Effects of gamma irradiation on antioxidant activities and chemical properties in *Agaricus bisporus* mushrooms

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Abstract. *Agaricus bisporus* is easily perishable and subject to microbial contamination. The present study aspires to follow antioxidant activities and chemical changes of *Agaricus bisporus* after being exposed to gamma radiation. Powder samples were irradiated at 2.5, 5, 7.5 and 10 kGy. Subsequently, antioxidant activities, total protein content and total soluble polysaccharide content of irradiated samples were evaluated. Result showed that gamma irradiation up to 10 kGy did not significantly affect antioxidant activities. 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity assay (DPPH) of non-irradiated and irradiated samples ranged from 2.07 ± 0.09 to 2.19 ± 0.14 mgAAE/g, whereas ferric reducing antioxidant power (FRAP) of irradiated samples varied from 32.74 ± 0.39 to 34.52 ± 1.45 μmol FeSO4/g. Total phenolic contents were between 7.24 ± 0.38 and 7.65 ± 0.53 mgGAE/g. In terms of chemical properties, gamma radiation at 10 kGy showed no significant differences in total protein content and total water soluble polysaccharide content. Total protein contents were found from 42.46 ± 0.28 to 43.18 ± 0.13 %w/w. Amount of total water soluble polysaccharide content in non-irradiated and irradiated samples varied from 5.49 ± 0.15 to 5.72 ± 0.14 %w/w.

1. Introduction

*Agaricus bisporus*, commonly known as button mushroom, is a favourite mushroom for consumers in many countries. It takes 30 percent market share of total mushroom production around the world [1]. It has white colour, umbrella shaped cap and cylindrical stipe. Mature stage of *A. bisporus* mushroom is called Portobello mushroom. It has brown colour and large cap size (2-6 inch). Portobello mushroom is also plentiful with vitamins (A, C and D), ergosterol, beta-carotene, minerals, phenolic compounds and free amino acid [2]. In addition, it also has pharmaceutical properties, which is supporting by its antioxidant, antimicrobial, immunomodulating and anticancer effects [3]. Fresh Portobello has a high moisture content of 85 – 95% that is easy to lose water content and to be attacked by microorganism after harvest [4]. There are 2 main factors of Portobello perishable. The first is internal factors related to Portobello itself (water activity, microbial activity and respiration rate). The second is external factor related to storage conditions (temperature and humidity) [5]. Several preservation methods could effectively reduce mushroom spoilage and extend mushroom shelf-life. Especially, drying process (i.e. sun drying, hot air and oven drying) is the efficient methods that could restrain temperature and water activity of mushroom [6]. Radiation process has also been applied as a decontamination technique, increasing shelf life and improving food safety. This technique is mainly intended to destroy microorganisms or insects, with the least impairment in sensory and nutritional quality [7]. This...
technology is suitable for post-harvest treatment, ensuring hygienic and sensory quality of mushrooms [8,9]. Gamma radiation is one of electromagnetic radiation type that is generated in nuclear decay processes. It has high energy due to its high frequency and consequently low wavelength, leading to high penetrating capacity [10]. The suggested dose for prolonging the shelf life of fresh mushroom is 1 – 3 kGy, whereas the suggested dose concerning the decontamination of dried mushrooms is 10 – 50 kGy [11]. Gamma radiation doses in the range of 2.5–10.0 kGy were selected for this study because irradiation at dose of 10 kGy has shown no toxicological hazards and nutritional problems [12]. The purpose of this study was to determine the effects of gamma irradiation on antioxidant activities and chemical compositions of dried Portobello mushroom powder.

2. Materials and methods

2.1. Samples preparation and samples irradiation

Agaricus bisporus Portobello samples were acquired from an Ang-Khang Royal Project in Chiang Mai, Thailand. Portobello samples were dried with a hot air oven (Memmert UFE 600, Schutzart, Germany) at 45 ºC for 24 hours. The dried mushrooms were ground into powder by a grinder. Then, 100 g of Portobello powder samples were packed in aluminum foil bag and irradiated with gamma radiation at room temperature. The treatment was performed in a Gamma chamber 5000 (Brit, India) at Thailand Institute of Nuclear Technology (Public Organization) Nakhon Nayok, Thailand. The Portobello powder samples were irradiated at 2.5, 5.0, 7.5 and 10.0 kGy with the dose rate of 2.96 kGy/h. After irradiation, the powder samples were stored at room temperature until used.

2.2. Analysis of antioxidant activity

The antioxidant activity was evaluated for its free radical scavenging with two assays: DPPH and FRAP.

2.2.1 DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. The scavenging activity of 1,1-diphenyl-2-picrylhydrazyl was performed according to the modified method of Khattak et al. [13]. The free radical scavenging activity using the free radical DPPH was analysed by detecting the decrease in absorbance at 517 nm. The experiment started by 100 µL of each extract was added to 900 µL of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol solution (150 µM) and the solution was shaken vigorously. After incubation at room temperature for 15 min in the darkness, the absorbance of each solution was determined at 517 nm by using a spectrophotometer (Shimadzu UV 1700, JAPAN). The free radical scavenging power was expressed as ascorbic acid equivalent (AAE)/g sample.

