Improvement of Bangka’s white pepper quality using gamma irradiation technology: microbial contamination reduction

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Abstract. The improvement of Bangka's white pepper quality in term of microbial contamination and physicochemical parameters has been done using gamma irradiation. Pepper samples were irradiated using gamma rays with dose of 2 to 10 kGy, and 0 kGy as unirradiated (control). The Harwell dosimeter was used to determine the absorbed dose. To evaluate the storage effect on the Bangka's white pepper quality, the optimum irradiation dose of 8 kGy was used and peppers were then kept at room temperature (27°C) for 3, 6, 9 and 12 months. The results showed that unirradiated sample was contaminated by bacteria and mold, both of them with concentration of 10^3 up to 10^4 cfu/g, while there was no contamination of yeast, E. coli and Salmonella. The physicochemical properties such as light berries, dark-colored berries, moisture, piperine and essential oil contents were 1.7, 0.7, 11, 5.5 and 2.8 (w/w), respectively. Gamma irradiation with dose of 2, 4, 6, 8 and 10 kGy reduced the number of bacteria to 3.5×10^1, 2.5×10^1, less than 10, less than 10 and 1.5×10^1 cfu/g, respectively. While for mold, irradiation from 2 up to 10 kGy reduce the number of molds to become less than 10 cfu/g. From the dose evaluation, irradiation dose of 8 kGy was selected to evaluate the effect of storage time. It was found that storage time up to 12 months, unirradiated sample still showed contamination of bacteria from 10^3 up to 10^4 cfu/g, while the irradiated pepper showed the contamination less than 10 cfu/g. The effect of storage time on physicochemical properties showed that there is no significant change for both unirradiated and irradiated pepper even after kept for 12 months. Piperine and essential oil contents of unirradiated pepper were 5.45 and 2.4%, respectively. Irradiated pepper showed slightly higher piperine content compared to un-irradiated. It can be concluded that gamma irradiation with a dose of 8 kGy is effective to improve the quality of Bangka's white pepper while maintaining its bioactive substances (piperine and essential oil) and other physicochemical properties as well.

1. Introduction
Pepper (Piper L.) is a well-known cultivated plant in the world mostly because of its pungency and flavor. This unique characteristic of pepper is attributed to the presence of alkaloid i.e., piperine and volatile essential oils [1]. Post-harvested pepper berries are processed into two kinds of commercial peppers i.e., black and white peppers. The difference lies in the berries; black pepper uses unripe berries and the blackening of the berries was done using the enzymatic reactions, while white pepper uses ripe berries processed by peeling the outer berries skin with a method called retting [2].
Recorded since 6000 BC, Pepper has been used for food ingredients and traditional medicine. As a food ingredient, pepper was used for seasoning and obscuring the flavor of preserved meat [3]. In traditional medicine, peppers are utilized for intermittent fevers and gastrointestinal disorders [4], treatment of epilepsy [3], alleviation of pain, muscular pain and influenza [1]. Nowadays, it has been known that the bioactive compound of pepper is piperine, which has therapeutic properties such as antioxidant activity [1], [5], anti-inflammatory activity [6], bio-enhancing ability [7], [8], antiemetic and antipyretic effects [9].

Pepper is one of the important export commodity of Indonesia. In 2007, Indonesia became the second-largest exporter of pepper in the world after Vietnam and the third producer of pepper after Vietnam and India [10]. One of world-widely known Indonesian pepper came from Bangka Belitung province, which is known as “Muntok white pepper”. It has a relatively high price compared to other white pepper (in 2015, $ 14.00/kg for Muntok white pepper and $ 13.70/kg for Vietnam white pepper) [11]. Thus, Muntok white pepper has a very important role in the national pepper export commodity. However, since 2008, Indonesia’s pepper production and export have been declining due to several reasons i.e., fluctuation of pepper world price [12], low productivity, loss of harvest yield because of pests and disease, low quality of pepper and lack of transfer technology [13].

