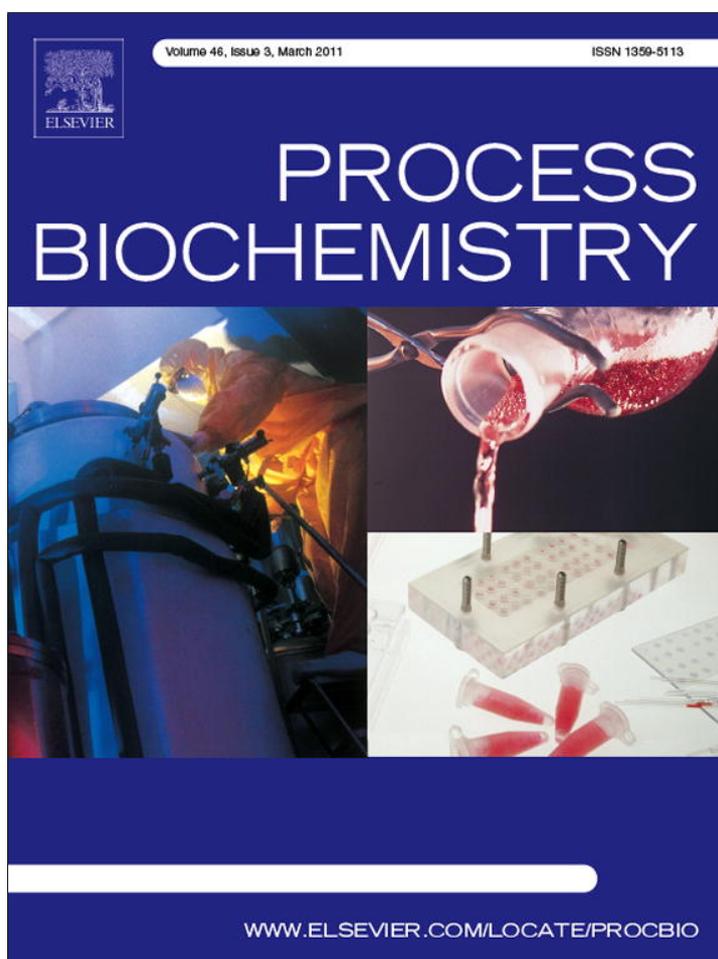


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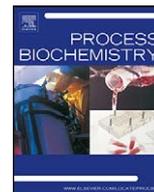
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Influence of gamma irradiation on microbial load and antioxidative characteristics of Polygoni Multiflori Radix

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ABSTRACT

Gamma radiation is a physical process commonly used for the eradication of microorganisms distributed in food ingredients, medicinal plants and other bioresearches. The aim of this study was to investigate the effect of radiation dosage on the microbial load, chemical compounds and antioxidative characteristics of Polygoni Multiflori Radix (POMU). Ten commercial POMUs were purchased from different herbal markets and treated with 2 kGy, 4 kGy, 6 kGy, 8 kGy and 10 kGy gamma radiation doses to evaluate the microbial burdens of irradiated and unirradiated POMUs.

Our results confirmed that 2 kGy was sufficient for the inactivation of enterobacteria; at 4 kGy, mold and yeast counts were obviously reduced; and at 6 kGy, neither yeasts nor fungi were observed any longer.

The antioxidative effects and major antioxidant components of 0 kGy, 5 kGy, 10 kGy and 15 kGy irradiated POMU samples were also examined. Our results confirmed that 5 kGy irradiated POMU had both the highest antioxidative activity and lowest value in IC₅₀ of DPPH radical-scavenging activity. The content of total phenols had no statistically significant changes. Therefore gamma irradiation at 5 kGy could be a potential method for decontaminate the microbial load of POMU to prolong shelf life and to improve hygienic quality.

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1. Introduction

Herbs quite commonly harbor large quantities of bacteria, fungi and spoilage-inducing organisms [1]. The most common bacteria are aerobic sporeformers, such as *Bacillus* and *Salmonella* species [2–4]. Today, three major methods are in use for the antiseptics of herbs, namely steam, fumigation (ethylene oxide and propylene oxide) and irradiation [5]. However, steam degrades light-weight leafy herbs, and ground products are difficult and sometimes impossible to handle in the steam system [5–8]. As for ethylene oxide gas, such disinfection method has been banned in the European Union and many other countries [9]. Gamma radiation within 3–10 kGy proved to be a viable alternative to fumigation or steam to ensure the hygienic quality of herbs; and there has been a steady

increase of radiation utilization in the last 10 years after the banning of ethylene oxide [1,10–12].

According to the standards established by World Health Organization (WHO) [13], most untreated herbs, harvested and handled under hygienic conditions and tested by appropriate methods of sampling and examination, should contain no more than 1×10^4 bacteria cfu/g. However, there are no literatures available on POMU regarding its variance in microbial burdens and chemical contents due to different doses of irradiation, especially when high doses (up to 15 kGy) are employed.

