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Improvement of Hygienic and Phytochemical Qualities of Mangosteen (*Garcinia Mangostana*) Peel by Irradiation

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Abstract

The aim of this study was to compare the effects of gamma ray and x-ray irradiation at different dose levels 95, 10, 15 and 20 kGy) on in mangosteen peel. The antioxidant activities were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP), while total phenolic contents and antibacterial activities against *Staphylococcus aureus* and *Escherichia coli* of 60 % ethanolic mangosteen powder extracts were analyzed. The results indicated that both types of ionizing radiations showed a non-significant decrease in measured parameters with increasing doses. The minimum inhibitory concentration (MIC) values of irradiated mangosteen peel extracted against *S. aureus* and *E. coli* ranged from 0.125 to 2.0 mg/mL. The quantity of bioactive compound mangostin determined via high-performance liquid chromatography (HPLC), did not show significant decrease with different irradiation sources and doses. In microbiological aspect, total yeast and mold from both irradiation sources were reduced by 1 log cycle after irradiation at 5 kGy. The irradiation induced color changes corresponding to Hunter color L a b value. X-ray irradiation caused slight color alteration compared to gamma rays. The advantage of x-ray irradiation was that it revealed non-significant changes in b values, whereas gamma irradiation showed the significant change in L, and b values with increasing dosage. The x-ray irradiation at the dose 5 kGy effectively decreased microbial contamination while causing minimal changes to phytochemical qualities and color.

Keywords: Antioxidant, Antimicrobial, Mangostin, Irradiation, Mangosteen peel, Microbial decontamination

Introduction

Mangosteen (*Garcinia mangostana* L.) peel has been widely used as an ingredient in traditional medical recipes due to its medicinal therapeutic activity. It has been subsequently studied and found that mangosteen peel contained a variety of bioactive compounds, especially phenolic compounds, such as xanthones, flavonoids, and phenolic acids. Among various active compounds, xanthones are predominant because of their high abundance. More than 30 xanthones are isolated from *G. mangostana* where the major constituents are α-mangostin and γ-mangostin [1]. Furthermore, mangosteen peel serves as a source of

natural pigment, namely anthocyanin. The major red-purple pigment is cyanidin-3-sophoroside. Prior research reported that the mangosteen peel is claimed to possess an antioxidant activity and it has been traditionally used to cure diseases, such as diarrhea, dysentery, cholera, skin infection, and respiratory disorder [2].

Nowadays, food safety is widely recognized as an important public health problem. For herbs, foodborne pathogens and insect pests are the major problems during food process, transportation and storage time. Conventional decontamination methods applying heat or chemicals can substantially reduce these contaminants. However, both treatments also result in change of flavor due to the degradation of the volatile aroma components, consequently devaluating quality of herbs. Food irradiation is an alternative and environmentally friendly technology. Irradiation processing was a non-thermal and non-chemicals substances method that's serves as an effective alternative to conventional treatments. This technology has been recognized as a method for reducing post-harvest food loss, decontamination, and increasing hygienic quality for medicinal herbs. Previous reports have proved that food irradiation with gamma rays, electron beams, and x-rays are effective methods for disinfestation, decontamination, food safety, and improving nutritional attributes and shelf-life extension [3].

In Thailand, mangosteen peel explored the potential in pharmaceutical and cosmetic application. The mangosteen peel powder may be contaminated with pathogens. Ionizing radiation was an effective method to reduce the microbial load in mangosteen peel to meet the standard. Gamma rays and x-rays have been widely used in food irradiation because of their share some characteristics with microwaves, but with much higher energy and penetration. It was in forms of high-frequency ionizing radiation, which means they have enough energy to remove an electron from atoms or molecules (ionization). Ionized molecules are unstable and quickly undergo chemical change. X-rays and gamma rays have the same basic properties but come from different parts of the atom. X-rays are emitted from processes outside the nucleus, whereas electrons striking a target or electrons rearrangement within an atom in x-ray tube. The x ray machine was operated like an electric appliance. While, gamma rays originate inside the nucleus. Gamma ray for irradiation process emitted from radionuclides of 60 Co and/or 137 Cs which complicated in operation and radioactive waste management. Both of ionizing radiation capable to inhibit microbial growth by direct and indirect mechanism. This mechanism was effect on metabolism and chemical reactions of microorganisms, leading to cell death or injury.

The mangosteen peel was widely used in pharmaceutical and health care industies. Plant materials are naturally contaminated with microorganisms found in soil, water and air. Irradiation was applied in quality control process. The irradiation dosage depends on the irradiation aspects. The U.S. Food and Drug Administration (FDA) set a limit for irradiation treatment of culinary herbs, seeds, spices, vegetable seasonings, and blends of these aromatic vegetable substances that must not exceed 30 kGy [4]. The effectiveness and safety of both ionizing radiations have been proved. However, questions concerning loss of phytochemical constituents, free radicals and radiolytic by-product formation, and change of antioxidant properties during irradiation are still being discussed [5].

Despite its high cost, recent research on x-ray irradiation has shown promise. The purpose of this study was to compare the effect of gamma ray and x-ray irradiation at various doses on the color,

microbiological loads, and stability of phytochemical constituents of mangosteen (*Garcinia mangostana* L.) peel powder.