Pepper quality is assessed by its physicochemical and microbial parameters. The physicochemical properties include light berries, dark-colored berries, moisture content, piperine and essential oil contents, while microbial parameters include total bacteria count (TPC), total mold and yeast (TMY), E. coli, and Salmonella contaminations.

The objective of this research is to overcome the low quality of white peppers, specifically Bangka’s white pepper, in terms of reduction of microorganisms by using radiation technology, while it still maintains their bioactive compounds and physicochemical properties. High energy radiation (ionizing radiation) such as gamma and electron beam is an effective way to inactivate microorganisms (bacteria, fungi, mold and yeast, and virus) [14]. Radiation will reach the microbe’s Deoxyribonucleic Acid (DNA) to break down its DNA strands resulting in the inability of microorganisms to proliferate and finally leads to the death of microorganisms. Radiation reacts with the DNA through the direct or indirect process. Direct processes involve the absorption of photon energy into a target molecule, resulting in damage to the target. Indirect processes occur when photon energy is absorbed by nearby molecule water resulting in the formation of highly reactive species (radicals), which in turn react with target molecules i.e. the microorganism [15], [16]. Gamma radiation is widely used for sterilization of pharmaceutical or medical devices [17], [18]. The advantages of radiation sterilization over other sterilization methods are better penetration, better certainty of sterility, effectiveness independent of temperature and pressure conditions [19]. In addition to sterilization, radiation is used for food preservation, crosslinking or degradation of polymer materials and mutation breeding in agriculture sector.

2. Material And Methods

2.1 Materials
White pepper was obtained from several areas of Bangka District, Bangka Belitung Province. Polyethylene plastic, Harwell Red 4034LK dosimeter, thermohygrometer were used. Gamma rays from Co-60 source (Gamma Cell 220) at the Center for Isotope and Radiation Application was used for irradiating the pepper samples.

2.2 Methodologies

2.2.1 Determination of optimum dose. Bangka’s white pepper was gathered and mixed to obtain homogeneous samples. The Bangka’s white pepper was then divided into one-kilogram sealed-packed polyethylene plastic bags. Every two samples were then irradiated using gamma rays with a dose of 0, 2, 4, 6, 8 and 10 kGy under a dose rate of 5 kGy/hours. Before irradiation, six Harwell Red 4034LK
dosimeters were placed in 3 parts of samples which every part consisted of 2 dosimeters on the top surface, middle and bottom surface of the samples. The absorbed dose was then measured using a UV-Vis spectrometer at a wavelength of 640 nm.

2.2.2 Effect of storage. Ten plastic bags of Bangka’s white pepper were irradiated with the obtained optimum dose and then each two bags were kept at room temperature (27°C) for 0, 3, 6, 9 and 12 months. Ten plastic bags of unirradiated Bangka’s white pepper were also kept as a control of storage time.

2.2.3 Microbiological evaluation. The microbial contamination of Bangka’s white peppers before and after irradiation, and after stored for 3, 6, 9 and 12 months were analyzed as total bacterial count, total mold count, total yeast count, E. Coli and Salmonella. Bacteriological Analytical Manual (BAM) Methods chapter 3-18 were used for enumeration of Total Bacterial Count (TBC) and Total Mold and Yeast (TMY) [20]–[23], while Indonesian National Standard 004 2013 was used for enumeration of E.coli and Salmonella [24]. The measurement of TBC and TMY was conducted by the Laboratory for Quality Testing of Goods, Directorate of Standardization and Quality Control, Jakarta.

2.2.4 Measurement of physicochemical properties of Bangka white pepper. Physicochemical characteristics of Bangka’s white pepper such as moisture content, light berries, moldy berries, piperine content and essential oil content before and after irradiation, and after stored for 3, 6, 9 and 12 months were evaluated. Indonesian National Standard 004 2013 [24] methods were used for the characterization of the physicochemical of pepper. UPTD Balai Sertifikasi dan Pengendalian Mutu Propinsi Bangka Belitung, Pangkal Pinang conducted the measurement of physicochemical properties of pepper.