Polygoni Multiflori Radix (POMU), “He Shou Wu” in Chinese, is the dried root of *Polygonum multiflorum* Thunb. (Polygonaceae). It is an herb that has been used in traditional Chinese medicine for the treatment of liver diseases, anemia, and hypopigmentary skin diseases, as well as for the prevention of hair-graying and other diseases associated with aging. POMU also counteracts toxicity, cures carbuncles and relaxes the bowels whereas processed POMU replenishes the liver and kidney with vital essence and blood, blackens the hair and strengthens the tendons and bones [14]. 2,3,5,4'-Tetra-hydroxystilbene-2-O-glucoside (THSG), a water-soluble active component extracted from dried tuberous

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root of POMU, can promote the release of nitric oxide (NO) from vascular endothelial cells and has a strong antioxidative effect [15]. Structurally, THSG belongs to hydroxystilbene, and its structure is similar to that of resveratrol in red wine which has significant protective effects on myocardial ischemia-reperfusion injury.

The present work was mainly undertaken to investigate the effect of different doses of gamma radiation on the elimination of microbes; whether they bring about any changes in the antioxidants composition of POMU and to find the lowest possible effective radiation dosage for POMU.

2. Materials and methods

2.1. Materials

BHT, GSH, potassium peroxodisulfate ($K_2S_2O_8$), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), Tris (hydroxymethyl) aminomethane, trypsin, potassium ferricyanide ($K_3Fe(CN)_6$), TCA, ferric chloride ($FeCl_3$), catechin, MTT, aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$), rutin, 2,2'-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), sodium bicarbonate (Na_2CO_3), sodium phosphate dibasic (Na_2HPO_4), sodium phosphate monobasic (NaH_2PO_4) and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Folin–Ciocalteu solution and 95% ethanol were purchased from Merck Co. (Santa Ana, CA, USA). THSG was purchased from the National Institute for the Control of Pharmaceutical and Biological Products.

2.2. Sampling and irradiation of *Polygoni Multiflori Radix*

Ten samples of *Polygoni Multiflori Radix* were all used in this manuscript. The samples were purchased from eight traditional Chinese medicine importers, one manufactory and one Chinese drugstore, of which all herbs originated from Mainland China. POMU was packaged as 15 g samples in PVC (poly-vinyl chloride) containers for irradiation. Gamma irradiation was performed at National Tsing Hua University according to the method of Wen et al. with slight changes [16]. Samples were placed on a hot cell stand and irradiated with cobalt-60 at room temperature. The irradiated stand rotated 10 rounds per minute to ensure a well-distribution of radiation applied to each sample. The samples were treated for various time intervals in order to achieve absorbed doses of 2, 4, 6, 8 and 10 kGy. Additional samples were treated with 5, 10 and 15 kGy of gamma radiation in the same way to evaluate any changes in antioxidant activities and active compounds. The absorbed dose was measured with silver dichromate dosimeter [17].

2.3. Enumeration of microbes

The microbial contents of POMU samples were measured immediately after irradiation. Direct observation and spread plate method were applied for the quantification of microorganisms [18]. The solid culture media used in this study included plate count agar (PCA; Difco, Detroit, USA), potato dextrose agar (PDA; Difco, Detroit, USA) and violet red bile glucose agar (VRBGA; Difco, Detroit, USA), and the liquid culture medium used in this experiment was plate count broth (PCB, Difco, Detroit, USA).

Appropriate dilutions of the homogenates with PBS were spread and plated onto PCA culture plates for total aerobic bacterial enumeration. Molds and yeasts were enumerated by the spread plate method using PDA. Each sample was examined on violet red bile glucose agar (VRBGA, Difco, Detroit, USA) to determine the total number of *Enterobacteriaceae* bacteria. Counts were recorded in colony forming units (cfu/g). The presented data was the average count in three petri dishes for each diluted suspension [16].

2.4. Preparation of the methanol extract of *Polygoni Multiflori Radix*

The POMU sample which purchased from traditional Chinese medicine importer was treated with different gamma radiation doses (0 kGy, 5 kGy, 10 kGy and 15 kGy) and macerated with methanol for 24 h at room temperature. Filtration and collection of the extracts were done three times. The methanol extracts were dried in vacuum at 40 °C. The dried extracts were weighed and dissolved in methanol (stock 10 mg/mL) and stored in –20 °C for further use.

2.5. Determination of antioxidant activity by ABTS*

ABTS* scavenging ability was determined according to the method of Chang et al. [19]. Aqueous solution of ABTS (7 mM) was oxidized with potassium peroxodisulfate (2.45 mM) for 16 h in the dark at room temperature. The ABTS* solution was diluted with 95% ethanol to an absorbance of 0.75 ± 0.05 at 734 nm (Beckman UV–vis spectrophotometer, Model DU640B). An aliquot (20 μ L) of each sample (125 μ g/mL) was mixed with 180 μ L ABTS* solution and the absorbance was read at 734 nm after 1 min. Trolox was used as the reference standard. A standard curve was constructed for Trolox at 0, 15.625, 31.25, 62.5, 125, 250, 500 μ M

concentrations. TEAC was expressed as millimolar concentration of Trolox solution with the antioxidant equivalent to a 1000 ppm solution of the sample under investigation.