Materials and methods

Sample preparation and radiation processing

Mangosteen peel powder was purchased from local distributers in Thailand. The moisture contents of mangosteen peel powder was approximately 11 %/wt. The samples were repacked in aluminum foil bags and irradiated according to commercial practice, using 60Co source and x-ray at the doses of 5, 10, 15 and 20 kGy. Irradiation processing was done at Thailand Institute of Nuclear Technology (Public Organization) with the multipurpose gamma irradiator (Paul Stephens, England) with the dose rate of 3.3 kGy/h and electron accelerator 50 kW (MB5-50, Mevex, Canada) that generates 5 MeV x-ray with the approximate dose rate 20 kGy/h was utilized. After irradiation, mangosteen peel powder was analyzed for microbial load and color alteration.

Extraction of mangosteen peel powder

The mangosteen peel powder was soaked in 60 % (v/v) ethanol (1:10 w/v) and sonicated at 25 °C for 1 h. following the method described by to Samuagam *et al*. [6]. The mixture was centrifuged at 8,000 rpm 10 min and filtered through filter paper (Whatman No.1). The supernatants, ethanolic extract of mangosteen peel was obtained. This extract was prepared for antioxidant, total phenolic, and mangostin analysis. For antibacterial test, the supernatants were collected and evaporated using a rotary evaporator Rotavapor (R-300, Büchi, Switzerland) at 45 °C until the volume of the evaporated filtrate was less than 10 % of the initial volume of the filtrate. Then the remaining water in concentrates was removed by lyophilization using a freeze dryer. (Labcongo, USA)

Microbiological analysis

Aerobic plate count and total yeast and mold were analyzed according to FDA's Bacteriological Analytic Manual (BAM) method [7]. Approximately 25 g of mangosteen peel powder was added to 0.1 % peptone water 225 mL to achieve 10^{-1} diluted solution and homogenized for 2 min. Then prepared a serial dilution in 0.1 % peptone water to the appropriate dilution. 0.1 mL of each dilution were dropped on solidified plate count agar and potato dextrose agar in triplicate and incubated at 35 °C for 48 h. and 25 °C for 5 days, respectively. Microbial colonies were counted and reported in colony forming units per gram of sample (CFU/g).

Determination of DPPH radical scavenging activity

The DPPH free radical scavenging activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as an electron receiver. DPPH radicals were analyzed following to the method of Khattak and Simpson [8]. The reaction of ethanolic extract solution and the stable DPPH was evaluated. In each sample, 100 µL extracted solution was added to 900 µL DPPH radical solution (0.2 mM in methanol). and the mixture was shaken vigorously. After incubation in dark condition at room temperature for 15 min. The

reaction is accompanied by color change from deep violet to light yellow. Each solution measured the absorbance at 517 nm with UV-visible spectrophotometer (UV-1700, Shimadzu, Japan). Ascorbic acid was used as a comparative compound, as a standard curve. Free radical scavenging activity was expressed as the ascorbic acid equivalent (AAE) per gram of extract.

Determination of ferric reducing antioxidant power

Antioxidant capability of the extract based on ferric reducing antioxidant power (FRAP) was evaluated according to the method described by Benzie and Strain [9] with some modifications. Stock solution included 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM ferric chloride ($Fe_2Cl_3 \cdot 6H_2O$) solution. The FRAP working solution was freshly prepared by mixing acetate buffer, TPTZ solution, and ferric chloride solution in a ratio of 10:1:1 and further incubated at 37 °C for 10 min prior to analysis. Mangosteen peel extracts (0.1 mL each) reacted with 2.9 mL FRAP solution for 30 min in dark condition. After incubation period, the solution resulted in dark blue complex of ferrous tripyridyl-s-triazine, was measured spectrophotometrically (UV-visible spectrophotometer (UV-1700, Shimadzu, Japan)) at λ_{max} = 593 nm. The antioxidant potential of the samples was determined based on a calibration curve plotted of $FeSO_4 \cdot 7H_2O$ with concentrations ranging from 400 to 2,000 μM.

Determination of total phenolic contents

Total phenolic content of the crude extracts was determined according to the Folin-Ciocalteu assay by Velioglu *et al*. [10]. In test tube, 100 μL of each extract was mixed with 750 μL of Folin-Ciocalteu reagent (previously 10-fold diluted with distilled water). The mixture was incubated at room temperature for 5 min. Then, 750 μL of 6 % (w/v) sodium carbonate solution was homogenized with the mixture. After incubation for 90 min at room temperature, absorbance was determined by UV - visible spectrophotometer (UV-1700, Shimadzu, Japan) at 725 nm. The standard calibration curve was plotted using gallic acid at the concentration of 20-100 mg/mL. The total phenolic content was expressed as gallic acid equivalent (GAE) mg/g.

Determination of α-mangostin contents

The contents of α-mangostin in all samples were analyzed by the high-performance liquid chromatography (HPLC) technique. The analysis was performed by use of an HPLC instrument (Waters e2695, USA) equipped with a UV-vis detector for UV detection at 320 nm with SunFire C18 Columns (4.5 cm×150 mm). A mobile phase contained 95 % methanol in ultrapure water. It was pumped at a flow rate of 1.2 mL/min at a temperature of 25 °C. Samples were determined in triplicate and calculated for α-mangostin content based on a linear regression equation for a standard curve of α-mangostin.

Color measurement

All samples were determined the color alteration in form of Hunter Lab using chromameter (CR300, Minolta Co., Ltd., Japan). This colorimeter was calibrated with standard white tile before measurement. The color of sample showed into tree dimension color scale which L measuring light to dark color components, a is a red to green scale and b is a yellow to blue scale.