3. Results and Discussion

3.1 Determination of radiation dose and microbiological properties of Bangka’s white pepper. Gamma irradiation not only react with microorganism contained in the Bangka’s white pepper but it also reacts with other components in the product when it passed through. Therefore, in order to improve the quality of white pepper, we have to consider that the content and quality of other components in pepper especially bioactive components such as piperine and essential oil should be maintained or not degraded.

![Figure 1. Effect of irradiation dose on the total bacteria of Bangka’s white pepper.](image)

Figure 1 showed the effect of irradiation dose on the total bacteria of Bangka’s white pepper. The initial contamination (without irradiation) of Bangka’s with pepper is in the range of $10^3$ to $10^4$ cfu/g with an average of $2.3 \times 10^4$ cfu/g. It was also found that Bangka’s white pepper is contaminated with mold in the range of $1.5 \times 10^3$ to $2 \times 10^4$ cfu/g, and no contamination of yeast, E. Coli, and Salmonella. Irradiation with a dose of 2, 4, 6, 8 and 10 kGy resulted in the reduction of total bacterial content to
3.5×10^4, 2.5×10^4, less than 10, less than 10 and 1.5×10^4 cfu/g, respectively. Meanwhile, the total mold was reduced to less than 10 cfu/g for all the irradiation dose and no contamination from yeast, *E. Coli*, and *Salmonella* was found. It can be said that gamma radiation is an effective ways to inactivate microorganisms contaminated in Bangka’s white pepper. Radiation dose of 6 kGy up to 10 kGy will inactivate bacteria by 3 log count decrease.

It was reported that gamma radiation is more suitable for the food preservation method than other alternative technologies for application in solid foods. Substantial reductions in the population of *E. Coli* in milk sterilization are possible using alternative technologies such as gamma radiation; these reductions are comparable to those achieved by heating at 63 °C for 16 s. Gamma radiation with a dose of 10 kGy is effective to reduce *E. coli* in milk by 7 log count decrease and a dose of 12 kGy can inactivate *Bacillus spp.* spores in frozen yogurt by 3 log count decrease [25]. Inactivation of microorganisms (microbes) such as bacteria, mold and yeast, and viruses by radiation is due to direct and or indirect interaction of high energy photon with DNA microbes. Direct inactivation of microbes by gamma radiation is believed to be mainly caused by radiolytic cleavage or crosslinking of genetic (DNA) microbes. Indirect process occurred when photon energy is absorbed by a nearby water molecule resulting in the formation of highly reactive radicals such as OH⁻ and aqueous electrons e⁻aq and also ozone, created from the radiolytic cleavage of O₂ to O²⁻ and its subsequent reaction with another O₂ molecule. These molecules can react with the microbe's nucleic acids as well as proteins. The destruction of nucleic acids replication, via both direct and indirect mechanisms, is believed to be the major mechanism of microorganisms inactivation by gamma irradiation [26], [27].

Radiation dose referred to as absorbed irradiation dose, is the amount of energy deposited in a given mass of a medium by ionizing radiation. To determine the gamma radiation dose on the Bangka’s white pepper, a Harwell Red 4034LK dosimeter was used. The results showed that the average absorbed irradiation dose for the above samples was 2.1, 4.2, 6.2, 8.3 and 10.9 kGy, respectively. To evaluate the effect of storage time on the characteristic of Bangka’s white pepper, an irradiation dose of 8 kGy was further used.

