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The Effect of Gamma Radiation on Microbial Content and Curcuminoids of *Curcuma amada* Roxb. Rhizomes

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ABSTRACT

The microbial contamination in the rhizomes of medicinal plants including *Curcuma amada* rhizomes is generally high. This due to the fact that rhizomes are the bottom parts that grow in the soil. Based on the Regulation of Head of the Indonesian National Agency of Drug and Food Control Number HK.00.06.1.52.4011, the limits of microbial contamination in herbal/medicinal plants are 10^6 cfu/g for the total microbial and 2×10^4 cfu/g for the total yeast and mold. Gamma irradiation is one of the methods to reduce microbial contamination in medicinal plants. In this research, the effectiveness of gamma irradiation in microbial reduction and its effects to curcuminoid contents was determined by irradiating *Curcuma amada* rhizomes at doses of 5 and 10 kGy. The initial contamination in this rhizome was 8.78×10^7 cfu/g and 5×10^1 cfu/g for the total microbial and for the total yeast and mould, respectively. The result indicates that at 5 kGy, the microbial contamination and the mould and yeast contamination were reduced from 8.78×10^7 cfu/g and 5×10^1 cfu/g to 1.39×10^4 cfu/g and under 1×10^1 cfu/g, respectively. Meanwhile the comparison of curcuminoids between the irradiated and non irradiated samples was performed by HPLC method and was found to actually increase from 0.26% to 0.36% after the 5-kGy irradiation. It can be concluded that an irradiation dose of 5 kGy is effective to reduce the content of microorganisms without lowering curcuminoids. Gamma radiation could be used as decontamination method in medicinal plants.

INTRODUCTION

The *Curcuma* is a plant genus, belonging to *Zingiberaceae*, that has been used widely as an alternative medicine (*jamu* in Indonesian) not only in Indonesia but also in many other countries [1-3]. One of the most known species in this family is the turmeric which has already been extensively investigated worldwide. In contrast, *Curcuma amada* is not explored yet even though it has been widely used traditionally. *Curcuma amada* or mango ginger has a unique aroma that makes its fresh rhizomes consumed in sauces and pickles [4,5].

The presence of microbial contamination (bioburden) in *Curcuma amada* rhizomes probably happens because the rhizomes are mostly located under the soil surface, and also because of environmental factors such as temperature, humidity, pre-/post-harvesting conditions, and the storage condition [6]. In accordance with the requirements of the Indonesian National Agency of Drug and Food Control (NA-DFC), herbal medicines must meet several requirements such as that the total number of microbes should not exceed 10^6 cfu/g and the total number of mould and yeast should not exceed 2×10^4 cfu/g [7]. Therefore processing *Curcuma amada* rhizomes as raw materials in medicinal plants has to be prerformed properly in accordance with Good Manufacturing Practices (GMP), and the rhizomes' microbiological

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contamination level has be reduced to below the required value.

The implementation of GMP will help to reduce the initial microbial contamination in rhizomes, but if the content of contaminants in the rhizomes are higher than requirement, it is necessary to decontaminate them to reach the permissible levels of contamination limit. The use of radiation with high energy such as gamma rays is one of the methods to reduce the amount of microbial contaminants in the rhizomes (decontamination). Several advantages of irradiation techniques compared to other decontamination techniques are that it is a fast, safe, convenient, and eco-friendly method which reduces the reliance on chemical fumigants and preservatives [8,9]. The product can be irradiated in the final packaging to reduce recontamination [8]. Numerous studies indicates that gamma radiation can be considered as a microbiologically and toxicologically safe technology; that is the reason this technique has been widely used in sterilizing medicinal plants [10-12].

Curcuma mango rhizomes (Fig.1) contain curcuminoids which are consist of three main chemical substances (diarylheptanoids group), namely curcumin (C), demethoxycurcumin (DTC) and bis-demethoxycurcumin (BDTC) [13,14]. Many literatures reported that curcumin, a phenolic compound that has been identified as the major constituent of Curcuma species, posseses several biological activities in animal and has been consumed for medical purposes such as cancer, neurodegenerative diseases, diabetes, inflammatory diseases, analgesic, antimicrobial, gastric effect and hepatoprotective[15-17]. Curcumin now used as a supplement in several countries such including the United States, Korea, China, Thailand, India, Japan and Pakistan [2,19].