Antibacterial activity assay

The antibacterial activity of 60 % ethanolic mangosteen peel extracts was evaluated against *Escherichia coli* DMST 4212 (ATCC 25923) and *Staphylococcus aureus* DMST 8840 (ATCC 25922) using agar disc diffusion method. The freeze dryer crude extract was dissolved in dimethyl sulfoxide (DMSO) at concentration of 0.125, 0.25, 0.5, 1.0, 2.0 mg/mL. Bacterial suspensions were spread on Muller-Hinton agar plates. The 20 µL of irradiated and non-irradiated mangosteen peel extract at various concentration were added to 0.6 mm paper disc and incubated at 37 °C for 24 h. DMSO and betadine were used as a negative and positive control. The antibacterial activity was evaluated by measuring the inhibition zone diameter after incubation at 37 °C for 24 h. The results are the means of 3 replicates.

Statistical analysis

Data analysis was obtained from triplicate samples. Values were expressed in form of mean ± standard deviation. Statistical analysis was interpreted by SPSS software (version 21.0). Analysis of variance (one-way ANOVA) in a completely randomized design was used to compare any significant difference between control and irradiated samples for all parameters. Duncan's multiple range test was performed at $p \leq 0.05$

Results and discussion

Microbiological analysis

Ionizing radiation, including gamma rays and x-rays has been recognized as a cold decontamination method of herbs and spices because of highly penetration attribute. Gamma radiation can demolish microorganisms by damaging DNA strand and reproductive system [11]. This experiment revealed that the irradiation with gamma ray and x-ray showed the same tendency for microbial decontamination aspect. The values of aerobic plate count and total yeast and mold from 2 sources at various doses are shown in **Table 1** as a log of colony forming unit per gram (CFU/g). The data indicated that the aerobic plate count of irradiated and non-irradiated samples was bellowed the detectable limit. This may be due to the good hygiene of post-harvest and drying process. In part of total yeast and mold, the values of non-irradiated samples were 3.3×10^2 CFU/g for gamma ray and 3.0×10^2 CFU/g for x-ray. These values exceeded the limitation of Thai Community Product Standard (TCPS. 480/2547) for dry herbs (moisture <12 %), that maximum permission was 1×10^2 CFU/g [12]. After irradiation at 5 kGy with gamma ray and x-ray, total yeast and mold were below the detectable levels. In accordance with the publication of Pewlong *et al*. [13], which reported that gamma irradiation was an effective decontamination method. Both total bacterial count and yeast and mold count of irradiated Tanaka at 5 kGy were significantly lower than those of the control sample.

In microbial decontamination aspect, ionizing radiation causes effective disruption of DNA strand of cells cause them to be inactivated [14]. The differences in sensitivity to radiation among microorganisms are related to the differences in their chemical and physical structures, and in their ability to recover from radiation injury. Therefore, the radiation dosage required for microbial decontamination in food depends on the resistance of the species, structure of cell, moisture contents, and initial load of microorganisms.

Normally, microbial contamination may occur in harvest, drying process, handling, and storage, etc. In this study, the mangosteen peel powder was slightly contaminated with microorganisms. Probably due to the quality of raw material, the non-soiled origin and good hygienic process. The gamma or x-ray irradiation at only 5 kGy was sufficient to reduce the microbial load to meet the Thai Community Product Standard. Both ionizing radiations expressed the same tendency in decreasing the microbial load.

Table 1 Microbial load of irradiated and non-irradiated samples from gamma and x-ray irradiation.

Dose (kGy)	Aerobic Plate Count (CFU/g)		Total Yeast and Mold Count (CFU/g)	
	Gamma	X-ray	Gamma	X-ray
0	<10	<10	3.3×10^2	3.0×10^2
5	<10	<10	<10	<10
10	<10	<10	<10	<10
15	<10	<10	<10	<10
20	<10	<10	<10	<10

<10 = belowed the detectable limit

Antioxidant activity (DPPH radical scavenging assay and FRAP)

Antioxidant activity is the basic function of foods and also plays as an important role in herbal medicine function. Its helps in neutralizing harmful free radicals and reduce oxidative stress, which can lead to health issues. Hence the antioxidant activity of many herbal resources has been explored. Antioxidant activity in term of DPPH and FRAP was evaluated. Both of DPPH and FRAP analyse are spectrophotometric methods, which are uncomplicated measurement the in vitro antioxidant activity of medicinal herbs. These techniques are based on the electron transfer reactions, which visually result on the reduction of a colored oxidant (DPPH or FRAP as oxidant). Therefore, the results obtained from both methods usually present an excellent correlation.

In the present study, the antioxidant activities of mangosteen peel extracts measured by DPPH and FRAP with the different radiation sources and doses are shown in **Table 2**. The DPPH values of gamma rays and x-rays irradiation varied in the range of 95.88 to 104.39 and 98.61 to 105.40 mg AAE/g, respectively. Effect of both sources revealed the same tendency. They resulted in a non-significant alteration of DPPH and FRAP values with increasing doses up to 20 kGy.

The results align with the previous studies. In Reza *et al*. [15], which reported that DPPH radical-scavenging effect was not significantly changed after irradiation up to 20 kGy in rosemary extract but higher dose (30 and 40 kGy) of irradiation caused a significantly increasing. This report was harmonized with the

previous studies that applied gamma irradiation to Korean ginseng powder. It was found that hydrogen donating activity was not drastically changed by gamma irradiation up to 10 kGy [16].

