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Effect of Processing Methods on the Proximate Composition, Total Phenols and Antioxidant Properties of Two Mushroom Varieties

Abena A. Asamoa, Esther A. Essel, Jacob K. Agbenorhevi*, Ibok N. Oduro

Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
*Corresponding author: jkagbenorhevi.cos@knust.edu.gh; jkagbenorhevi@yahoo.com

Abstract Mushrooms are traditionally consumed as food and are known to possess nutritional and medicinal properties. However, the choice of processing methods for mushrooms are often based on preference rather than the impact on nutritional or health benefits. The effect of solar drying, steaming and roasting on the proximate, phytochemicals and antioxidant activity of two mushroom varieties (*Termitomyces schimperi* and *Volvariella volvacea)* were investigated. Proximate analysis and phytochemical screening were carried out using standard protocols. The total phenols content and antioxidant activity were determined by means of Folin-Ciocalteu method and 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) assay, respectively. Results indicate that both processing methods and varietal differences had effects on nutrient composition of mushrooms. Solar drying retained most nutrients in both varieties. Dried *T. schimperi* had the highest protein content of 29.09 % whereas dried *V. volvacea* had the highest carbohydrate and ash contents of 60.71% and 5.56 %, respectively. Steamed mushrooms retained moisture and had the lowest carbohydrate content with steamed *V. volvacea* having the highest moisture and lowest carbohydrate values of 91.15% and 54.71%, respectively. Roasting also showed a high protein content of 28.65% in *T. schimperi* and a high carbohydrate of 58.65% in *V. volvacea*. None of the processing methods however had a significant effect on fat and fibre contents of both varieties used. The phytochemicals tested were present in both varieties in the processed and unprocessed forms. The steamed extract was the strongest scavenger of DPPH with 50 % inhibitory concentration (IC_{50}) value of 3.03 ± 0.40 mg/mL whereas the unprocessed extract had the least effect with IC_{50} value of 9.35 ± 0.42 mg/ml. Similarly, the steamed extract recorded the highest total phenol content with value of 1644 ± 39 mg GAE/100 g whereas the unprocessed extract was the lowest with value of 1336 ± 93 mg GAE/100 g respectively. The present findings suggest that steamed mushrooms possess the highest antioxidant activity.

Keywords: *Termitomyces schimperi, Volvariella volvacea, nutrients, phytochemicals*

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1. Introduction

Mushrooms are a special group of fungi which are considered as healthy food because they are rich in nutrients such as minerals, vitamins and proteins as well as phytochemicals such as alkaloid, flavonoids, saponins and tannins [1]. The mineral content of mushrooms is higher than that of meat, fish and most vegetables. Edible mushroom proteins have also been shown to contain all nine essential amino acids, comparable to egg and human milk, which are lacking in most staple foods [2]. Mushrooms have been found to contain compounds with the property of scavenging free radicals which cause diseases such as cancer, diabetes, etc. They also contain β-glucans, polysaccharides and other bioactive compounds which are responsible for immune-stimulating, analgesic and neuro-protective activities [3,4].

Generally, vegetables are processed on the basis of taste preference and convenience of preparation rather than retention of nutrients [5]. Research has shown that processing results in significant changes in the chemical composition of mushrooms depending on the processing time [6]. Higher antioxidant activity has been reported for boiled, microwaved, steam cooked and autoclaved mushrooms compared to raw mushrooms [7,8,9]. Drying has been reported to increase in the antioxidant activity of food substances [10]. Roasting, which accelerates caramelization and Maillard reactions in foods, could increase the total phenolics content of foods as well as Maillard reaction by-products, some of which have health promoting properties.

In Ghana, *Volvariella volvacea* and *Termitomyces schimperi*, locally known as '*domo*' and '*sibre*' respectively, are the most popularly consumed mushroom varieties, with their consumption being high when they are in season [2]. Since processing is known to have an effect on the chemical composition of the food [11], it is necessary to investigate the effect of the various processing methods applied to foods in order to help consumers make an informed decision on their choice of processing methods based on their health and nutritional needs.

