

Research Article

Radiation sterilization of Honey and Honey-Alginate Wound Dressing from Stingless Bee (*Tetragonula biroi*) Collected from Sta. Maria, Laguna, Philippines

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ABSTRACT

This study was conducted to determine the effect of radiation sterilization on alginate wound dressing containing honey from the Philippine stingless bee, *Tetragonula biroi*. Our results show that a radiation dose of 30 kGy did not affect the antibacterial property of honey against *Staphylococcus aureus*. Electron-beam irradiation did not produce significant alterations in the physicochemical properties (pH, total soluble solids, and flavonoids); however, the total phenolics was significantly increased in honey with higher irradiation doses. Demonstrating that irradiation can be applied to honey with negligible physicochemical effects, honey was incorporated in alginate and exposed to a sterilization dose of 25 kGy using an electron beam facility. Irradiation did not affect the physicochemical properties (pH, moisture content, gel fraction, moisture vapor transmission rate (MVTR), and fluid handling capacity) of the honey alginate wound dressing (HAWD). The perspectives for the potential use of irradiated HAWD as a natural product-based substitute for commercial wound care products may be considered.

Keywords: Alginate wound dressing, Radiation sterilization, Stingless bee honey.

Introduction

For thousands of years, honey has been used to treat bacterial infections [1]. The antimicrobial properties of honey and its ability to assist wound healing have been well documented. Its ability to generate hydrogen peroxide, osmotic effect, low pH, and abundances of phytochemical constituents, such as phenolic acids and flavonoids, are the possible reasons behind its antimicrobial and wound healing properties [2–4]. Honey containing wound care products are already in the market. Manuka honey is considered the most popular ingredient because of its high level of polyphenols and documented wound healing properties [5, 6]. On the other hand, stingless bee honey is also gaining a lot of attention because of its comparable properties with the Manuka honey, such as high levels of polyphenols and high antimicrobial activities [7, 8].

In the present study, we prepared alginate blend dressing combined with honey for the development of wound dressing that has both antimicrobial and wound healing properties. When formed into a wound dressing, alginate absorbs wound fluid to form a gel and provides a physiologically moist wound environment, thereby promoting rapid wound healing and at the same time minimizes bacterial infection [9]. Wound healing properties of honey alginate wound dressing (HAWD) have been studied for incisional and sutured wound healing using an animal model. Results of recent studies show that dressing promotes faster wound healing compared to the traditional hydrogen peroxide and povidone-iodine wound treatment method [10, 11]. For clinical application, it is essential to have an overall understanding of their properties and how

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sterilization conditions of HAWD affect the property of the material.

One of the well-established technologies is radiation sterilization. This process uses gamma rays and high energy electron in sterilizing health care products in their final packaging form. The recommended sterilization dose for health care products is 25 kGy. This dose was set considering the bioburden and radiation resistance of microorganisms generally present in health care products. However, in the case of health care products manufactured under clean conditions, where the bioburden is usually low, a dose of 25 kGy may be safe enough [12]. Thus, the study aims to investigate the effects of various radiation treatments on the properties of honey from the *Tetragonula biroi* and the HAWD.

Material and Methods

Collection, preparation, and irradiation of *Tetragonula biroi* honey

Tetragonula biroi honey was collected from beehives in Sta. Maria, Laguna, Philippines. Approximately, 35 grams of honey from the collected sample were placed in laminated PET-foil-PE packs and irradiated at 10, 20, and 30 kGy using the electron beam facility (1.0 – 2.5 MeV ELV-8 EB Tech Co., Korea) of the Philippine Nuclear Research Institute (Quezon City, Philippines). After irradiation, the honey samples were stored in a chiller maintained at $4 \pm 2^\circ\text{C}$.

Production of honey alginate wound dressing

T. biroi honey that was used in the production of HAWD came from the same beehives in Sta. Maria, Laguna, Philippines. To produce it, a layer of gel composed of sodium alginate that was purchased from Mioka Biosystem (a local supplier of alginate), stingless bee honey, and water were spread on top of a cotton gauze, sprayed with 2% calcium chloride (HiMedia) solution twice, and dried in an oven set at 50°C overnight. Dried dressing was cut into sizes (2"x2"), packed, and irradiated to a dose of 25 kGy for sterilization using the electron beam facility of PNRI. Honey alginate wound dressings were stored in an airtight container, at room temperature and away from direct sunlight.