Table 2 DPPH and FRAP radical-scavenging of 2 ionizing irradiated samples at various doses.

Source	Dose (kGy)	DPPH (mg AAE/g)	FRAP (mmol $FeSO_4$/g)
Gamma	0	103.35 ± 3.14[a]	921.73 ± 16.18[a]
	5	104.39 ± 9.36[a]	884.69 ± 31.88[a]
	10	103.50 ± 4.31[a]	919.26 ± 16.09[a]
	15	102.98 ± 2.84[a]	909.21 ± 38.10[a]
	20	95.88 ± 4.75[a]	869.70 ± 30.25[a]
x-ray	0	103.35 ± 3.14[a]	921.73 ± 16.18[a]
	5	101.79 ± 1.62[a]	911.85 ± 6.67[a]
	10	98.61 ± 7.58[a]	929.66 ± 25.90[a]
	15	105.40 ± 8.60[a]	929.66 ± 25.10[a]
	20	103.10 ± 6.00[a]	902.15 ± 34.48[a]

In each column, means of 3 independent experiments ± standard deviations, the same superscript letter are not significantly different ($p < 0.05$).

For positive results, the ethanolic extracts of irradiated cinnamon powder at doses of 10, 15, 20 and 25 kGy was evaluated. The radical scavenging activity (DPPH) of the cinnamon extract were increase after irradiation more than 10 kGy. With regard to the effect of irradiation up to 25 kGy on the antimicrobial activity compared to the control value, there was no statistically significant difference in the activity of irradiated samples [17]. For the higher dose of irradiation, Alloun *et al*. [17] reported that gamma irradiation at the dose up to 30 kGy was affects the antioxidant activity of *Thymus pallescens* essential oil [18]. On the other hands, the other experiments revealed that the DPPH scavenging activities of black pepper, black seed, Ginger, and Garlic were significantly decreased with increasing doses [5].

The FRAP values were estimated from the ability to reduce the TPTZ-Fe (III) complex to the TPTZ-Fe (II) complex. In **Table 2**, the values of FRAP of gamma and x-ray at various doses fluctuated in the range of 869.70 - 921.73 and 902.15 - 929.66 μmol $FeSO_4$/g, respectively. The results also expressed that the FRAP values were not significantly change at different doses and ionizing radiations sources. In consistency with Kitazura's study, which reported that the 5 - 25 kGy of gamma irradiation showed no effect on the antioxidant potential of cinnamon compounds [19].

In positive results, Eamsiri *et al*. [20] reported that gamma irradiation at 20 kGy resulted in significantly increase in FRAP value of *Coscinicum fenestratum* powder. Contrarily, irradiation below 15 kGy showed non-significant difference from the control. This data indicated that the higher irradiation dose affected the increase in FRAP value.

Evidently, gamma irradiation could enhance the antioxidant activity only in some foods and no general pattern of gamma irradiation on antioxidant activity was found. The irradiation influencing factors

on active components, antioxidant, and volatile compounds can depend on various factors, such as radiation dose, dose rate, components of raw materials, samples state (solid or dry), extraction solvent, extraction process, packaging, time delay between the irradiation process and the measurement, conditions (temperature and oxygen exposure during and after irradiation), and storage time [8,21-23].

Total phenolic contents

Phenolic compounds are secondary metabolites that found in plant tissue. Mangosteen peel serves as a source of natural pigment and phenolic compounds. The effect of gamma and x-rays irradiation at 5, 10, 15 and 20 kGy on total phenolic contents is demonstrated in **Figure 1**. The results indicated that the levels of total phenolic contents were varied between 80.19 and 86.99 for gamma ray and between 83.75 and 91.06 mg GAE/g for x-ray. The statistical analysis expressed that no significant effect was marked in total phenolic contents of the irradiated samples from 2 different ionizing irradiations. In consistency with report of Eamsiri *et al*. [20], which stated that no significant effect was perceived in total phenolic contents in irradiated *Coscinium fenestratum* (Goetgh.) Colebr at the dose of 20 kGy and Chatterjee *et al.* [24] reported that no difference in the total phenolic content of irradiated turmeric (*Curcuma longa*) and fenugreek (*Trigonella foneum*) samples when compared to non-irradiated samples.

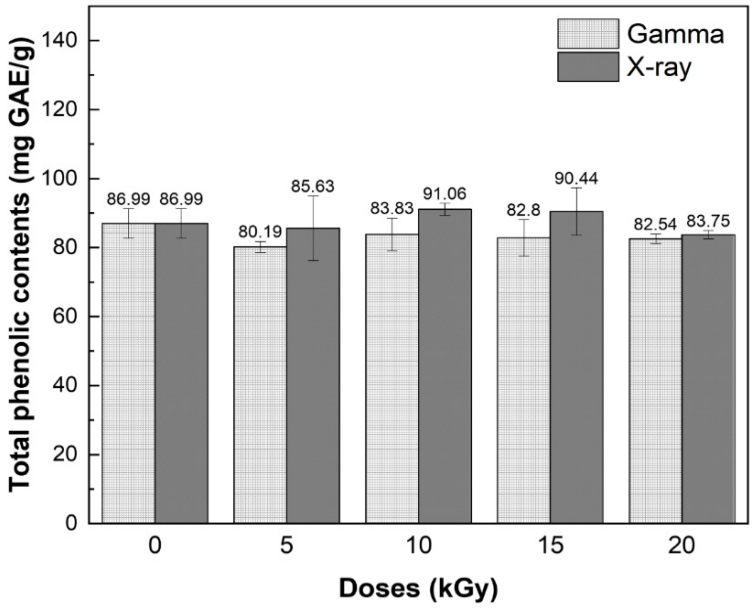

Figure 1 Total phenolic contents of 2 ionizing irradiated samples at various doses.