3.2 Effect of storage time on Bangka white pepper properties.
Bangka’s white peppers were gamma-irradiated with dose of 8 kGy (average absorbed dose 8.3 kGy) and kept at room temperature for interval of 3, 6, 9 and 12 months. The physicochemical properties and microbial contamination were analyzed and the results are as follow:

a. Physicochemical properties of Bangka’s white pepper

Extraneous matter, light berries, dark color berries, and water content are among physical properties of pepper which should be complied according to Indonesia National standard (INS) for white pepper [24] and International Pepper Community (IPC) [28]. INS classify whole white pepper into 2 standard categories i.e. Grade I, and Grade II as can be seen in Table 1. In Table 2, the physical properties of Bangka’s white pepper are shown. From Table 2, we can determine that Bangka’s white pepper is categorized as Grade II, which is observed from the result of moisture content i.e., in the range of 13.25 to 14%. And it can be categorized as Grade I from the point of view of extraneous matter and light berries content i.e., less than 0.8 and 1%, respectively.

| No | Specification                   | Unit | Requirement         |
|----|---------------------------------|------|---------------------|
|    |                                 |      | Quality I         | Quality II       |
| 1  | Bulk Density                    | g/l  | Min 600            | Min 600          |
| 2  | Moisture Content (w/w)          | %    | Max 13.0           | Max 14.0         |
| 3  | Light Berries (w/w)             | %    | Max 1.0            | Max 2.0          |
| 4  | Extraneous matter (w/w)         | %    | Max 1.0            | Max 2.0          |
| 5  | Dark Coloured Berries(w/w)      | %    | Max 1.0            | Max 3.0          |
| 6  | Mould contamination(w/w)        | %    | Max 1.0            | Max 3.0          |
Table 2. Physical properties of Bangka’s white pepper before and after irradiation and storage at room temperature up to 12 months.

| Storage interval (month) | Physical properties |
|--------------------------|---------------------|
|                          | extraneous matter (w/w), max | light berries (w/w), max | moldy berries (w/w), max | dark coloured berries (w/w), max | moisture content (w/w), max |
|                          | Dose (kGy) | Dose (kGy) | Dose (kGy) | Dose (kGy) | Dose (kGy) |
| 0                        | 0.25       | 0.5        | 1.65       | 1.65       | 0.5       | 0.25       | 13.6       | 13.85     |
| 3                        | 0.25       | 0.2        | 0.35       | 0.25       | 0.0       | 0.3        | 14.25      | 14.25     |
| 6                        | 0.55       | 0.5        | 1.9        | 2.25       | 0.0       | 0.7        | 13.7       | 13.85     |
| 9                        | 0.2        | 0.75       | 1.2        | 1.15       | 0.0       | 0.5        | 13.65      | 13.55     |
| 12                       | 0.25       | 0.5        | 0.95       | 1.1        | 0.0       | 0.35       | 14         | 13.5      |

Piperine and essential oil are bioactive components of pepper. Piperine, a naturally occurring alkaloid, has numerous health effects and beneficial therapeutic properties such as antioxidant, anti-inflammatory activity, bio-enhancing ability, [1][3], [4], [5], [7]. It stimulates the circulatory system, possesses a broad-spectrum antimicrobial activity, analgesic, antipyretic action [7], It has a beneficial influence on lipid metabolism efficacy, as antidiabetic and reduces painful swelling caused by tissue injury [7].

Table 3. Piperine and essential oil content of Bangka’s white pepper before and after irradiation and storage at room temperature up to 12 months.

| Storage interval (month) | Piperine content (w/w) max | Essential oil content (w/w) max |
|--------------------------|-----------------------------|---------------------------------|
|                          | Dose (kGy)                  | Dose (kGy)                      |
| 0                        | 5.2                        | 5.45                            | 2.65 | 2.4      |
| 3                        | 5.6                        | 5.55                            | 2.65 | 2.7      |
| 6                        | 5.85                       | 5.85                            | 2.6  | 2.7      |
| 9                        | 5.55                       | 5.95                            | 2.8  | 2.75     |
| 12                       | 5.45                       | 6.7                             | 2.6  | 2.5      |

Essential oils are volatile and liquid aroma compounds from natural resources, usually plants [29]. It has been reported that plant essential oils have antioxidant, antimicrobial and anticancer, antifungal, antileishmanial and anti-Trichomonas properties [30], [31]. Piperine and essential oil content of Bangka’s White pepper are 5.45 and 2.4% as depicted in Table 3. Based on IPC,
it is classified as Grade I i.e., the minimum value of piperine is 4.0 and the essential oil is 1.5%. The high content of piperine and essential oils of Bangka’ white pepper has many benefits in the medical application as described above.