2.6. Determination of antioxidant activity by DPPH radical scavenging ability

The effect of crude extracts and positive controls (GSH and BHT) on DPPH radicals was estimated according to the method of Huang et al. [20]. 20 μ L of sample extract was mixed with 100 mM Tris–HCl buffer (80 μ L, pH 7.4) and 100 μ L of DPPH in ethanol to a final concentration of 250 μ M. The mixture was shaken vigorously and left to stand at room temperature for 20 min in the dark. The absorbance of the reaction solution was measured spectrophotometrically at 517 nm. The percentage of DPPH decolorization was calculated according to the equation: % decolorization = $[1 - (ABS_{\text{sample}}/ABS_{\text{control}})] \times 100$. IC_{50} value is the effective concentration by which 50% of DPPH radicals are scavenged and was obtained by interpolation with linear regression analysis. A lower IC_{50} value indicated a greater antioxidant activity.

2.7. Determination of antioxidant activity by reducing power measurement

The reducing power of the crude extracts and BHT was determined according to the method of Yen and Chen [21]. POMU samples (0, 0.2, 0.4, 0.6, 0.8 and 1 mg/mL) and BHT were each mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6, and 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min, during which time ferricyanide was reduced to ferrocyanide. Then an equal volume of 1% trichloroacetic acid was added to the mixture before centrifugation at 6000 \times g for 10 min. The upper layer of the solution was mixed with distilled water and 0.1% $FeCl_3$ at a ratio of 1:1:2, then the absorbance was measured at 700 nm to determine the amount of ferric ferrocyanide (Prussian Blue) formed. Increased absorbance of the reaction mixture indicated increased reducing power of the sample.

2.8. Determination of total polyphenol content

The total polyphenol content of the crude extracts was determined according to the method of Huang et al. [22]. 20 μ L of sample extract (125 μ g/mL) was added to 200 μ L distilled water and 40 μ L of Folin–Ciocalteu reagent. The mixture was allowed to stand at room temperature for 5 min, and then 40 μ L of 20% sodium carbonate was added to the mixture. The resulting blue complex was measured at 680 nm. (+)-Catechin was used as standard for the calibration curve. The polyphenol content was calibrated using a linear equation based on the calibration curve. The total polyphenol content was expressed as μ g (+)-catechin equiv./mg dry weight (μ g CE/mg). The dry weight indicated was the sample dry weight.

2.9. Determination of total flavonoid content

The total flavonoid content of the crude extracts was determined according to the method of Huang et al. [22]. 1.5 mL aliquot of each extract was added to an equal volume of 2% $AlCl_3 \cdot 6H_2O$ (2 g in 100 mL methanol) solution. The mixture was vigorously shaken, and the absorbance at 430 nm was read after 10 min of incubation. Rutin was used as the standard for the calibration curve. The total flavonoid content was calibrated using a linear equation based on the calibration curve. The total flavonoid content was expressed as μ g rutin equiv./mg dry weight (μ g RE/mg). The dry weight indicated was the sample dry weight.

2.10. Determination of total flavonol content

The total flavonol content of the crude extracts was determined according to the method of Chang et al. [23]. 200 μ L of sample extract was added to 1 mL of 0.1% *p*-dimethylaminocinnamaldehyde (DMACA) in methanol/HCl (3:1, v/v). The mixture was vigorously shaken, and the absorbance was read after 10 min of incubation at 640 nm. (+)-Catechin was used as standard for the calibration curve. The total flavonol content was calibrated using a linear equation based on the calibration curve, and expressed as μ g (+)-catechin equiv./mg dry weight (μ g CE/mg).

2.11. Analyses of THSG by HPLC

Moderate amount of the methanol extracts from POMU was weighed and dissolved in methanol. At first, the solutions were filtered through 0.45 μ m PVDF filters. HPLC (Waters 2695 separations module; detector: Waters 996 photodiode array detector) analysis was carried out under the following conditions: Waters XTerra RP18 column (5 μ m, 4.6 mm \times 250 mm) was used with deionized water as mobile phase A, and acetonitrile was used as mobile phase B; in a ratio of 25:75 and ran for 30 min at a flow rate of 0.6 mL/min. The injection volume was 10 μ L, and a wavelength of 320 nm was used for detection. THSG was also analyzed by HPLC under the same conditions, and the retention time was used to identify THSG contents in the samples.

2.12. Statistical analyses

Experimental results were presented as the mean \pm standard deviation (SD) of three parallel measurements. Statistical analyses were performed by one-way

Table 1
Effect of gamma irradiation on the microbial count (CFU/g \pm SD) of *Polygoni Multiflori Radix* samples.