Microbial analysis

The minimum inhibitory concentration (MIC) of the honey was determined using the broth

dilution method with some modification [13]. Honey samples were diluted to 50%, 25%, 12.5%, 6.25%, and 3.13% using sterile Mueller Hinton Broth (HiMedia). Ten ml from each honey dilutions were transferred to sterile test tubes aseptically. Three test tubes per dilution were used. Two tubes were inoculated with a fresh bacterial broth culture of *Staphylococcus aureus* (ATCC 6538) previously adjusted to a concentration approximately equivalent to 1×10^8 cfu/ml and the remaining tube as negative control. Bacteria were allowed to grow at 37°C for 18-24 hours. The presence of turbidity in the inoculated tubes indicates growth of bacteria. The lowest concentration of honey without turbidity (no bacterial growth) was recorded as MIC. All tubes (with and without visible bacterial growth) were streaked on a Mueller Hinton Agar (HiMedia) and incubated at 37°C for 18-24 hours. The lowest concentration of honey without visible bacterial growth was recorded as Minimum Bactericidal Concentration (MBC).

Sterility testing

Dressings were opened aseptically under the laminar flow hood. Each dressing was transferred and submerged to a sterilized soybean casein broth (TM Media) in media bottle, and incubated at $30 \pm 2^\circ\text{C}$ for a period of 14 days. After incubation, samples were observed for microbial growth. The sterility test showing positive results are those samples exhibiting detectable microbial growth. Samples with no detectable microbial growth are considered sterile after 14 days of incubation. This procedure was performed in ten replicates.

Analysis of physicochemical properties.

Total soluble solids (TSS). TSS of the honey was determined by dissolving 10 grams of honey in 30 ml of carbon dioxide-free distilled water. The solution was filtered using Whatman filter paper. Total soluble solids of the filtrate were measured using a digital Refractometer (Atago PR-32 α). This procedure was performed in triplicates.

pH. The pH of honey samples was measured using a pH meter (Eutech Instruments PC700). For HAWD, pH was measured directly from a homogenized mixture of 25% HAWD (w/v) using carbon dioxide-free distilled water. This procedure was performed in triplicates.

Total Flavonoid content (TFC). For the honey

samples, TFC was determined using the Dowd method with some modifications [14]. Twelve and a half grams of honey was dissolved using distilled water to a volume of 50 ml. The solution was filtered using Whatman filter paper. A 1.5 ml aliquot of the filtrate was mixed with 0.3 ml of 10% aluminum chloride, 0.3 ml of 1M potassium acetate and 8.4 ml of distilled water. The solution was incubated for 30 minutes at room temperature. Absorption readings at 415 nm using a spectrophotometer (OPTIZEN POP, Mecasys Co. Ltd., Korea) were taken against a blank sample consisting of 1.5 ml honey solution, 0.3 ml of water without aluminum chloride, 0.3 ml of 1M potassium acetate, and 8.4 ml of distilled water. The total flavonoid content was determined using a standard curve of quercetin ($\geq 95\%$ (HPLC), Sigma) dissolved in 80% ethanol (RCI Labscan) of different concentrations (0-50 mg/L). Total flavonoid content was expressed as mg of Quercetin Equivalence (QE)/100g of honey. This procedure was performed in triplicates.

Total phenolic Content (TPC). Total phenolic content of the samples was determined using Folin-Ciocalteu method with some modifications [14]. A 0.1 ml aliquot of the filtrate prepared following the same procedure in the determination of total flavonoid content was mixed with 0.4 ml water (DF = 5). This solution was then mixed with 2.5 ml of 0.2 N Folin-Ciocalteu reagent (Sigma-Aldrich) for 5 minutes, and 2.0 ml of 75 g/L sodium carbonate (Qualikems) was then added. The solution was incubated for 120 minutes at room temperature. Absorption readings at 760 nm using a spectrophotometer were taken against a blank sample consisting of 0.1 ml honey solution mixed with 0.4 ml water, 2.5 ml of water without Folin-Ciocalteu reagent, and 2.0 ml of 75 g/L sodium carbonate. The total phenolic content was determined using a standard curve of gallic acid (TM Media) solutions from different concentrations (0-100 mg/L). Total phenolic content was expressed as mg of Gallic Acid Equivalence (GAE)/100g of honey. This procedure was performed in triplicates.

Fluid Handling Capacity (FHC). The fluid handling capacity of the dressing was defined as the sum of the absorbency and Moisture Vapor Transmission Rate (MVTR). The FHC was examined according to BS EN 13726-1 & 2:2002 with some modification [15]. Dressing was first cut into circles having a diameter of 3.37 cm and

weighed. Dressing was then applied to a cup (modified Paddington cup) containing 10 ml of Solution A., a solution of calcium chloride and sodium chloride containing 142 mmol/liter of sodium ions and 2.5 mmol/liter of calcium ions. Cup assembly was then weighed and placed in an inverted position (Solution A in contact with the dressing) inside an airtight container containing 2 kg of freshly dried silica gel. The container was then placed inside an incubator for 24 hours at $37 \pm 2.0^\circ\text{C}$. Assemblies were removed from the incubator, allowed to equilibrate to room temperature, and reweighed to determine moisture vapor loss of the assembly. Each dressing was detached from the assembly and portions of the dressing that were not exposed to air were removed and remaining dressing was weighed to determine absorbency. MVTR was calculated based on the moisture vapor loss of the assembly as a function of the area of the dressing exposed to air. FHC was calculated based on moisture vapor loss and absorbency. These procedures were performed in eight replicates.