The objective of this work was to investigate the effect of drying, roasting and steaming on the nutrient, some phytochemical constituents and the antioxidant and total phenols content of the two major mushroom varieties consumed in Ghana.

2. Materials and Method

2.1. Materials and Sample Preparation

Termitomyces schimperi (sibre) was obtained from Afari in the Nkawie district of the Ashanti region of Ghana. *Volvariella volvacea* (domo) was purchased from a mushroom cultivation farm located at Kwabre District in the Ashanti Region of Ghana.

The mushroom samples were washed under running water and the roots sliced off. Each cap was sliced into uniform sizes of about 3 cm each and the stems were all halved. Samples were divided into four 450g portions per variety, one each was kept as the control and other three were then processed by roasting, drying or steaming.

2.2. Roasting of Mushroom

Sliced mushrooms were arranged on sticks and roasted on a grill above heated charcoal. The distance between the grill and charcoal was about 4 cm. Roasting was completed within 4 - 6 min and samples were turned regularly within this period.

2.3. Drying of Mushroom

Sliced mushrooms were spread on an aluminium tray in batches of 100g per tray and placed in a solar box dryer to dry in the sun. Drying was done 6 h for 3 consecutive days.

2.4. Steaming of Mushroom

Sliced mushrooms were placed in a colander placed over a sauce pan containing about 300 mL of boiling water. Mushrooms were steamed for 4 – 6 min in batches of 100 g sample each.

2.5. Proximate Analysis

Proximate composition (moisture, crude protein, fat, fibre, ash and carbohydrate) was determined using the method of AOAC [12].

2.6. Phytochemical Screening

Phytochemical screening for alkaloids, tannins, flavonoids, sterols, saponins and anthraquinones was performed as previously reported [13].

2.7. Determination of Antioxidant Activity

For the determination of antioxidant activity, 2 g of the sample was weighed into an Erlenmeyer flask and 28 mL of 70 % methanol was added to dissolve the sample. The flask was covered with aluminium foil and stored in a dark chamber for 4 days. Antioxidant activity was determined using the 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) method with Trolox as a standard compound. DPPH of 0.01 g was dissolved in 100 mL of 100 % methanol solution and stored in a dark chamber. Aliquots (1 mL) of DPPH solution was added to 2 mL of each extract and the standard Trolox solution in a cuvette. The mixtures were shaken for 1 min and left for 30 min in the dark. Oxidation of the mixtures was monitored spectrophotometrically with absorbance reading at 517 nm. A control consisted of 2 mL of distilled water with the DPPH solution. Antioxidant activity was expressed as percentage DPPH inhibition and inhibitory concentration.

2.8. Determination of Total Phenols Content

Total phenols content was determined using the Folin-Ciocalteu reagent with slight modification [13]. The mushroom samples of 0.5 g each were homogenized in 10 mL of 50 % ethanol and the extract was obtained by sieving. One hundred microlitres of the extract was diluted to 6.0 mL with distilled water and 0.5 mL of Folin-Ciocalteu reagent was added. After 5 min, 1.5 mL of 20 % sodium carbonate was added and the solution made up to the 10 mL mark with distilled water; the contents were mixed thoroughly. The resulting solution was incubated at 20 – 23°C for 2 h in the dark. Absorbance was measured at 765 nm in a spectrophotometer using Gallic acid as the standard. The results were expressed as mg Gallic acid equivalent/100 g of dry weight mushroom samples.

2.9. Statistical Analysis

Analysis of variance was performed by one-way ANOVA followed by post hoc multiple comparison (Tukey's test) using GenStat Discovery Edition 4. Differences between the mean values of the treatments were determined at a significance defined at p-value < 0.05.