In previous reports, the increase and decrease in total phenolic contents can occur. The difference effects of irradiation on total phenolic content might be depended on various factors such as the type and parts of the plant, phenolic content composition, environmental condition, solvent and method of extraction, storage conditions and irradiation dose [25]. For examples, Gerolis *et al*. [26] reported that the gamma irradiation caused a reduction in total phenol of mate and green teas. On the other hands, gamma irradiation at the dose of 7 kGy also caused slight increase in *Nigella sativa* and *Carulluma tubercullata* [27]. Similarly

in case of *Curcuma alismatifolia* (Zingiberaceae) leaves, total phenolic content was increased after irradiation [28]. The increase of total phenolic may occurred because of irradiation lead to destructive processes of oxidation and breaking the chemical bonds of polyphenols [29].

α-Mangostin contents

The natural mangosteen peel contains several bioactive compounds, especially phenolic compounds, such as xanthones, flavonoids, and phenolic acids. The major xanthone in mangosteen peel is α-mangostin. In this investigation, the α-mangostin contents in irradiated and non-irradiated mangosteen peel extracts were measured by HPLC technique. The effects of 2 different radiations, gamma and x-ray, were compared as well as irradiation doses. The results given in **Figure 2** indicated that the levels of α-mangostin in the gamma ray and x-ray irradiated samples at various doses were in the range of 34.53 - 37.88 and 34.48 - 36.42 mg/g sample, respectively. The maximum reductions of α-mangostin contents were observed at the irradiation of 20 kGy for gamma ray and 10 kGy for x-ray. However, the means of α-mangostin contents were not significantly difference among 2 ionizing radiation treatments at various doses ($p \leq 0.05$). These results agreed with report of Shigemura *et al*. [30], which stated that the irradiation of ground black and white pepper at the dose of 10 kGy showed no effect on volatile oil and piperine content.

Nevertheless, the effect of ionizing radiation on herbs and spices depends on various factors, including varieties, moisture, dose rate and absorbed doses. For Tanaka, gamma radiation at doses higher than 10 up to 20 kGy significantly affected these values. The increasing of irradiation dose led to increasing the arbutin content [13]. For turmeric, Dhanya *et al.* [31] reported the gamma irradiation of freshly peeled turmeric rhizomes at the doses of 1, 3 and 5 kGy slightly increased in curcuminoid content with increasing doses, but no statistically significant change in the volatile oil constituent.

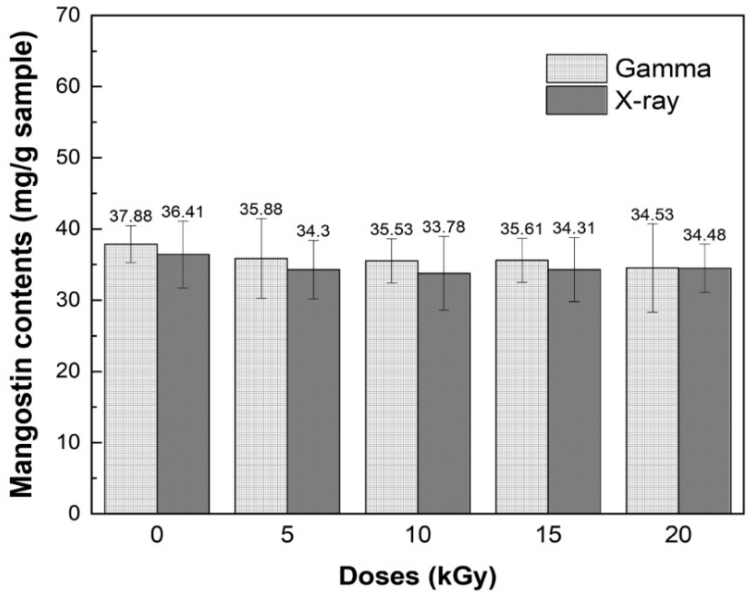

Figure 2 The α-mangostin contents of gamma and x-ray irradiation at different doses.

Color measurement in Hunter color L a b

Color of spices and herbs is one of the most important qualitative parameters. Normally, the mangosteen peel powder has a brown to red color characteristic. The red to brown color is mainly due to the presence of anthocyanin. The color alteration was determined in term of Hunter color L, a, b. The L a b values exhibited the lightness, the redness to greenness and the yellowness to blueness, respectively. The Hunter L, a, b of mangosteen peel powder from gamma and x-ray irradiation at various doses are demonstrated in **Table 3**. The data exhibited that both of radiation caused a significant decreased in lightness (L). Only gamma irradiation at the dose of 15 kGy caused a significantly changes on b value. Our results are in agreement with Najafabadi *et al*. [32], who studied about effect of radiation on anthocyanin contents. The result expressed that lightness (L*) value of Jujube juice anthocyanins decreased significantly after irradiated more than 0.6 kGy. The irradiation at 5 kGy was slightly changed in the a* and b* value. For other herbs and spices, Polovka and Suhaj reported that that gamma irradiation up to 30 kGy was slightly affected the color deviation in commercial herbs and spices, such as caraway, ginger, bay leaf, clove, oregano and black pepper [33].