When *Curcuma amada* is irradiated with gamma rays, the gamma energy not only interacts with microbes but also interacts with other compounds in *Curcuma amada* , and the interaction might alter the characteristics and composition of the curcuminoids content which may reduce the eficacy of the rhizome.

Fig. 1. *Curcuma amada* rhizomes.

To our knowledge, irradiated *Curcuma amada* has not been investigated intensively. Therefore, the present study is aimed to evaluate the effect of gamma irradiation on microbial content of *Curcuma amada* and curcuminoids as well.

EXPERIMENTAL METHODS

Plant and chemical materials

Rhizomes of *Curcuma amada* (family *Zingiberaceae*) were obtained from farmers in Yogyakarta, Central of Java, Indonesia. They were analyzed at the Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia. Ethanol 96%, methanol p.a, HPLC-grade acetic acid, and HPLC-grade acetonitrile were obtained from Merck (Germany), Standard of curcuminoid was purchased from Cromadex (USA), while Sabouraud dextrose agar (SDA) and Trypticase soy agar (TSA) were obtained from Difco (France).

Irradiation of *curcuma amada* rhizome

One hundred grams of *Curcuma amada* rhizome (later indicated as samples) were sealed in polyethylene plastic bags (0.02 mm) and irradiated using gamma rays with dose of 5 and 10 kGy and dose rate of 10 kGy at room temperature. The irradiation was carried out at the Irpasena Cobalt-60 irradiator Center for Isotopes and Radiation Application (CIRA), BATAN, Pasar Jumat, Jakarta. Unirradiated samples (0 kGy) were used as control.

Microbial analysis

Preparation of stock solution

The microbial test was done by slight modification (adding Polysorbate 80, or known as Tween 80) of a microbiological analysis method [20]. Stock solutions of *Curcuma amada* were prepared by weighing 10 grams of the irradiated sample and then pouring it into 90 ml of sterile Pepton Dilution Fluid (PDF) to which 0.01% Tween 80 was already added as the suspending agent. It was then homogenized for 30 seconds to obtain a homogenuous suspension. To each 9 ml of PDF solution, one mL of suspension was then added. A series of dilutions were probably needed if the number of microbes are still high. The same procedure was done for unirradiated sample.

Total viable count (TVC)

The microbial load (viable microorganisms) of the samples was analyzed quantitatively using the pour plate technique. To obtain the viable count, 1 ml of diluted samples was transferred into duplicate sterile plates. Into each plate, 15 ml of TSA medium was added and incubated at 36°C for 24 h.The results were presented as number of colony-forming units (cfu) [21].

Total yeast and mould count (TYMC)

To determine the total yeast and mould count, 1 ml of diluted samples was transferred into duplicate sterile plates. Into each plate of 15 ml of medium, SDA was added, and then the plates were incubated for 3-4 days at room temperature (25°C). The colonies were counted and data were expressed as the number of cfu [21].

Preparation of ethanolic extract

Five hundred grams of samples were macerated using 2.5 L of ethanol and were allowed to shake then left overnight for 48 hours. The macerate was filtered and the samples were re-macerated for 4 times. All macerates were collected then evaporated using vacuum evaporator (Hahnshin Scientific Co.).

Curcuminoids analysis

Quantitative analysis of curcuminoids was done using HPLC (Hitachi L2000). Ethanolic extracts of *Curcuma amada* were injected into HPLC. The mobile phase consisted of acetonitrile 2% acetic acid (45:55, v/v) mixture. The absorbance for each solution was determined at 425 nm [22].

RESULTS AND DISCUSSION

Effects of irradiation on the microbial load

It was found that total viable bacteria of *Curcuma amada* rhizome is 8.78×10^7 cfu/g. This value exceeds the threshold that is allowed by the Indonesian National Agency of Drug and Food Control (NA-DFC) which is under 10^6 cfu/g [7]. The high bioburden of *Curcuma amada* rhizome might be due to the fact that rhizome is a part of the *Curcuma* plant that grows in the soil, which generally contains a lot of microbes.

The result of this study reveals that gamma rays reduce microbial load significantly, proportional to the dose delivered. As shown in Fig. 2, the irradiation doses of 5 kGy and 10 kGy reduce the microbial contamination to 1.39×10^4 cfu/g and 2.47×10^3 cfu/g, respectively.