3. Results and Discussion

Results for proximate composition of the processed mushroom varieties are shown in Table 1. Results indicated a general decrease in protein content for all three processing methods. However, statistical analysis ($p < 0.05$) showed the decrease was non-significant in solar dried and roasted *T. schimperi*. Steaming of samples showed non-significant difference in moisture content of both *T. schimperi* and *V. volvacea*. However, there were significant differences in moisture content for both species after drying and roasting ($p < 0.05$). Analysis also showed a significant increase in carbohydrate content of all species after processing using the three methods ($p < 0.05$). There were also significant decreases in ash content of *V. volvacea* after been processed by the three methods ($p < 0.05$). All processing methods had non-significant difference on fibre and fat contents ($p > 0.05$).

Results obtained for unprocessed samples clearly showed that mushrooms are rich in protein, fibre, ash and moisture and low in fat which have also been reported in other studies on proximate composition of mushrooms by

Ezeibekwe *et al.* [14], Barros *et al*., [15] and Hung and Nhi, [16]. The variations in the results obtained for both mushrooms clearly indicate the influence of type of specie on the nutrient content of mushrooms. Other factors such as temperature, type of substrate, method of cultivation and other growing conditions also contribute to the variations in the nutrient content [15,16].

The reduction in nutrient content may be due to the nature and sensitivity of the nutrient to the level of heat during processing [11, 17]. Moisture content decreased in solar dried and roasted samples because both were processed methods used to reduce moisture content of foods. A study by Ayodele *et al.*, *[17]* also confirms the reduction in moisture content after drying of mushrooms. The effect of roasting on moisture content is also reported by Adetunde *et al*., [18]. Steaming had no effect on moisture content because steam was used to process the samples. Steam contains moisture and hence does not dehydrate food samples. Carbohydrate content increased in all samples with the highest increase in dried *V. volvacea*. The increase in carbohydrate after drying is also reported by Barros *et al*., [15] on other species of mushroom. The increase in carbohydrate content maybe due to concentration of nutrients in the dry forms of foods compared to the same amount of it in the wet forms [15].

V. volvacea showed a higher ash content in the raw form but decreased after processing for all three methods. However, after processing, *V. volvacea*, had higher ash content compared to *T. schimperi* in all cases. This suggests *V. volvacea* to be a better source of minerals due to its high ash content. The effect of processing on mineral content is also reported by Ayodele *et al*., [17]. According to them processing can increase or decrease mineral content of mushrooms. Some minerals increase in content after processing whereas others also decrease.

The amount of fats and fibre in both species showed non-significant differences after processing using the three methods. Ebru *et al*., [19] also reported a non-significant difference in fat content of mushrooms after processing compared to the unprocessed samples.

Phytochemical screening of the samples also indicated the presence of saponins, flavanoids, alkaloids, sterols, tannins and anthraquinones in both unprocessed and processed forms of *Termitomyces schimperi* and *Volvariella volvacea* shown in Table 2. This is also reported by Shamaki *et al.*, *[20]*. However, a similar work by Egwin *et al*., [21] showed the absence of anthraquinones and sterols which do not agree with other works on mushroom phytochemicals.

The presence of these compounds explains the addition of mushroom to diets may to help fight against diseases such as cancer [22]. The presence of alkaloids for instance explains the antibacterial activity of mushrooms since alkaloids are known to have a high anti-bacterial activity. Flavanoids are also known to contain antioxidants which help protect body tissues Shamaki *et al.*, *[20]*. Saponins, flavanoids, sterols and anthraquinones are all phytochemicals whose presence contributes to classifying mushrooms as a rich source of phytochemicals. Their stability after processing promotes the addition of mushrooms to most of our meals to increase intake of these health promoting compounds.