In this research, we did not determine the kind of essential oil in the Bangka’s white pepper, however, Morshed et al reported that the major component of essential oils in Chittagong pepper was caryophyllene (19.12%), limonene (9.74%), and camphene (8.44%) [30], and Rmili et al reported that the major components of essential oil of black pepper from China extracted using hydrodistillation process were 3-α-carene (31.9%), limonene (19.3%), caryophyllene (18.4%), β-pinene (13.0%), α-pinene (5.8%).

The effect of storage up to 1 year on the physicochemical properties of Bangka’s white pepper is not changed significantly. Gamma irradiation with the dose of 8 kGy did not give significant change (degrade) on the physicochemical properties of Bangka’s white pepper. It can be concluded that irradiation at 8 kGy is effective in maintaining and inactivate microorganisms in Bangka’s white pepper.

b. Microbial contamination

According to INS and IPC, white pepper has to be free from pathogenic bacteria such as E. Coli and Salmonella. However, other non-pathogenic contamination i.e., bacteria, mold and yeast, should also be considered because they can cause deterioration in pepper quality. In addition, contaminated pepper can also serve as disease transmission vehicles and cause of death if contaminated with harmful microorganisms, microbial toxins or environmental contaminants [32].

Table 4. Effect of irradiation dose of 8 kGy and storage interval up to 12 months on the microbial content of Bangka’s white pepper.

| Storage interval (month) | Total bacteria (cfu/g) | Total mold (cfu/g) | Total yeast (cfu/g) | Salmonella spp. per 25 g | E. Coli APM/g |
|-------------------------|------------------------|--------------------|--------------------|-------------------------|--------------|
| Dose (kGy) | Dose (kGy) | Dose (kGy) | Dose (kGy) | Dose (kGy) | Dose (kGy) | Dose (kGy) | Dose (kGy) | Dose (kGy) |
| 0 | 23,040 | <10 | 27,000 | 0 | <10 | <10 | <10 | negati f | negati f | negati f | negati f |
| 3 | 9,700 | <10 | 3,150 | <10 | <10 | <10 | negati f | negati f | negati f | negati f | negati f |
| 6 | 21,275 | <10 | 20,700 | 0 | <10 | <10 | negati f | negati f | negati f | negati f | negati f |
| 9 | 21,900 | <10 | 13,000 | 0 | <10 | <10 | negati f | negati f | negati f | negati f | negati f |
| 12 | 20,000 | <10 | 3,100 | <10 | <10 | <10 | negati f | negati f | negati f | negati f | negati f |

As can be seen in Table 3 that Bangka’s white pepper was contaminated with bacteria and mold with a quite high amount reaching up to 10⁷ cfu/g, while it was free from yeast, E. Coli and Salmonella contamination. Irradiation with a dose of 8 kGy resulted in the reduction of bacteria and mold contamination less than 10 cfu/g. The effect of storage time up to 12 months in the unirradiated pepper still contained high bacteria and mold contamination. To be noted that the mold contamination after 3 and 12 months storage of unirradiated pepper was relatively low compared to other storage time, this is probably due to the independent pepper batch sample meaning that this particular batch had low contamination of mold even before storage. The effect of storage time on
the irradiated pepper showed that there was no increasing contamination of all microorganisms. This result reflected that the irradiation of white pepper at the dose of 8 kGy is effective in improving the quality in term of reduction of microorganism and can be kept up to at least 12 months.

3. Conclusions
Gamma irradiation with a dose of 8 kGy can reduce microbes from $10^4$ cfu/g to less than 10 cfu/g, showing its effectiveness to improve the quality of Bangka's white pepper in term of microorganism reduction while maintaining its bioactive substances (piperine and essential oil) and other physicochemical properties as well. These bioactive substances of white pepper are a very important value in medical and pharmacy.

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