Moisture Content. Moisture content was determined using the oven drying method. Samples of pre-weighed dressings were placed in a dish and dried in an oven set at $100\text{--}103^\circ\text{C}$ for one hour. After drying, sample dressings were reweighed to determine weight loss, which was used to calculate moisture content. This procedure was performed in ten replicates.

Gel Fraction. The gel fraction is the fraction of insoluble materials in the dressing. This was determined by soaking the dry dressing in 40 ml Solution A repeatedly for four times in an environment maintained at $37 \pm 2.0^\circ\text{C}$. After soaking, dressing was dried and weighed. The final weight is the amount of insoluble materials in the dressing. This procedure was done in ten replicates.

Statistical Analysis

Results were expressed as mean $\pm$ standard deviation. Analysis of Variance was employed using least significant difference (LSD) as post-hoc tests at $p < 0.05$.

Results and Discussions

T. biroi is one of the stingless bee species in the Philippines. This bee is locally known as "lukot" and has abundant wild populations [16]. Stingless bees can collect more diversified bio-

Table 1. Physicochemical properties of irradiated and non-irradiated stingless bee honey

Irradiation Dose (kGy)	Total Soluble Solids (%)	pH	Total Flavonoids, (QE mg/100g)	Total Phenolics, (GAE mg/100g)
0	70.0 ± 0.0 ^a	3.3 ± 0.0 ^a	7.20 ± 0.42 ^a	67.47 ± 2.40 ^d
10	69.5 ± 0.4 ^a	3.3 ± 0.0 ^a	7.05 ± 0.15 ^a	72.16 ± 1.04 ^c
20	69.8 ± 0.5 ^a	3.3 ± 0.0 ^a	6.84 ± 0.14 ^a	79.41 ± 1.43 ^b
30	70.2 ± 0.3 ^a	3.3 ± 0.0 ^a	6.73 ± 0.30 ^a	83.48 ± 2.55 ^a

Note: Values represent means ± standard deviation. Values with different superscript along the same column are significantly different ($p < 0.05$).

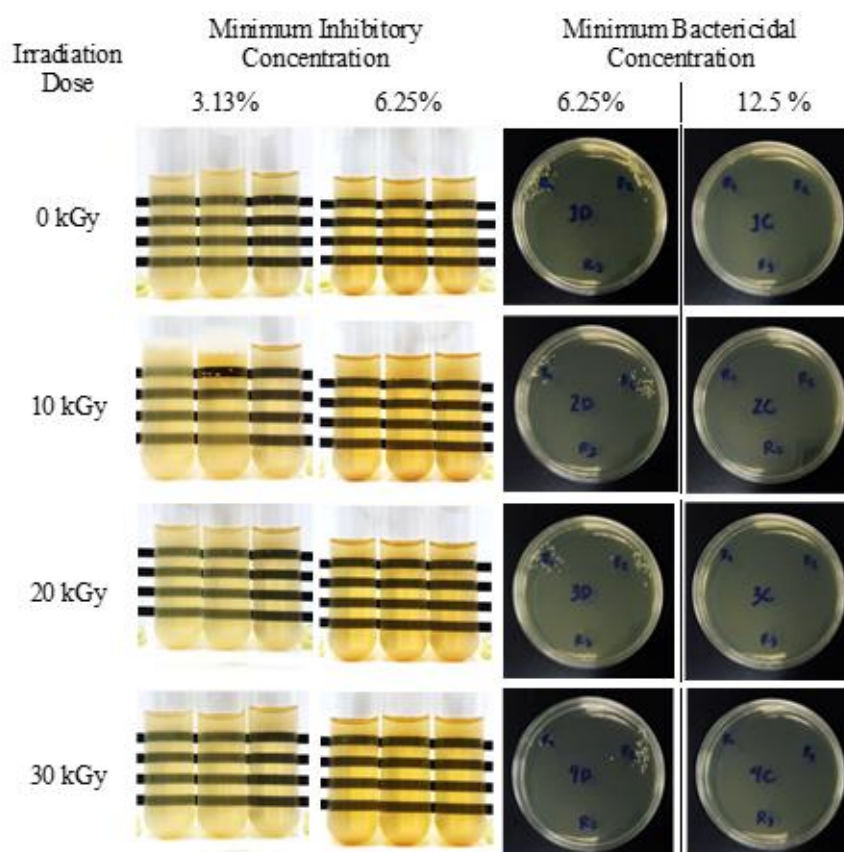


Figure 1. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration on *S. aureus* after treatment with *T. biroi* honey treated with different radiation doses

active compounds due to their small body size. This property enables them to produce honey with higher content of polyphenol and thus, with better more potent bioactivity [17]. In this study, we explored the application of *T. biroi* honey as component of alginate-based wound dressing. Since wound dressings require to be sterile in the clinical setting, we studied some properties of the honey and a derivative honey alginate wound dressing (HAWD) after exposure to various radiation doses.