Results for the antioxidant activity were expressed in terms of inhibitory concentration as shown in Table 3. The statistical analysis showed a significant difference ($p < 0.01$) between the extracts, both processed and unprocessed in comparison with the standard Trolox. This implies that processing has an effect on the antioxidant activity of the mushroom samples. The solar dried, steamed and roasted extracts were statistically analysed and there was no significant difference between them. The order of increasing antioxidant activity of the processed extracts is as follows: solar dried extract < roasted extract < steamed extract. The unprocessed extract had the lowest antioxidant activity. The anti-oxidative properties of Volvariella *volvacea* can be attributed to the abundance of free phenolics [16], flavonoids, ascorbic acid, lycopene, tocopherols and carotenoids [23]. Rechkemmer [24] posits that the plant cell wall can also undergo structural changes to cause an increase in antioxidant activity. The highest anti-oxidative activity observed in the steamed extract can be attributed to the release of compounds such as ascorbic acid and alpha-tocopherol or their break down products. These compounds contribute to antioxidant activity through the various chemical transformations they experience in the cellular matrix due to the heat treatment [25]. Antioxidant activity in the roasted extract might have resulted from the products of the Maillard reaction which occurred during roasting and the thermal inactivation of the oxidative enzymes present in the mushroom thus yielding a lower antioxidant activity [26, 27]. Of the processed extracts, the solar dried recorded the lowest value and this could be attributed to the exposure of the mushroom to solar ultraviolet radiation which might have caused oxidative damage in its cells.

Table 1. Proximate composition of raw and processed forms of two mushroom varieties

Process	Specie name	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	Moisture (%) (w.b.)	Carbohydrate (%)
Raw	*T. schimperi*	30.19±0.00a	0.50±0.00a	2.25±0.35a	4.46±0.12a	86.22±0.84a	51.04±1.12b
	V. volvacea	31.06±0.00a	0.50±0.00a	2.75±0.35a	7.22±0.11a	91.50±0.70a	44.39±0.17b
Solar Dried	*T. schimperi*	29.10±0.30a	0.75±0.35a	2.50±0.00a	3.87±0.71a	7.89±2.63c	55.89±1.97a
	V. volvacea	19.25±0.62b	0.50±0.00a	1.50±0.00a	5.57±0.61b	12.47±0.16c	60.71±1.38a
Steamed	*T. schimperi*	25.38±0.00b	0.50±0.00a	2.75±0.35a	4.25±0.17a	84.85±0.45ab	55.60±0.30a
	V. volvacea	22.10±1.55b	0.50±0.00a	2.00±0.00a	5.52±0.25b	91.16±0.629a	54.72±1.56a
Roasted	*T. schimperi*	28.66±0.93a	0.35±0.21a	1.75±0.35a	4.42±0.08a	77.06±0.88b	55.67±1.06a
	V. volvacea	22.32±0.62b	0.60±0.00a	1.75±0.35a	5.62±0.37b	84.54±0.09b	58.66±0.55a

All values are mean values of duplicates ± standard deviations. Values in the same column with different letters are significantly different ($p<0.05$).

Table 2. Phytochemical screening of unprocessed and processed forms of the two selected mushrooms

Samples		Phytochemical compounds					
		Saponins	Tannins	Alkaloids	Sterols	Flavonoids	Anthraquinone
Raw	*T. schimperi*	+++	+++	+++	+++	+++	+++
	V. volvacea	+++	+++	+++	+++	+++	+++
Dried	*T. schimperi*	+++	+++	+++	+++	+++	+++
	V. volvacea	+++	+++	+++	+++	+++	+++
Steamed	*T. schimperi*	+++	+++	+++	+++	+++	+++
	V. volvacea	+++	+++	+++	+++	+++	++-
Roasted	*T. schimperi*	+++	+++	+++	+++	+++	+++
	V. volvacea	+++	+++	+++	+++	+++	+++

(+): detected, (-): not detected.