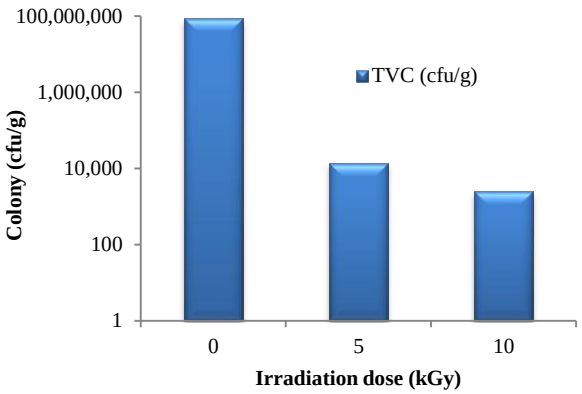

Fig. 2. Total viable count of *Curcuma amada* after irradiation.

The total yeast and mould in the unirradiated sample was 5.0×10^1 cfu/g. As shown in Fig. 3, irradiation with a dose of 5-10 kGy reduced the yeast and mould load to below 10 cfu/g. To the best of our knowledge, no report was available on the effect of gamma irradiation to the *Curcuma amada*. However, Suchandra *et al*. (2015) reported that a radiation dose of 7.5 kGy was sufficient to maintan microbial quality up to 18 months of storage [23]. From our results, it can be said that irradiation dose of 5 kGy is effective to reduce total count of yeast and mould, so it complies with the NA-DFC requirements.

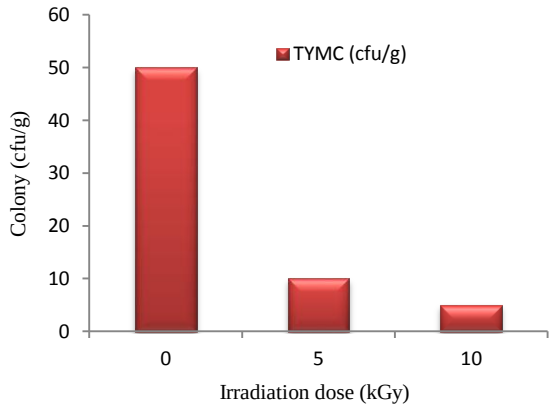

Fig. 3. Total yeast and mould count of *Curcuma amada* after irradiation.

Effect of irradiation on curcuminoids

In this study, we analyzed the curcuminoid contents of *Curcuma amada* quantitatively after exposing it to gamma rays with doses of 5 and

10 kGy. The results were then compared to the results for the unirradiated *Curcuma amada* rhizomes. From the HPLC analysis, it was found that the curcuminoid compounds in *Curcuma amada* consisted of curcumin (C), desmethoxycurcumin (DTC), and bisdemethoxycurcumin (BDTC). This results was in accordance with curcumin, desmethoxycurcumin and bisdemethoxycurcumin standards. DTC was detected as the main component in *Curcuma amada* and followed by two minor peaks, representing C and BDTC, in all samples (Fig. 4).