The effects of irradiation on various physicochemical parameters of honey are shown in Table 1. TSS values were not significantly affected by

irradiation. These values indicate that the moisture content of the honey was around 30% (w/v), which is the typical moisture content of a stingless bee honey [18]. However, our samples' moisture content is higher compared to a previous study on stingless bee honey from the Philippines [19], which may be explained by botanical, geographic or even weather variations [20, 21]. For stingless bee honey to be qualified as good quality honey, its moisture content must not exceed 35% (w/w), based on Malaysian Standard [22]. The pH of the non-irradiated honey was comparable with that of a previous study [19]. There was also no significant change in the pH among the irradiated sam-

Table 2. Physicochemical properties of HAWD at radiation sterilization dose

Irradiation Dose (kGy)	Sterility Test	pH	Moisture (%w/w)	Gel Fraction, (%w/w)	MVTR, (g/m ² /24 hr)	FHC, (g/10 cm ² /24 hr)
0	Positive (+)	3.8 ± 0.0 ^a	6.19 ± 0.42 ^a	21.36 ± 1.37 ^a	6206 ± 83 ^a	6.50 ± 0.09 ^a
25	Negative (-)	3.8 ± 0.0 ^a	5.95 ± 0.30 ^a	22.42 ± 1.51 ^a	6278 ± 183 ^a	6.51 ± 0.19 ^a

Note: Values represent means ± standard deviation. Values with different superscript along the same column are significantly different ($p < 0.05$).

ples.

There was no significant change in our samples' flavonoid content in contrast to the result of a previous study where the flavonoid content of honey increased after irradiation [23]. This may be due to the presence of condensed tannins in their honey sample that degraded into smaller flavonoid molecules after irradiation [24, 25].

In contrast to the effect seen with flavonoid content, irradiation significantly increased the total phenolic content. Our results agree with the previously observed effect of radiation on honey samples from Malaysia. This observation may have been attributed to the radiolytic degradation of hydrolysable tannins, thereby increasing the total phenolic content in the samples [23, 26].

The radiation sterilization profile of *T. biroi* honey is shown in Figure 1. There was no significant turbidity at 6.25% (w/v) honey dilution in the tubes. Growth of *S. aureus* was no longer present at 12.5% (w/v) honey dilution.

Regardless of radiation dose, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of honey were 6.25% and 12.5%, respectively. The antimicrobial activity is comparable with stingless bee honey from Ethiopia, which showed similar values for MIC and MBC against *Staphylococcus aureus* (ATCC 25923) [27].

Given that the 0-30 kGy radiation dose range did not adversely affect the physicochemical and antimicrobial property of *T. biroi* honey, we incorporated the natural product in an alginate matrix. The HAWD, which was previously shown to have wound healing properties [10, 11], was exposed to a sterilization dose of 25 kGy using the same electron beam facility that was used in the irradiation of honey.

Sterility test shows that non-irradiated dressings were initially contaminated with microorganisms. This, therefore, limits the clinical utility of the HAWD since it can exacerbate infection of the wound bed. These contaminants must be removed to have sterile dressing that is safe for its intended

application [12]. After exposure to 25 kGy, the material became sterile. Notably, at the sterilization dose of 25 kGy, the physicochemical properties of HAWD remained unaltered (Table 2).

The in vitro moisture vapor transmission rate and the fluid handling capacity of the sterile HAWD were 6278 ± 183 g/m²/24 hr and 6.51 ± 0.19 g/10 cm²/24 hr, respectively. These values indicate that the fluid handling property of the HAWD is comparable to a hydrophilic polyurethane foam dressing that belongs to the category of extremely high permeable dressings and most suitable to be used for heavy exudate wounds [28, 29].

Conclusion

Honey alginate wound dressing (HAWD) for heavy exudate wounds can be developed using honey from *T. biroi*. The antimicrobial activity against *S. aureus* and most physicochemical properties of honey at various irradiation doses remained unaltered. Electron beam sterilization at 25 kGy is sufficient to remove microbial contamination in the HAWD while avoiding any significant effect on the physicochemical properties. The increased phenolic content as a result of radiation sterilization is noteworthy and future studies to investigate the radiolytic species is warranted.

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