Table 3 The Hunter L a b of gamma and x-ray irradiation at varied doses.

Source	Dose (kGy)	L	a	b
Gamma	0	47.44 ± 0.63^{e}	10.27 ± 0.24^{a}	17.89 ± 0.09^{e}
	5	44.94 ± 0.98^{c}	10.88 ± 0.35^{a}	17.48 ± 0.50^{bce}
	10	45.34 ± 1.03^{cd}	11.01 ± 0.17^{a}	17.38 ± 0.42^{bce}
	15	45.17 ± 0.50^{a}	10.72 ± 0.72^{a}	16.09 ± 0.28^{a}
	20	45.82 ± 0.51^{cd}	10.79 ± 0.27^{a}	17.63 ± 0.19^{bce}
X-ray	0	47.44 ± 0.63^{e}	10.27 ± 0.24^{a}	17.89 ± 0.09^{e}
	5	46.18 ± 1.08^{d}	10.45 ± 0.19^{a}	17.79 ± 0.58^{ce}
	10	45.34 ± 0.53^{cd}	11.01 ± 0.36^{a}	17.38 ± 0.29^{bce}
	15	44.99 ± 0.54^{c}	10.79 ± 0.43^{a}	17.54 ± 0.34^{bce}
	20	43.73 ± 0.53^{b}	10.75 ± 0.70^{a}	17.20 ± 0.36^{b}

In each column, means of 3 independent experiments ± standard deviations, the same superscript letter are not significantly different ($p < 0.05$).

The color alteration of medicinal herbs was previously discussed to involved in the formation of colored compound by Maillard reaction, non-enzymatic browning or via enzymatic oxidation of some phenolic compounds in food [34,35]. In addition, color change of irradiation samples may be occurred from oxidation of pigment, free radicles formation that induced the degradation of anthocyanin [36].

Antibacterial activity

In this experiment, the antibacterial activity was expresses in term of the minimum inhibitory concentration (MIC) values. Minimum inhibitory concentration was the lowest concentration of antimicrobial agent that will inhibit the visible of bacterial growth. In this research, we used *S. aureus* and

E. coli as a representative of gram-positive and gram-negative bacteria, respectively. The MIC of mangosteen peel extracts against *S. aureus* and *E. coli* were 0.125 and 2.0 mg/mL, respectively. Inhibition zone of gamma irradiated samples was slightly higher than x-ray irradiation samples and increasing with the irradiated dose. The diameter of inhibition zone from various crude extract concentration against *S. aureus* and *E. coli* was showed in **Tables 4** and **5**, respectively.

Table 4 Diameter of inhibition zone (cm) against *Staphylococcus aureus* and MIC of irradiated and non-irradiated mangosteen peel extract.

Sample	Concentration (mg/mL)					Minimum Inhibitory Concentration
	2	1	0.5	0.25	0.125	
G-0	1.10	1.11	1.17	0.89	0.69	0.125
G-5	1.00	1.07	1.07	0.84	0.64	0.125
G-10	1.23	1.15	1.17	0.84	0.69	0.125
G-15	1.13	1.17	1.15	0.81	NI	0.25
G-20	1.23	1.13	1.03	0.85	0.66	0.125
X-0	1.10	1.13	1.17	0.89	0.69	0.125
X-5	1.01	0.80	1.03	0.90	0.70	0.125
X-10	1.13	0.97	1.13	0.88	0.70	0.125
X-15	1.17	1.03	0.97	0.81	NI	0.25
X-20	1.17	1.16	0.97	0.89	0.67	0.125

NI = no inhibition, paper disc diameter 0.6 cm.

Table 5 Diameter of inhibition zone(cm) against *Escherichia coli* and MIC of irradiated and non-irradiated mangosteen peel extract.

Sample	Concentration (mg/mL)					Minimum Inhibitory Concentration
	2	1	0.5	0.25	0.125	
G-0	0.80	NI	NI	NI	NI	2.0
G-5	0.80	NI	NI	NI	NI	2.0
G-10	0.70	NI	NI	NI	NI	2.0
G-15	0.80	NI	NI	NI	NI	2.0
G-20	0.70	NI	NI	NI	NI	2.0
X-0	0.80	NI	NI	NI	NI	2.0
X-5	0.70	NI	NI	NI	NI	2.0
X-10	0.70	NI	NI	NI	NI	2.0
X-15	0.70	NI	NI	NI	NI	2.0
X-20	0.70	NI	NI	NI	NI	2.0

NI = no inhibition, paper disc diameter 0.6 cm.

The results indicate that the inhibition zone was depended on the concentration of crude extract and the bacterial strains. Both types of irradiated mangosteen peel extracts demonstrated nonsignificant effect on antibacterial activity against *S. aureus* and *E. coli*. In consistency, the gamma irradiation of lemon verbena, peppermint and thyme extracts at 1, 5 and 10 kGy did not affect antibacterial activity against Gram-positive (*S. aureus*, *B. cereus*, *L. monocytogenes* and *E. faecalis*) and Gram-negative (*E. coli* and *S. enterica* serotype Typhimurium) bacteria [37]. The mangosteen peel extracts express a stronger antibacterial activity against gram-positive bacteria (*S. aureus*) than gram-negative bacteria (*E. coli*). The results were in agreement with previous study of Iinuma *et al*. [38] and Sakagami *et al*. [39], who reported that gram-positive bacteria were sensitive to this extracted more than gram- negative bacteria because of the complicated of cell wall structure. The cell wall of gram-negative bacteria is comprised of thin peptidoglycan and cell wall surrounded by an outer membrane that was in turn enveloped by a capsule. The complicated structure of gram-negative bacteria resulting in the resistance to chemical substances and antibiotics [40]. The α-mangostin is the main component of xanthone in mangosteen peel which demonstrated antimicrobial activities. Thus, the ability of microbial inhibition might be depended on α-mangostin contents in accordance with our results. The α-mangostin contents and antibacterial activity were not significantly difference among 2 ionizing radiation treatments at various doses.