Table 3. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (IC_{50}) by extracts of *Volvariella volvacea* subjected to different heat treatments

Sample	IC_{50} (mg/ml)
Unprocessed	9.35 ± 0.42
Solar Dried	3.40 ± 0.39
Roasted	3.20 ± 0.21
Steamed	3.04 ± 0.40
Trolox	0.03 ± 0.01

Values are mean ± standard deviation; n=3 for each sample. There was significant difference in DPPH scavenging activity of the various extracts as compared to the standard Trolox ($p < 0.01$).

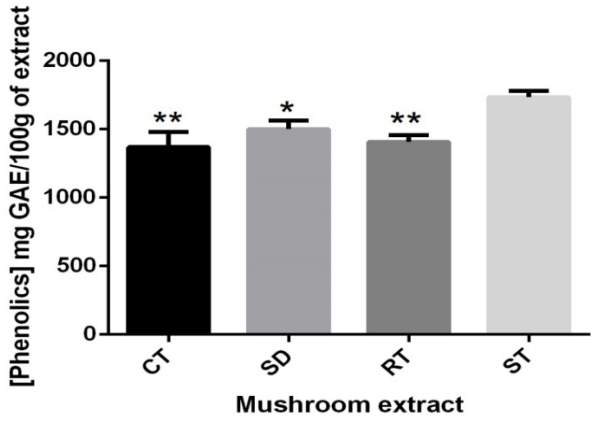

Figure 1. Total phenols content of extracts of *Volvariella volvacea* processed under different heat conditions. CT: unprocessed extract, SD: solar dried extract, RT: roasted extract and ST: steamed extract. Bars with different asterisk (*) indicate significantly different ($p < 0.05$)

The results for the total phenols content of the mushroom samples are shown in Figure 1. The results showed increasing total phenols content in all the processed mushroom extracts. The statistical analysis showed no significant difference between the roasted extract and unprocessed extracts. However, there was a significant difference between the steamed extract and the other processed extracts ($p < 0.05$).

The high phenols content is an indication that the mushroom extract has antioxidative properties as the phenolic compounds are responsible for its antioxidative activity due to their redox properties and ability to act as hydrogen donors and singlet oxygen quenchers [27]. The different heat treatments applied to the mushroom may be the cause of the varying levels of total phenol content observed. Thermal treatment disrupts the cellular matrix of plants which contains bound phenols; in the process, the cellular matrix breaks down and liberates the bound phenol compounds into the extracting solvent [13]. The highest phenol content obtained from the steamed extract is as a result of the exposure of the mushrooms to heat (hot water) to cause a disruption of its cellular matrix and liberating the phenolic compounds which reacted with the Folin-Ciocalteu reagent [29,30]. The solar dried extract having lower phenol content could be due to oxidative damage which occurred in the mushroom during exposure to solar ultraviolet radiation. Polyphenol oxidase and peroxidase are oxidative enzymes and contribute to the production of free radicals in the body. When these enzymes are exposed to heat, they are denatured and become inactive; this stabilizes the phenolic compounds and account for low total phenol content in the roasted extract [31].

4. Conclusion

Proximate analysis of the nutrients in solar dried, steamed and roasted samples of *V. volvacea* and *T. schimperi* showed that processing affects nutrient composition of mushrooms. Results suggest that, for high protein and low carbohydrate mushroom diet, solar dried *T. schimperi* or roasted *T. schimperi* could be recommended whereas for a low protein, high carbohydrate diet, solar dried *V. volvacea* could be recommended. Both solar dried and steamed *V. volvacea* could also be recommended for a high mineral diet and dried *T. schimperi* for a low mineral diet. The low moisture content of the dried samples suggest solar drying to be the best processing method which extends shelf life of mushrooms best and retains most of the nutrients. However, one can choose between *T. schimperi* and *V. volvacea* based on the individual's nutrient requirements. The results proved that the anti-oxidative properties were improved depending on the type of heat treatment applied. This indicates that mushrooms could be processed by way of steaming in order to obtain a high level of antioxidant activity in the body when consumed.

Statement of Competing Interest

The authors have no competing interests

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