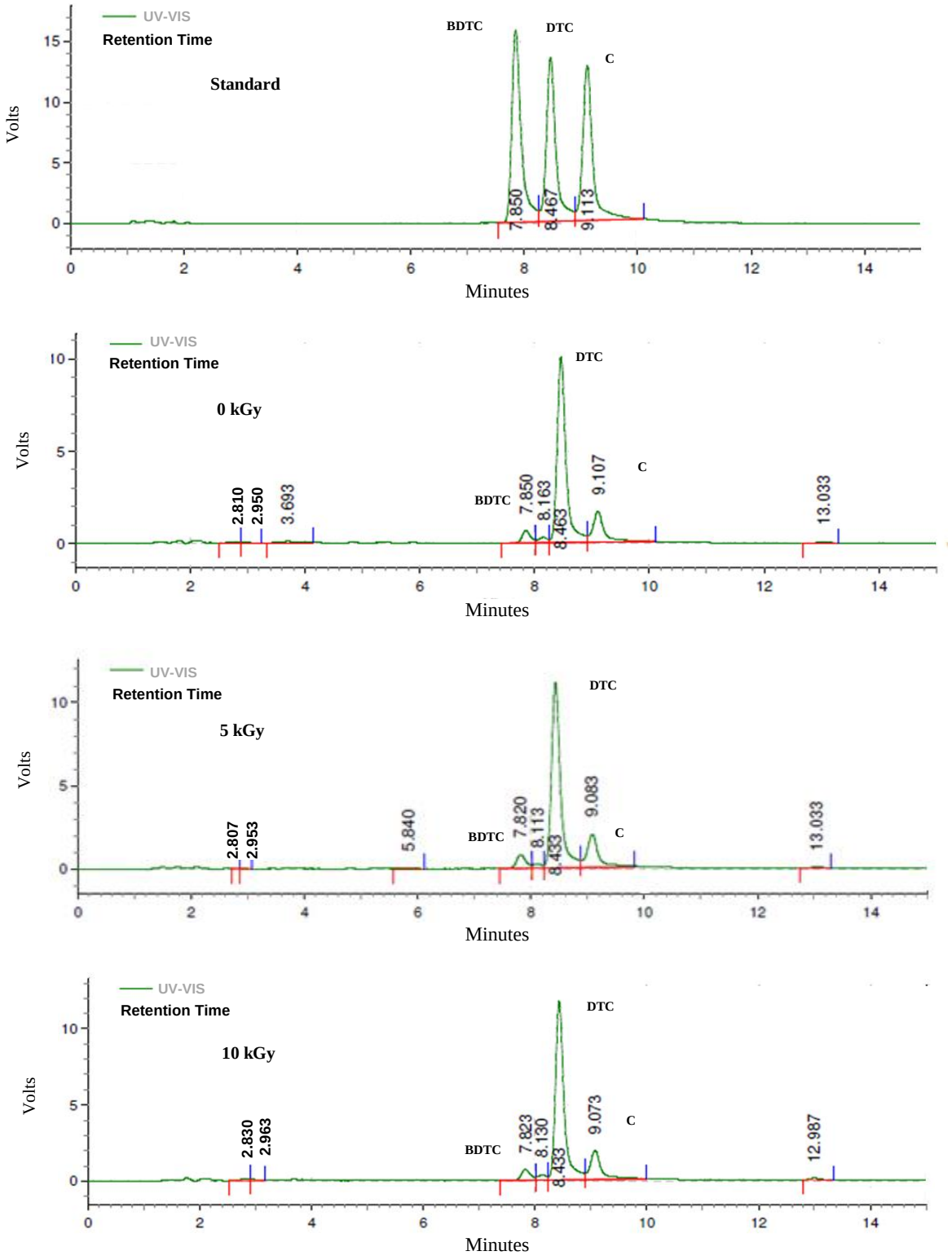

Fig. 4. HPLC profile of Curcuminoids on *Curcuma amada*. Curcumin (C), Demethoxycurcumin (DTC) and Bis-demethoxycurcumin (BDTC).

From Table 1. It seems that there is an increase in the total curcuminoids at irradiation dose of 5 kGy. Unexpectedly, irradiation at 10 kGy had no effect on total curcuminoid compared with the control. However, some studies reported that irradiation at certain doses can enchance or decrease the active components. Similar to our findings, Dhanya *et al*. (2011) reported that low doses of gamma radiation (3 and 5 kGy) on turmeric (*Curcuma longa*) slightly increased the curcuminoid content due to radiation-induced damage of the turmeric's cell membrane thus increased the extractability of medicinal compounds [24]. The increasing number of curcuminoids by radiation has an advantage because curcumin is an efficient antioxidant, as also reported by Sagirroglu *et al*. (2012) and Khalil *et al*. (2012) [25,26].

Table 1. The average of total curcuminoids of *Curcuma amada* at various irradiation doses

Irradiation Dose (kGy)	Total Curcuminoid (%)
0	0.26
5	0.36
10	0.26

According to Zhu *et al*. (2010), gamma radiation may modify some components and change the post-harvest physiology during storage at room temperature, resulting in enhanced or diminished synthesis of phenolic compounds. Thus, it explains that gamma radiation may disrupt the phenolic compounds resulting in a reduction, and/or activate the synthesis of the phenolic compounds [27]. The balance of the disruption and synthesis may depend on the irradiation dose, such as at 5 kGy which showed an increase or no change total curcuminoids at 10 kGy. However, further biochemical data are needed to explain this phenomenon. From our study, it can be considered that the irradiation dose of 5 kGy is the effective dose for *Curcuma amada*.

CONCLUSION

Gamma irradiation with dose of 5 kGy is effective to reduce microbial load in *Curcuma amada* to a mandated level of standard. The major component of curcuminoids in *Curcuma amada* is desmethoxycurcumin (DTC).

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