Sample no.	Irradiation dose (kGy)				
	0	2	4	6	8
1					
T ^a	$(1.2 \pm 0.4) \times 10^3$	$(1.3 \pm 0.4) \times 10^2$	$(5.0 \pm 0.6) \times 10^1$	ND ^b	ND
M ^c	$(6.2 \pm 0.7) \times 10^2$	$(4.0 \pm 0.6) \times 10^1$	$(2.0 \pm 0.3) \times 10^1$	ND	ND
E/Pa ^d	ND	ND	ND	ND	ND
2					
T	$(2.1 \pm 0.3) \times 10^2$	$(2.0 \pm 0.4) \times 10^1$	$(2.0 \pm 0.5) \times 10^1$	ND	ND
M	$(1.7 \pm 0.4) \times 10^2$	ND	ND	ND	ND
E/Pa	ND	ND	ND	ND	ND
3					
T	$(5.1 \pm 0.9) \times 10^2$	$(7.0 \pm 1.4) \times 10^1$	ND	ND	ND
M	$(1.3 \pm 0.3) \times 10^2$	$(2.0 \pm 0.4) \times 10^1$	$(1.0 \pm 0.2) \times 10^1$	ND	ND
E/Pa	ND	ND	ND	ND	ND
4					
T	$(2.9 \pm 0.3) \times 10^3$	$(3.5 \pm 0.5) \times 10^2$	$(6.9 \pm 1.1) \times 10^1$	ND	ND
M	$(4.5 \pm 0.4) \times 10^1$	$(1.9 \pm 0.3) \times 10^1$	$(1.0 \pm 0.2) \times 10^1$	ND	ND
E/Pa	ND	ND	ND	ND	ND
5					
T	$(6.5 \pm 1.2) \times 10^1$	$(4.0 \pm 0.7) \times 10^1$	$(2.5 \pm 0.3) \times 10^1$	ND	ND
M	$(5.0 \pm 0.8) \times 10^1$	$(2.5 \pm 0.2) \times 10^1$	$(1.5 \pm 0.3) \times 10^1$	ND	ND
E/Pa	ND	ND	ND	ND	ND
6					
T	$(3.0 \pm 0.4) \times 10^2$	$(7.5 \pm 0.5) \times 10^1$	$(1.5 \pm 0.2) \times 10^1$	ND	ND
M	$(5.0 \pm 0.9) \times 10^1$	$(1.5 \pm 0.4) \times 10^1$	ND	ND	ND
E/Pa	$(1.0 \pm 0.4) \times 10^1$	ND	ND	ND	ND
7					
T	$(5.0 \pm 0.5) \times 10^1$	ND	ND	ND	ND
M	$(2.5 \pm 0.4) \times 10^1$	$(1.5 \pm 0.2) \times 10^1$	$(1.0 \pm 0.2) \times 10^1$	ND	ND
E/Pa	ND	ND	ND	ND	ND
8					
T	$(1.5 \pm 0.3) \times 10^2$	$(5.0 \pm 0.5) \times 10^1$	ND	ND	ND
M	$(5.0 \pm 1.3) \times 10^1$	$(2.0 \pm 0.3) \times 10^1$	ND	ND	ND
E/Pa	ND	ND	ND	ND	ND
9					
T	$(5.0 \pm 0.7) \times 10^1$	$(3.0 \pm 0.4) \times 10^1$	$(2.0 \pm 0.3) \times 10^1$	ND	ND
M	$(5.0 \pm 0.3) \times 10^1$	$(2.0 \pm 0.3) \times 10^1$	ND	ND	ND
E/Pa	ND	ND	ND	ND	ND
10					
T	$(2.6 \pm 0.4) \times 10^2$	$(5.0 \pm 0.6) \times 10^1$	$(3.0 \pm 0.3) \times 10^1$	$(2.0 \pm 0.2) \times 10^1$	ND
M	$(5.0 \pm 0.5) \times 10^1$	$(2.0 \pm 0.3) \times 10^1$	ND	ND	ND
E/Pa	ND	ND	ND	ND	ND

^a T: Total aerobic microbes.

^b ND: No microbe detected on plates.

^c M: Molds.

^d E: Enterobacteriaceae; Pa: *Pseudomonas aeruginosa*.

ANOVA, followed by Dunnett's *t* test. The difference was considered to be statistically significant when *p* value was less than 0.05.

3. Results

3.1. Effect of gamma irradiation on the microbial burden of *Polygoni Multiflori Radix*

The microbial loads in POMU specimens of different origins were analyzed immediately after irradiation by the spread plate method. The viability of microorganisms in POMU specimens irradiated with different gamma-ray doses of 0, 2, 4, 6, 8 and 10 kGy are shown in Table 1. POMU specimens contained total mesophilic bacterial counts of 5.0×10^1 to 2.9×10^3 cfu/g, and mold and yeast counts of 1.0×10^1 to 6.2×10^2 cfu/g. Total bacterial counts were much higher than the mold and yeast counts in all examined samples. Enterobacteria were also found in one of the ten POMU specimens, and ranged from not detected to 1.0×10^1 cfu/g. Although the microbial profiles of specimens from different sources varied markedly,

following irradiation at 2 kGy, enterobacteria were inactivated; at 4 kGy, mold and yeast counts were obviously reduced; and yeast and fungi were no longer observed in POMU specimens after irradiation at 6 kGy. As the radiation dosage increased, microbial profiles in the POMU specimens changed dramatically; the microbial counts decreased as the irradiation dose increased.

3.2. Detecting changes in antioxidant activity after gamma irradiation by ABTS assay

ABTS assay is often used for the evaluation of total antioxidant power of single compounds and complex mixtures of various plants [24]. In this assay, ABTS radical monocation was generated directly in stable form from potassium peroxodisulfate. Radicals were generated before antioxidants were added; this modification made the assay less susceptible to interruptions and prevented overestimation of antioxidant power [25]. The sample was added to the reaction medium when stable absorbance was obtained, and the antioxidant activity was measured in terms of decolorization.

Table 2
Total antioxidant activity assessed by TEAC.