Conclusions

Regarding to the pharmaceutical and cosmetic safety, application of 2 ionizing radiations clearly eliminated microbial contamination. The irradiation at the dose of 5 kGy could result in the reduction of total yeast and mold 1 log cycle that passed the Thai Community Product Standard. Furthermore, phytochemical quality such as antioxidant activities, total phenolic, α-mangostin contents and antibacterial activity against *S. aureus* and *E. coli* from both ionizing radiations showed the inclination of non-significant decrease with the doses. The color was significantly decreased in lightness in both of radiation source, only gamma irradiation at 15 kGy was significantly affected on the yellowness value. Thus, the advantage of x-ray irradiation was revealed the non-significant alteration in b values, whereas gamma irradiation showed the significant change in L and b values after increasing the dose.

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References

[1] D Obolskiy, I Pischel, N Siriwatanametanon and M Heinrich. *Garcinia mangostana* L.: A phytochemical and pharmacological review. *Phytother. Res.* 2009; **23**, 1047-65.

[2] MB Osman and R Milan. *Fruits for the future 9: Mangosteen (Garcinia mangostana*). Southampton Centre for Underutilised Crops, Southampton, 2006, p. 170.

[3] YH Hong, JY Park, JH Park, MS Chung, KS Kwon, K Chung and KB Song. Inactivation of *Enterobacter sakazakii*, *Bacillus cereus*, and *Salmonella typhimurium* in powdered weaning food by electron-beam irradiation. *Radiat. Phys. Chem.* 2008; **77**, 1097-100.

[4] A Bendini, TG Toschi and G Lercker. Influence of gamma irradiation and microwaves on the linear unsaturated hydrocarbon fraction in spices. *Zeitschrift für Lebensmittel Untersuchung und For-schung A* 1998; **207**, 214-8.

[5] M Suhaj, J Rácová, M Polovka and V Brezová. Effect of γ-irradiation on antioxidant activity of black pepper (*Piper nigrum* L.). *Food Chem.* 2006; **97**, 696-704.

[6] L Samuagam, CM Sia, GA Akowuah, PN Okechukwu and HS Yim. The effect of extraction conditions on total phenolic content and free radical scavenging capacity of selected tropical fruits' peel. *Health Environ. J.* 2013; **4**, 80-102.

[7] FDA's Bacteriological Analytical Manual (BAM). Aerobic plate count and yeasts, molds and mycotoxins, Available at: https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam, accessed January 2024.

[8] KF Khattak,TJ Simpson and Ihasnullah. Effect of gamma irradiation on the extraction yield, total phenolic content and free radical-scavenging activity of *Nigella staiva* seed. *Food Chem.* 2008; **110**, 967-72.

[9] IFF Benzie and JJ Strain. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* 1996; **239**, 70-6.

[10] YS Velioglu, G Mazza, L Gao and BD Oomah. Antioxidant capacity and total phenolics in selected fruits, vegetable and grain products. *J. Agric. Food Chem.* 1998; **46**, 4113-7.

[11] GM Belbe and M Tofană. Effects of ionizing radiation on microbiological contaminants of foods. *Bull. UASVM Agric.* 2010; **67**, 178-85.

[12] Thai Industrial Standards Institute. Community Products Standards 480/2547 for dry herbs, Available at: http://tcps.tisi.go.th/pub%5Ctcps480_47.pdf, accessed January 2024.

[13] W Pewlong, S Sajjabut, S Chookaew, J Eamsiri, K Khemthong and L Maikaew. Improvement of Thanaka powder by gamma radiation: Microbial and chemical properties. *Chiang Mai Univ. J. Nat. Sci.* 2021; **20**, e2021041.

[14] JF Diehl. *Safety of irradiated foods.* 2nd eds. Marcel Dekker, New York, 1995, p. 454.

[15] R Rezanejad, M Heidarieh, SM Ojagh, M Rezaei, M Raeisiand and A Alishahi. Values of antioxidant activities (ABTS a nd DPPH) and ferric reducing and chelating powers of gamma-irradiated rosemary extract. *Radiochim. Acta* 2019; **108**, 477-82.

[16] MW Byun, HS Yook, OJ Kwon and IJ Kang. Effects of gamma irradiation on physicochemical properties of Korean red ginseng powder. *Radiat. Phys. Chem.* 1997; **49**, 483-9.

[17] M Jamshidi, M Barzegar and MA Sahari. Effect of gamma irradiation on the antioxidant and antimicrobial activities of cinnamon powder. *J. Nutr. Sci. Food Tech.* 2013; **7**, 73-82.

[18] K Alloun, O Benchabane, M Hazzit, F Mouhouche, A Baaliouamer, A Chikhoune and A Benchabane. Effect of gamma ray irradiation on chemical composition, antioxidant, antimicrobial and insecticidal activities of *Thymus pallescens* essential oil. *Acta Chromatogr.* 2019; **31**, 57-62.