2.2.2 FRAP (ferric reducing antioxidant power) assay. Ferric reducing ability was determined as previously described by Benzei and Strain [14]. The FRAP reagent contained 16.7 mM 8.3 mM FeCl₃.6H₂O and 8.3 mM 2,4,6-tripyridyl)-s-triazine (TPTZ) with 250 mM acetate buffer, pH 3.6. A total of 75 µL sample and 225 µL of distilled water were added to 2.25 mL of freshly prepared FRAP reagent in a test tube. The mixture was incubated at room temperature throughout the reaction. The absorbance was measured at 596 nm using a spectrophotometer immediately and 30 min after mixing against blank. The antioxidant potential of samples were calculated based on a calibration curve plotted using FeSO₄.7H₂O at concentration ranging from 400 to 2000 µM.

2.3. Total phenolic content

The total phenolic content was measured by the Folin–Ciocalteu assay according to the method developed by Velioglu et al. [15]. Phenols react with phosphomolybdic–phosphotungustic components in the Folin–Ciocalteu reagent and produce a blue colored complex which is measured at 725 nm. First, 0.75 mL of 10 fold diluted Folin–Ciocalteu reagent and 100 µL of the methanolic extract were placed in a test tube. The mixture was mixed and allowed to stand at room temperature for 5 min. Then, 0.75 mL of 6% (w/v) sodium carbonate solution was added. The mixture was homogenized and allowed to stand at room temperature for 90 min. The total phenolic content was determined via the absorbance
measurements at 725 nm against blank control. All spectrometric work was performed using a spectrophotometer (Shimadzu UV 1700, JAPAN). The total phenolic contents were calculated on the basis of a calibration curve of gallic acid at the concentrations of 0.02-0.10 mg/mL. The results were expressed as mg gallic acid equivalents (GAE) per gram of dry weight of sample.

2.4. Analysis of chemical composition

2.4.1 Protein content. Protein content of sample was analysed by Kjeldahl method, using Gerhardt equipment (Germany). Dried sample (2.0 g) was digested with 20 ml of 98% H2SO4, using 7 g of the potassium sulphate and 0.7 g of copper sulphate mixture as the catalyst. The digestion was continued for three hours after the digestion mixture turned clear green. Then 80 mL of 40% sodium hydroxide solution was added, and the mixture was distilled for 3 minutes. The distillate was collected in a flask containing 100 mL of 5% boric acid solution, with methylene blue and methyl red as the indicators. The distillate was then titrated with 0.1 N H2SO4 solution; the end point was pink. Crude protein was calculated as the percentage on the wet weight basis (N × 6.25).

2.4.2 Total water soluble polysaccharide. Crude water polysaccharide extracts were prepared from dried mushroom powder that has been boiled in distilled water from 30 min under reflux. The total polysaccharide content of the extracts was analysed by the phenol-sulfuric acid method according to Xiunggang et al. [16]. The absorbance of 0.1 mg/mL solution mixed with 6% phenol and concentrated sulfuric acid was measured at 490 nm. The total polysaccharide content was calculated by the standard curve of glucose.

2.5. Statistical analysis

All experiments were performed in triplicate and the results are expressed as means ± standard deviation. The data were analysed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests at 5% significance level.

3. Results and discussion

3.1. Antioxidant activities of Portobello mushroom

DPPH is a stable radical that has been extensively used to determine the free radical scavenging ability of natural antioxidants [17]. The free radical scavenging assay is based on the decolouration of the compound when reduced by a free radical scavenger [13]. Positive DPPH showed that the samples were free radical scavengers. In terms of FRAP assay, the ability to reduce Fe3+ to Fe2+ is considered a good indicator of antioxidant activity. The mechanism for this reducing power is interrupting the free radical chain by donating hydrogen atoms [18]. From previously mentioned, the antioxidant activities of Portobello mushroom powder in this study were measured by DPPH and FRAP assay. The results indicated that there were no significant differences in the DPPH activity as the irradiation dose increased from 2.5 to 10 kGy as shown in Table 1. The DPPH activity of control was 2.19 ± 0.06 mgAAE/g while the irradiated samples were 2.21 ± 0.06, 2.17 ± 0.02, 2.21 ± 0.06 and 2.14 ± 0.06 mgAAE/g, respectively. Similarly, gamma radiation did not affect antioxidant activity of ferric reducing antioxidant power (FRAP). The FRAP values of non-irradiated and irradiated samples were in range 33.12 ± 0.31 – 34.18 ± 0.45 µmolFeSO4/g. The FRAP value of control was 34.18 ± 0.45 µmolFeSO4/g while the irradiated samples varied in the range of 33.12 ± 0.31 to 33.45 ± 0.33 µmolFeSO4/g. Our results are in agreement with Huang and Mau [19] who also reported that methanol extract of irradiated dried mushrooms did not show significant modifications in their scavenging activity as a result of irradiation doses between 2.5 and 20 kGy. Byun et al. [20] reported no significant changes in the scavenging abilities of non-irradiated and 5, 10 and 20 kGy of Chungkookjang and Doenjang samples. Most of the Portobello mushroom powder samples were able to scavenge DPPH radicals. This activity may be resulted from the hydroxyl groups of the polysaccharides that can donate hydrogen to reduce the DPPH radical [21].
Table 1. Analysis of antioxidant activities by DPPH, FRAP and total phenolic content of Portobello mushroom powder at varied irradiation doses.