Samples and positive control	TEAC ($\mu\text{M Trolox}/\text{mg} \pm \text{SD}$) ^a
0 kGy	52.08 \pm 0.79
5 kGy	63.62 \pm 0.84
10 kGy	59.77 \pm 1.03
15 kGy	55.92 \pm 0.80
THSG	1871.53 \pm 15.38
BHT	1869.41 \pm 34.63
GSH	1827.68 \pm 76.84

^a All values are expressed as mean \pm S.D. of triplicate tests ($n=3$). Values represented mean \pm S.D. of three parallel measurements ($p < 0.05$) when analyzed by ANOVA and Duncan's multiple-range tests.

The results of ABTS assay were expressed in TEAC values. A higher TEAC value meant that the sample had a stronger antioxidant activity. TEAC values determined from the calibration curve for POMU are shown in Table 2. Antioxidant activities of the POMU extracts were in the following decreasing order: 5 kGy (63.62 \pm 0.84 $\mu\text{M}/\text{mg}$ extract) > 10 kGy (59.77 \pm 1.03 $\mu\text{M}/\text{mg}$ extract) > 15 kGy (55.92 \pm 0.80 $\mu\text{M}/\text{mg}$ extract) > 0 kGy (52.08 \pm 0.79 $\mu\text{M}/\text{mg}$ extract). The antioxidant potency of THSG (positive control) was 1871.53 \pm 15.38 $\mu\text{M}/\text{mg}$ extract.

ABTS assay was used to estimate the total antioxidant power because it is quick and simple to perform, and reactions are reproducible and linearly related to the molar concentration of antioxidants [26]. Furthermore it can also be used to measure the antioxidant capacity of a wide range of biological samples, pure compounds, fruits, wines, and animal tissues [27].

3.3. Detecting the effect of gamma irradiation on antioxidant activity by DPPH assay

The relatively stable organic radical DPPH is widely used in modeling systems to investigate the scavenging activity of several natural compounds, such as phenolics and anthocyanins, or crude mixtures. DPPH radical is scavenged by antioxidants through the donation of a proton to form reduced DPPH. The color changes from purple to yellow after reduction, and can be quantified by the decrease in absorbance at wavelength 517 nm. Table 3 shows IC₅₀ values for the radical-scavenging activities of POMU, THSG, and BHT using the DPPH colorimetric method. It was found that 0 kGy had the lowest IC₅₀ value (513.04 \pm 0.01 $\mu\text{g}/\text{mL}$), followed by 5 kGy (514.28 \pm 0.01 $\mu\text{g}/\text{mL}$), 10 kGy (526.79 \pm 0.01 $\mu\text{g}/\text{mL}$) and 15 kGy (546.03 \pm 0.02 $\mu\text{g}/\text{mL}$). The four extracts showed significant differences ($p < 0.05$) in radical-scavenging activity. As shown from the above results, the sample without any treatment of gamma radiation (0 kGy) had the highest DPPH radical scavenging activity. However, its capacity was still much lower compared to the positive controls of BHT and THSG (41.06 \pm 0.76 and 37.51 \pm 0.31 $\mu\text{g}/\text{mL}$).

Table 3
IC₅₀ values of the DPPH radical scavenging activities of the methanol extracts of Polygoni Multiflori Radix that had been irradiated with different dosages of gamma ray.

The dosage of gamma radiation	IC ₅₀ ($\mu\text{g}/\text{mL}$) ^a
0 kGy	513.04 \pm 0.01
5 kGy	514.28 \pm 0.01
10 kGy	526.79 \pm 0.01
15 kGy	546.03 \pm 0.02
THSG	37.51 \pm 0.31
BHT	41.06 \pm 0.76

^a Values represented mean \pm S.D. of three parallel measurements ($p < 0.05$).

Table 4
Total polyphenol, flavonoid and flavonol contents of the methanol extracts of Polygoni Multiflori Radix that had been exposed to different doses of gamma radiation.

Dose of gamma radiation	Total polyphenols ($\mu\text{g CE}/\text{mg}$) ^b	Total flavonoids ($\mu\text{g RE}/\text{mg}$) ^c	Total flavonols ($\mu\text{g CE}/\text{mg}$) ^b
0 kGy	27.84 \pm 0.06	6.99 \pm 0.02	5.74 \pm 0.27
5 kGy	28.21 \pm 0.02	7.14 \pm 0.04	5.78 \pm 0.06
10 kGy	27.77 \pm 0.01	7.01 \pm 0.03	5.79 \pm 0.03
15 kGy	26.82 \pm 0.01	5.12 \pm 0.02 ^d	5.71 \pm 0.07

^a Values represented mean \pm S.D. of three parallel measurements.

^b Data expressed in μg (+)-catechin equiv./mg dry weight ($\mu\text{g CE}/\text{mg}$).

^c Data expressed in μg rutin equiv./mg dry weight ($\mu\text{g RE}/\text{mg}$).

^d Values significantly differing from others at 5%.