[19] ER Kitazuru, AVB Moreira, J Mancini-Filho, H Delincee and ALCH Villavicencio. Effects of irradiation on natural antioxidants of cinnamon (*Cinnamomum zeylanicum* N.). *Radiat. Phys. Chem.* 2004; **71**, 39-41.

[20] J Eamsiri, S Chookaew, W Pewlong, S Sajjabut and P Orpong. Effects of irradiation on antioxidant and antimicrobial activities of *Coscinium fenestratum* (Goetgh.) Colebr. *J. Phys. Conf. Ser.* 2019; **1285**, 012006.

[21] LM Crawford and EH Ruff. A review of the safety of cold pasteurization through irradiation. *Food Control* 1996; **7**, 87-97.

[22] M Alothman, R Bhat and AA Karim. Effects of radiation processing on phytochemicals and antioxidants in plant produce. *Trends Food Sci. Tech.* 2009; **20**, 201-12.

[23] M Polovka and M Suhaj. The effect of irradiation and heat treatment on composition and antioxidant properties of culinary herbs and spices - A review. *Food Revi. Int.* 2010; **26**, 138-161.

[24] S Chatterjee, PS Variyar and A Sharma. Stability of lipid constituents in radiation processed fenugreek seeds and turmeric: role of phenolic antioxidants. *J. Agric. Food Chem.* 2009; **57**, 9226-33.

[25] KF Khattak and TJ Simpson. Effect of gamma irradiation on the antimicrobial and free radical scavenging activities of *Glycyrrhiza glabra* root. *Radiat. Phys. Chem.* 2010; **79**, 507-512.

[26] LGL Gerolis, FS Lameiras, K Krambrock and Maria Jose. Effect of gamma radiation on antioxidant capacity of green tea, yerba mate, and chamomile tea as evaluated by different methods. *Radiat. Phys. Chem*. 2017; **138**, 177-85.

[27] N Shahzad, R Elahi and S Ali. Effect of gamma irradiation on phytochemical content and antimicrobial activities of selected herbs. *Austin J. Nutri. Food Sci.* 2017; **5**, 1093.

[28] S Taheri, TL Abdullah, E Karimi, E Oskoueian and M Ebrahimi. Antioxidant capacities and total phenolic contents enhancement with acute gamma irradiation in *Curcuma alismatifolia* (Zingiberaceae) leaves. *Int. J. Mol. Sci.* 2014; **15**, 13077-90.

[29] M Adamo, D Capitani, L Mannina, M Cristinzio, P Ragni, A Tata and R Coppola. Truffles decontamination treatment by ionizing radiation. *Radiat. Phys. Chem.* 2004; **71**, 165-8.

[30] R Shigemura, DL Gerdes and WR Hall. Effect of gamma processing on prepackaged black and white pepper (*Piper nigrum* L.). *Food Sci. Tech.* 1991; **24**, 135-8.

[31] R Dhanya, BB Mishra and KM Khaleel. Effect of gamma irradiation on curcuminoids and volatile oils of fresh turmeric (*Curcuma longa*). *Radiat. Phys. Chem.* 2011; **80**, 1247-9.

[32] NS Najafabadi, MA Sahari, M Barzegar and ZH Esfahani. Effect of gamma irradiation on some physicochemical properties and bioactive compounds of jujube (*Ziziphus jujube* var. vulgaris) fruit. *Radiat. Phys. Chem.* 2017; **130**, 62-8.

[33] M Polovka and M Suhaj. Classification and prediction of γ-irradiation of ten commercial herbs and spices by multivariate evaluation of properties of their extracts. *J Food Nutr. Res.* 2013; **52**, 45-60.

[34] KG Lee and T Shibamoto. Toxicology and antioxidant activities of non-enzymatic browning reaction products. *Food Rev. Int.* 2002; **18**, 151-75.

[35] MC Nicoli, MA Casadei, ME Guerzoni and CR Lerici. Non-enzymatic browning reactions in irradiated glucose-glycine aqueous model systems. *Appl. Radiat. Isot.* 1994; **45**, 389.

[36] DM Holcroft, MI Gil and AA Kader. Effect of carbon dioxide on anthocyanins, phenylalanine ammonia lyase and glycosyltransferase in the arils of stored pomegranates. *J. Am. Soc. Hortic. Sci.* 1998; **123**, 136-40.

[37] E Pereira, AI Pimenta, L Barros, RC Calhelha, AL Antonio, SC Verde and ICFR Ferreira. Effects of gamma radiation on the bioactivity of medicinal and aromatic plants: *Mentha* × *piperita* L., *Thymus vulgaris* L. and *Aloysia citrodora* Paláu as case studies. *Food Funct*. 2018; **9**, 12.

[38] M Iinuma, H Tosa, T Tanaka, F Asai, Y Kobayashi, R Shimano and K Miyauchi. Antibacterial activity of xanthones from guttiferaeous plants against methicillin-resistant *Staphylococcus aureus*. *J. Pharm. Pharmacol.* 1996; **48**, 861-5.

[39] Y Sakagami, M Iinuma, KGNP Piyasena and HRW Dharmaratne. Antibacterial activity of α-mangostin against vancomycin resistant Enterococci (VRE) and synergism with antibiotics. *Phytomedicine* 2005; **12**, 203-8.

[40] R Merkl, I Hrádková, V Filip and J Šmidrkal. Antimicrobial and antioxidant properties of phenolic acids alkyl esters. *Czech J. Food Sci.* 2010; **28**, 275-9.