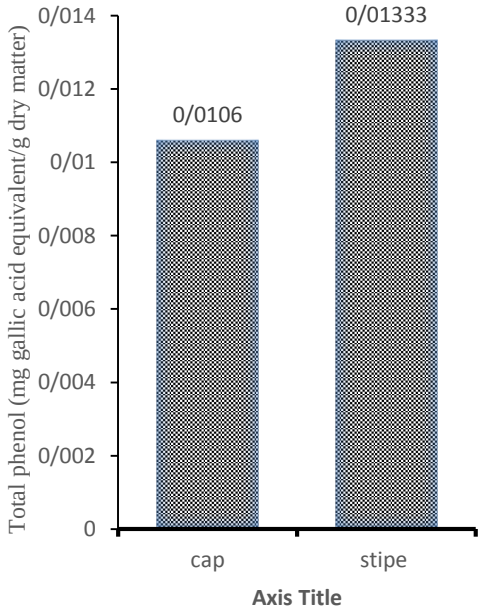

Figure 11. Effect of mushroom size on the total phenol content

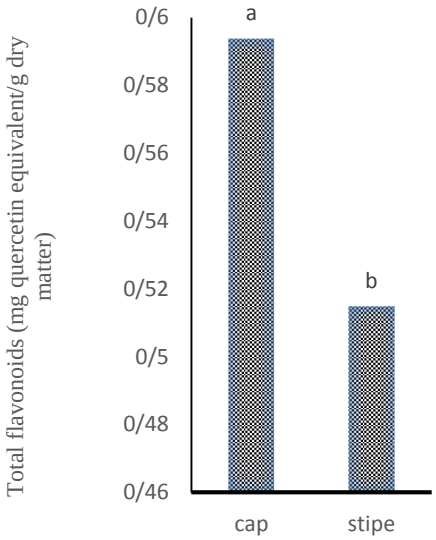

Figure 12. Effect of mushroom size on the total flavonoid content

2.4. Cap development stage

The opened and closed cap mushrooms also revealed different antioxidant characteristics. Results showed that closed cap mushrooms had significantly more dry matter and total phenol content, while no significant difference was observed in antioxidant and flavonoids contents. Results of this experiment contradicted the public idea about dietary worthless of opened cap mushrooms. It is so worth noting that different methods for extracting antioxidant substances are available like green extraction which was successfully used by numerous researchers (30). So, it can be suggested that other extraction methods be utilized for extracting antioxidant active substances of mushrooms.

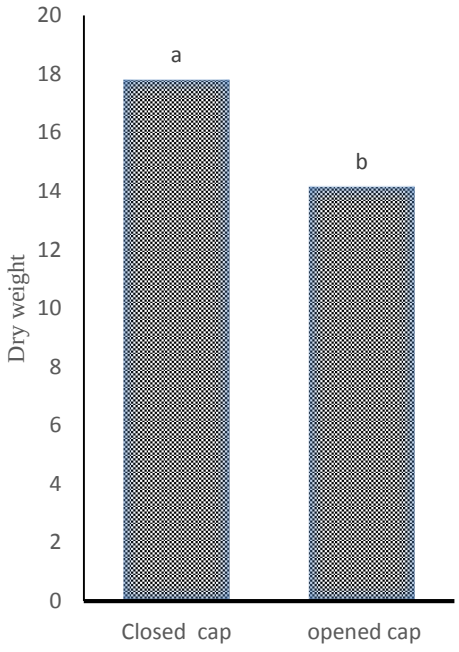

Figure 13. Effect of mushroom size on dry matter

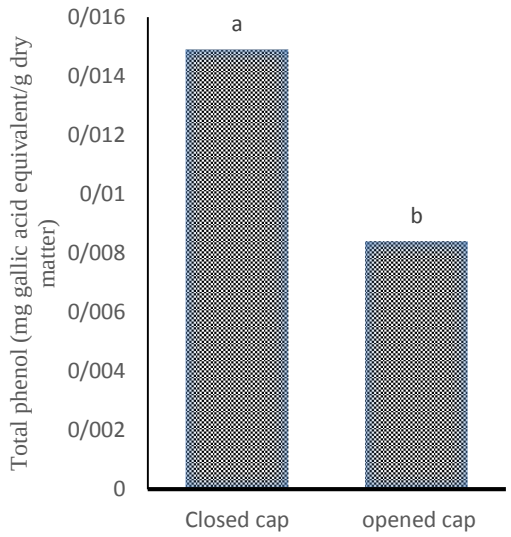

Figure 14. Effect of mushroom size on the phenol content

3. Experimental

Button mushroom cultivated in mushroom farm of Sari Agricultural Sciences and Natural Recourses University (SANRU) was harvested to evaluate in three separated examinations. The first examination was carried out in factorial format based on completely randomized design with three replications. Evaluated factors comprised flush number (flush 1, 2 and 3 with ten-day interval) and cap size (small 1-4 cm, medium 4-8 cm and large 8-12 cm). In the second experiment two factors consisting cap size (small 1-4 cm, medium 4-8 cm and large 8-12 cm) and edible parts (cap and stipe) was examined. In the third experiment, the same size mushrooms categorized in two groups of open and closed cap and then tested for dietary value. All mushroom utilized for second and third experiment were harvested in 2nd flush.

For determination of total antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as a free radical. Briefly, DPPH was added to the methanol extraction of the mushroom samples; then prepared tubes preserved in dark conditions for 15 min and finally absorbance was recorded at 517 nm (23). The power of antioxidant was reported by percentage of inhibition at the concentration of 3.2 mg/mL. Based on method outlined by Nabavi et al. (24), estimation of total phenol and flavonoid content was derermined by Folin-Ciocalteu method. The extract samples of mushrooms (0.5 mL) were mixed with 2.5 mL 0.2 N Folin-Ciocalteu reagents for 5 min, and

then 2.0 mL of sodium carbonate (1 M) was added. Having incubated at ambient temperature, the absorbance was read at 760 nm and results were reported as mg gallic acid /g extract. For determination of flavonoids contents, 0.5 ml of methanol extract was mixed with 1.5 ml methanol, 0.1 mL 10% aluminum chloride, 0.1 mL 1M potassium acetate and 2.8 ml distilled water. In the end, the obtained mixture left at ambient temperature for 30 min and absorbance was measured at 415 nm with a

spectrophotometer. Total flavonoid contents were expressed as mgr quercetin /g extract (24).

Data analysis was carried out by SAS software version 9.1 and mean comparison was conducted by Duncan multiple test at 1 and 5% level of probability.

4. Conclusion

Results of this research showed that mushrooms harvested in third flush have lower dietary quality than those harvested in first and second flushes. On the other hand, keeping compost for a long time in the growth room can increase the risk of infections, so in some cases it is logical to focus on only two flushes instead of three. Although total antioxidant content in large-size mushrooms was the highest, other mushroom sizes showed some positive attributes as well. Cap part of button mushroom is better than stipe at least in the terms of antioxidant and flavonoid contents. Therefore, smaller stipe of the mushroom can be preferable for consumers. Opposite to the public opinion about low dietary value of opened cap mushrooms, no significant difference was seen between opened and closed cap mushrooms in total antioxidant capacity as a primary nutritional attribute, although total phenol of closed mushrooms was significantly higher.

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