3.4. Detecting changes in antioxidant activity after gamma irradiation by reducing power measurement

We investigated Fe³⁺–Fe²⁺ transformation in POMU samples for the measurement of their reducing capacity. The reducing capacity of a compound may serve as an important indicator of its potential antioxidant activity [28]. The antioxidant activity of putative antioxidants have been attributed to various mechanisms, such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging [29]. In this assay, potassium ferricyanide was added to POMU samples and positive controls of THSG and BHT for the determination of any changes in reducing power after gamma irradiation. The mixtures were allowed to incubate during which time ferricyanide was reduced to ferrocyanide.

The reducing power was measured in terms of absorbance of the reaction mixtures at 700 nm. A higher absorbance indicated a stronger reducing power, vice versa. The reducing powers of the samples were in the following decreasing order: 0 kGy (0.79 \pm 0.02) > 5 kGy (0.55 \pm 0.02) > 10 kGy (0.52 \pm 0.01) > 15 kGy (0.46 \pm 0.01) at the dose of 1 mg/mL of POMU. The reducing powers of THSG and BHT were 1.98 \pm 0.04 and 1.30 \pm 0.02 respectively (data not shown).

3.5. Effect of gamma irradiation on total polyphenol, flavonoid, and flavonol contents

The total polyphenol, total flavonoid, and total flavonol contents of POMU are shown in Table 4. The total polyphenol content of POMU extracts ranged from 26.82 to 28.21 $\mu\text{g CE}/\text{mg}$. 0 kGy was 27.84, 5 kGy was 28.21, 10 kGy was 27.77 and 15 kGy was 26.82 $\mu\text{g CE}/\text{mg}$. 5 kGy had the highest polyphenolic content and 15 kGy was the lowest.

The total flavonoid content was expressed as μg rutin equiv./mg dry weight, and ranged from 7.14 to 5.12 $\mu\text{g RE}/\text{mg}$. 0 kGy was 6.99, 5 kGy was 7.14, 10 kGy was 7.01 and 15 kGy was 5.12 $\mu\text{g RE}/\text{mg}$. 5 kGy had the highest flavonoid content. 15 kGy was the lowest and significant different from others.

The total flavonol content was expressed as μg catechin equiv./mg dry weight. The total flavonol content of POMU extracts varied slightly and ranged from 5.79 to 5.71 $\mu\text{g CE}/\text{mg}$.

Both flavonoids and flavonols are polyphenolic compounds. Polyphenolic compounds have important roles in stabilizing lipid oxidation and are associated with antioxidant activities [30]. Phenolic compounds may contribute directly to antioxidative actions [19]. It is suggested that when as much as 1.0 g of polyphenolic compounds is ingested from a daily diet rich in fruits and vegetables, there may be inhibitory effect on mutagenesis and carcinogenesis in humans [31]. The antioxidative activities observed could

be ascribed both to variant mechanisms exerted by different phenolic compounds and to their synergistic effects. The antioxidant assays used in this study measured the oxidative products at the early and final stages of oxidation. Antioxidants have different functional properties, such as reactive oxygen species scavenging, e.g. quercetin, rutin, and catechin [32]; and inhibition of free radical generation and chain-breaking activity, e.g. *p*-coumaric acids [33] and metal chelation [34]. These compounds are normally phenolic compounds, which are effective proton donors, such as tocopherols, flavonoids and other organic acids. However, the components responsible for the antioxidative activity of POMU are still currently unclear.

3.6. Compositional changes of *Polygoni Multiflori Radix* after gamma irradiation

The effect of different doses of gamma radiation on the compositional changes of POMU was assessed. Quantitative analyses of POMU were conducted with HPLC–UV. THSG content of unirradiated and irradiated POMU was quantified based on a previously constructed HPLC–UV calibration curve for THSG standard ($R^2 = 0.996$). The content ranged from 2.13 to 3.05 mg/g of dry weight (DW) for unirradiated or irradiated POMU. Irradiation treatment resulted in significant changes in THSG content at different doses.

In this database, THSG showed statistically significant decreases from 3.05 mg/g (0 kGy, $p < 0.05$) to 2.60 mg/g after 5 kGy of irradiation ($p < 0.05$), 2.50 mg/g at 10 kGy ($p < 0.05$) and 2.13 mg/g at 15 kGy ($p < 0.01$) (Fig. 1; $N = 3$). Therefore, it was indicated that gamma irradiation caused damage to THSG content in POMU.

4. Discussion

POMU has long been used as a medicinal herb in Asia. Insect infestation and microbial contamination are serious problems for POMU storage. Gamma irradiation is able to penetrate deeply, and has been found to be useful for the hygienization of herbal products even when they are already in packages or sacks. The choice of a suitable radiation dosage is particularly important for decontaminate the microbial load of stored POMU. In this research, the optimal dosage for the inactivation of microorganisms in POMU was evaluated.

2 kGy was sufficient for the hygienization of enterobacteria, whereas a dosage of 6 kGy was required for the hygienization of yeasts and fungi. Since the dosage required removing microbial contamination is higher than that required killing insects, insects would be killed at the same time [35].

Our results confirmed that at the dose of 4 kGy, mold and yeast counts were obviously reduced; and at 6 kGy, neither yeasts nor fungi were observed any longer in POMU specimens. Thus in our research, it was demonstrated that 4–6 kGy of treatment was effective for the inactivation of microorganisms and 8 kGy treatment could induce complete inactivation.