| Irradiation dose (kGy) | DPPH (mgAAE/g) | FRAP (µmol FeSO4/g) | Total phenolic content (mgGAE/g) |
|------------------------|--------------|---------------------|-------------------------------|
| Non-irradiated         | 2.19 ± 0.06  | 34.18 ± 0.45        | 7.47 ± 0.08                   |
| 2.5                    | 2.21 ± 0.06  | 33.30 ± 0.56        | 7.24 ± 0.38                   |
| 5.0                    | 2.17 ± 0.02  | 33.12 ± 0.31        | 7.65 ± 0.53                   |
| 7.5                    | 2.21 ± 0.06  | 33.33 ± 0.24        | 7.42 ± 0.07                   |
| 10.0                   | 2.14 ± 0.06  | 33.45 ± 0.33        | 7.46 ± 0.23                   |

Different letters in the same column represent significant differences (p ≤ 0.05).

3.2. Total phenolic content
Phenolic compounds are one of the most responsible about food antioxidant activity when it is measured by scavenging free radicals. Hydroxylated derivatives of benzoic and cinnamic acids are major component that conduce to overall antioxidant activities in the plants [13]. The total phenolic content of irradiated and non-irradiated samples of Portobello powder was measured by the Folin-Ciocalteu’s phenol reagent. The results are expressed as mg equivalents of gallic acid/g dry weight of sample and illustrated in Table 1. Portobello powder irradiated at dose 2.5, 5.0, 7.5 and 10 kGy did not show any significant difference in total phenolic content comparing with control. These results were similar to Chatterjee et al. [22] who also presented the gamma radiation at dose 5 and 10 kGy did not affect significant change in total phenolic content of turmeric (Curcuma longa) and fenugreek (Trigonella foneum) samples. In the same way of Sommer et al. [23] reported that gamma irradiation at 5 kGy did not influence to phenolic content of Shiitake mushroom.

Table 2. Total polysaccharide content of dried Portobello mushroom powder at varied irradiation doses.

| Irradiation dose (kGy) | Total protein content (% w/w) | Total polysaccharide content (% w/w) |
|------------------------|-------------------------------|-------------------------------------|
| Non-irradiated         | 43.18 ± 0.13a                 | 5.72 ± 0.14a                        |
| 2.5                    | 42.97 ± 0.25a                 | 5.66 ± 0.22a                        |
| 5.0                    | 42.46 ± 0.28a                 | 5.53 ± 0.19a                        |
| 7.5                    | 42.63 ± 0.41a                 | 5.49 ± 0.15a                        |
| 10.0                   | 42.83 ± 0.29a                 | 5.65 ± 0.63a                        |

Different letters in the same column represent significant differences (p ≤ 0.05).

3.3. Chemical properties of Portobello mushroom powder
Mushrooms are an important source of carbohydrates and proteins [24]. The higher protein content of the crude polysaccharide and polysaccharide fractions isolated from Lentinula edodes, Grifola frondosa and Trametes versicolor mushroom are correlated with antioxidant activity [25]. Relating to chemical properties, total protein and total polysaccharide content were examine d in this study. The protein content of non-irradiated and irradiated samples was shown in Table 2. The results showed that there were no significant differences in protein content between non-irradiated and irradiated samples. The protein contents were ranked from 42.46 ± 0.28 to 43.18 ± 0.13 %w/w. This result is contrast to Cardoso et al. [10] who reported that gamma irradiation at dose of 5 kGy tend to increase the protein content of fresh Portobello mushroom. Polysaccharide has an important role in immunosuppressive activity protecting against tumour growth by promoting the host's natural immune protections. Total polysaccharide also has the effect of regulating body immunity and inhibiting tumour growth [26]. In this study, the total polysaccharide content of Portobello mushroom powder was determined by phenol-
sulfuric acid method. Gamma irradiation at all the tested doses (2.5, 5.0, 7.5 and 10.0 kGy) did not significantly change in total polysaccharide content in comparison with non-irradiated samples. The amount of total polysaccharide ranged from 5.49 ± 0.15 to 5.72 ± 0.14%w/w (Table 2).

4. Conclusion
These results indicated that the gamma irradiation at dose in the range of 2.5 – 10 kGy did not affect antioxidant activity, total phenolic content, total protein content including total polysaccharide content of Portobello mushroom powder. In addition, it is clearly shown that the gamma irradiation is a good process to preserve the Portobello mushroom in order to produce a mushroom product such as food seasoning, dietary supplement etc.

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