Phenols are regarded as main contributors to antioxidant activities, the measurements are based on radical scavenging. Antioxidant capacity depends on various factors, such as the number and location of hydroxyl groups on the aromatic ring, as well as their mutual positions [36]. Our results demonstrated that the irradiation dosage on POMU (5 kGy, $63.62 \pm 0.84 \mu\text{M}/\text{mg}$ extract) had both the highest antioxidative activity and the lowest value in IC_{50} of DPPH radical-scavenging activity, and as expected, an increase of radiolytic products, which agreed with literary reports for other foods and medicines. In this study, 5 kGy γ -irradiation increased radiolytic products of POMU; however it seemed to increase the antioxidant effect of POMU in the ABTS assay.

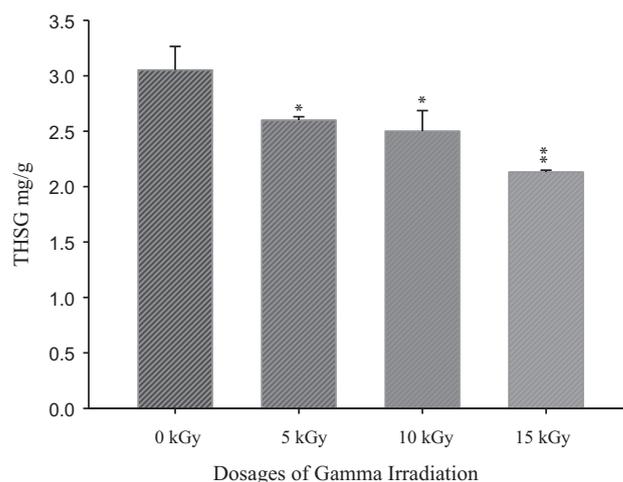


Fig. 1. Quantitation of THSG contents in the methanol extracts of *Polygoni Multiflori Radix* by HPLC.

The content of total phenols, expressed as μg (+)-catechin equiv./mg dry weight value, did not vary distinctively in irradiated POMU samples. The values were slightly lower; however variations were not statistically significant (Table 4). THSG content of POMU had decreased as the γ -radiation increased (Fig. 1). However, the total phenolic compounds and antioxidative activities were increased, probably because other phenolic compounds were formed during irradiation. It has to be mentioned that irradiation of aqueous systems containing aromatic compounds can implicate the formation of phenols.

5. Conclusion

The results of this study indicated that 5 kGy of gamma irradiation was effective for the hygienization of POMU. It did not alter the appearance of POMU before and after gamma irradiation. Though, after 5 kGy of irradiation, some physicochemical properties were slightly changed or compensated for the improved hygiene of this medicinal herb, such as the decrease in THSG content. However, the total phenolic compounds and antioxidative activities were increased. Therefore gamma irradiation at 5 kGy could be a potential method for decontaminate the microbial load of POMU to prolong shelf life and improve hygienic quality.

References

- [1] Farkas J. Irradiation of dry food ingredients. Florida: CRC Press Inc.; 1988. p. 2.
- [2] McKee LH. Microbial contamination of spices and herbs: a review. *Lebensm Wiss Technol* 1995;28:1–11.
- [3] Kneifel W, Berger E. Microbiological criteria of random samples of spices and herbs retailed on the Austrian market. *J Food Prot* 1993;57:893–901.
- [4] Pafumi J. Assessment of the microbiological quality of spices and herbs. *J Food Prot* 1986;49(12):958–63.
- [5] Eiss I. Growing impact of irradiation on global production of and trade in spices. Irradiation for food safety and quality. In: Loaharanu P, Thomas P, editors. *FAO/IAEA/WHO international conference on ensuring the safety and quality of food through radiation processing*. Turkey: American Spice Trade Association; 2001. p. 178–91.
- [6] Ehrenberg L, Hussain S. Genetic toxicity of some important epoxides. *Mutat Res* 1981;86:112–3.
- [7] Kligerman AD, Erexson GL, Phelps ME, Wilmer JL. Sister-chromatid exchange induction of peripheral blood lymphocytes of rats exposed to ethylene oxide by inhalation. *Mutat Res* 1983;120:37–44.
- [8] Pfeiffer EH, Dunkelberg H. Mutagenicity of ethylene oxide and propylene oxide and of the glycols and halohydrins formed from them during the fumigation of foodstuffs. *Food Cosmet Toxicol* 1980;18(2):115–8.
- [9] Intergovernmental Negotiating Committee. Operation of the interim prior informed consent procedure for banned or severely restricted chemicals in international trade. In: Debois M, Karmen K, editors. *Proceedings of the Rotterdam convention on the prior informed consent procedure for certain hazardous*

- chemicals and pesticides in international trade. Rotterdam: United Nations Environment Program; 2001. p. 12.
- [10] Farkas J, Andrassy E. Increased sensitivity of surviving bacterial spores in irradiated spices. In: Dring GJ, Ellar DJ, Gould GW, editors. Fundamental and applied aspects of bacterial spores. London: Academic Press, London; 1985. p. 397–407.
- [11] Farkas J. Irradiation as a method for decontaminating food—a review. *Int J Food Microbiol* 1998;44(3):189–204.
- [12] Ito H, Kamakura G, Sekita H, Kamiyoga S. Distribution of microorganisms in herb medicines and their decontamination by gamma-irradiation. *Shokuhin-shosha* 1999;34(1–2):16–22.
- [13] World Health, Organization. Report of an ICGFI consultation on microbiological criteria for foods to be further processed including irradiation. Geneva: WHO/EHE/FOS/89.5 WHO; 1989. p. 1.
- [14] Chinese Pharmacopeia, Commission. Pharmacopoeia of the People's Republic of China, vol. 1. Beijing: People's Medical Publishing House; 2010. p. 164–5.
- [15] Ye S, Tang L, Xu J, Liu Q, Wang J. Postconditioning's protection of THSG on cardiac ischemia-reperfusion injury and mechanism. *J Huazhong Univ Sci Technol Med Sci* 2006;26(1):13–6.
- [16] Wen HW, Wang YT, Chung HP. Efficacy of gamma irradiation for protection against postharvest insect damage and microbial contamination of the adlay. *Postharvest Biol Technol* 2008;50(2–3):208–15.
- [17] Janovsky I. Response of three types of aqueous chemical dosimeters to gamma and electron radiations in the dose range 0.1–10 kGy. *Int J Radiat Appl Instrum* 1986;37(1):61–3.
- [18] Wen HW, Chung HP, Chou FI, Lin IH, Hsieh PC. Effect of gamma irradiation on microbial decontamination, and chemical and sensory characteristic of Lycium Fruit. *Radiat Phys Chem* 2006;75:593–603.
- [19] Chang TN, Huang GJ, Ho YL, Huang SS, Chang HY, Chang YS. Antioxidant and antiproliferative activities of *Crossostephium chinensis* (L.) Makino. *Am J Chin Med* 2009;37:797–814.
- [20] Huang DJ, Chen HJ, Lin CD, Lin YH. Antioxidant and antiproliferative activities of water spinach (*Ipomoea aquatica* Forsk) constituents. *Bot Bull Acad Sin* 2005;46:99–106.
- [21] Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J Agric Food Chem* 1995;43:27–32.
- [22] Huang DJ, Lin CD, Chen HJ, Lin YH. Antioxidant and antiproliferative activities of sweet potato (*Ipomoea batatas* [L.] Lam 'Tainong 57') constituents. *Bot Bull Acad Sin* 2004;45:179–86.
- [23] Chang HC, Huang GJ, Agrawal DC, Kuo CL, Wu CR, Tsay HS. Antioxidant activities and polyphenol contents of six folk medicinal ferns used as Gusuibu. *Bot Stud* 2007;48:397–406.
- [24] Jagetia GC, Baliga MS. Evaluation of the radioprotective action of geriforte in mice exposed to different doses of γ -irradiation. *Am J Chin Med* 2004;32(4):551–67.
- [25] Sanchez-Moreno C. Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Sci Technol Int* 2002;8:121–37.
- [26] Benzie IFF, Wai Y, Strain JJ. Antioxidant (reducing) efficiency of ascorbate in plasma is not affected by concentration. *J Nutr Biochem* 1999;10:146–50.
- [27] Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem* 2006;94:550–7.
- [28] Meir S, Kanner J, Akiri B, Hadas SP. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *J Agric Food Chem* 1995;43:1813–9.
- [29] Diplock AT. Will the 'good fairies' please prove to us that vitamin E lessens human degenerative of disease? *Free Radic Res* 1997;27:511–32.
- [30] Oh YC, Kang OH, Choi JG, Chae HS, Lee YS, Brice OO, et al. Anti-inflammatory effect of resveratrol by inhibition of IL-8 production in LPS-induced THP-1 cells. *Am J Chin Med* 2009;37(6):1203–14.
- [31] Tanaka M, Kuei CW, Nagashima Y, Taguchi T. Application of antioxidative maillard reaction products from histidine and glucose to sardine products. *Nippon Suisan Gakkai Shi* 1988;54:1409–14.
- [32] Hatano T, Edamatsu R, Hiramatsu M, Moti A, Fujita Y, Yasuhara T, et al. Effects of tannins and related polyphenols on superoxide anion radical, and on 1,1-diphenyl-2-picrylhydrazyl radical. *Chem Pharm Bull* 1989;37:2016–21.
- [33] Laranjinha J, Vieira O, Madeira V, Almeida L. Two related phenolic antioxidants with opposite effects on vitamin E content in low density lipoproteins oxidized by ferrylmyoglobin: consumption versus regeneration. *Arch Biochem Biophys* 1995;323(2):373–81.
- [34] Van-Acker SABB, Van-Balen GP, Van-den-Berg Bast A, Van der vijgh WJF. Influence of iron chelation on the antioxidant activity of flavonoids. *Biochem Pharmacol* 1998;56(8):935–43.
- [35] Younes MWF, Shehata NF, Mahmoud YA. Histopathological effects of gamma irradiation of the peach fruit fly, *Bactrocera zonata* (Saund.) female gonads. *J Appl Sci Res* 2009;5(3):305–10.
- [36] Rahman A, Rahman MM, Mominul IS, Mashiar R, Sheik MMI, Rahman MM, et al. Free radical scavenging activity and phenolic content of *Cassia sophera* L. *Afr J Biotechnol* 2008;7(10):1591–3.