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Utility of UV-C radiation as anti-*Salmonella* decontamination treatment for desiccated coconut flakes

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ABSTRACT

This study established the ultraviolet-C (UV-C)-mediated reduction of a cocktail of *Salmonella enterica* serovars, artificially inoculated onto desiccated coconut flakes. Inoculated cells exhibited biphasic inactivation behavior, characterized by an initial, log-linear population reduction, followed by a slower log-linear population decline where sublethal injury accumulated. Decimal reduction times in the faster inactivation phase (D_{fast}) ranged from 0.65 to 0.82 min, equivalent to UV-C energy dose of 86.58 −109.22 mJ/cm^2. The D_{slow} values ranged from 21.19 to 24.21 min, equivalent to energy dose of 2822.51 −3224.78 mJ/cm^2. A total of 3-log cycles reduction in inoculated *Salmonella* were observed after 40 min exposure of desiccated coconut to UV-C. Further, this 40-min process resulted in changes in the Hunter L^*, a^* and b^* color parameter values, but were not detected by a test consumer panel as evident in the non-significant difference in the color acceptability of UV-C treated and untreated coconut flakes. The UV-C process also did not affect the general acceptability of baked coconut macaroons made from UV-C treated coconut flakes. The results obtained in this work may serve as baseline information in the development of an in- or post-process integration of a UV-C radiation step against *Salmonella* spp. in the desiccated coconut production process flow.

1. Introduction

Coconut products are among the chief export products of the Philippines (Alave, 2011). There has been an increased interest in coconut liquid endosperm-based beverages in many parts of the world due to rising consumer demands for food products with potential health benefits. Since the compositional and physico-chemical properties of coconut liquid endosperm are conducive for microbial survival and growth; and handling and processing may easily introduce microbial hazards to raw materials, a number of studies have been conducted in our lab, involving traditional and emerging food processing technologies to ensure safety and quality of such commodities (Gabriel, 2015; Gabriel, Aguila, & Tupe, 2015; Gabriel & Arellano, 2014; Gabriel & Estilo, 2015; Gabriel & Pineda, 2014; Gabriel & Salazar, 2014).

Other chief export commodities of the Philippines are products obtained from the solid endosperm or coconut meat, most commonly, desiccated coconut flakes. Desiccated coconut is defined by the Codex Alimentarius (2011) as a product prepared from the white kernel of whole coconut (*Cocos nucifera* L.), and processed by paring, pasteurizing, drying, comminuting, and sifting operations. It is used mainly in the bakery and confectionary industries (Kumar, Senanayake, Visvanathan, & Basu, 2003). Microbiological standards for desiccated coconut require that the product is free from *Salmonella* and *Staphylococcus* (Bureau of Product Standards, 2007; Food and Drug Administration, FDA Philippines, 2013b). The complexities in its production process, however, make it prone to contamination with these microorganisms. Possible sources of contamination in the process include inferior quality of raw materials, mishandling during transport and storage, and unclean equipment and factory conditions during manufacture (Kumar et al., 2003). Unfortunately, there have been recent cases of Philippine desiccated coconut being confirmed positive for the presence of *Salmonella*, which were only detected when the products were already in the importing countries (FDA Philippines, 2013a; Rapid Alert System for Food and Feed, RASFF, 2007).

Thermal processing remains the most widely used technology

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to ensure microbiological safety, but its application may cause severe physical and chemical changes that negatively affect sensory and nutritional quality (Basyal, Molva, & Unluturk, 2013; Rawson et al., 2011). Thus, demand for alternative processing techniques, which can inactivate microorganisms but maintain the quality of the product, have been increasing in recent years. Ultraviolet-C (UV-C) light, in particular, has been shown to have lethality effects on bacteria, yeasts, molds, and viruses, and as such is approved for the treatment of food surfaces and some food systems (Basyal et al., 2013). This is due to the ability of UV-C light to penetrate through the cell wall, blocking DNA transcription and replication, thus eventually halting the microorganism's ability to grow and reproduce (Azimi, Allen, & Farnood, 2012; Chun, Kim, Lee, Yu, & Song, 2010). It is a promising technology for food preservation and safety because it is cheap and easy to use, does not generate large amounts of chemical residue, and is lethal to most microorganisms (Basyal et al., 2013). Gayán, Serrano, Pagán, Álvarez, and Condón (2015) explained that in order for the UV-C technology to be accepted by and transferred to the industry, the need to further understand UV-C resistance and inactivation behavior of pertinent foodborne pathogens must first be addressed. Moreover, as with other food processing techniques, UV-C treatments may also result in physicochemical changes, emphasizing the need to further investigate the effects of this process on quality (Pala & Toklucu, 2013).

This report presents works conducted to evaluate the utility of UV-C radiation as a decontamination treatment against *Salmonella enterica* serovars in artificially inoculated desiccated coconut flakes. Specifically, the study characterized the inactivation behavior of a cocktail of *S. enterica* serovars in UV-C treated desiccated coconut. The effects of UV-C treatment on the color quality of desiccated coconut were also measured objectively and subjectively through instrumental analyses using a colorimeter and sensory evaluation that employed a consumer panel, respectively. The treated desiccated coconut flakes were used to prepare baked coconut macaroons, which were similarly subjected to consumer acceptability test. The results obtained in this work may serve as basis in planning for integration of a UV-C radiation step against *Salmonella* spp. in the desiccated coconut production process flow.

2. Materials and methods

2.1. Decontamination of background microflora of desiccated coconut

All desiccated coconut samples used in this study were obtained from a local supplier (Fiesta Desiccated Coconut; Fresh Fruit Ingredients Inc., Makati City, Philippines). The coconut flakes were produced from mature coconut meat of about 12 mos. Prior to inoculation, the samples were subjected to decontamination by moist heat under pressure. The choice of decontamination procedure was based on the results of an initial study that compared the efficacies of decontaminating processes as applied to the desiccated coconut sample (data not presented). In the decontamination process, the samples were placed in glass media bottles, and heated in a pressure canner (Hall America Pressure Canner/Cooker Model No. 930, USA) to 121 °C at 15 psi for 20 min.

2.2. Test organisms

The study utilized a cocktail of seven strains of *Salmonella enterica*, which included American Type Culture Collection (ATCC) serovars Typhimurium (ATCC 14028), Diarizonae (ATCC 12325 and 29934), and Abortuse Equi (ATCC 9842). Furthermore, Hiroshima University, Laboratory of Food Microbiology and Hygiene Culture Collection *S. enterica* serovars Montevideo and Infantis were also tested. The study also tested *Salmonella* serovar Enteritidis strain B11, obtained from the Hiroshima City Institute of Public Health. A cocktail was used to simulate the multi-strain contamination that occurs in nature, and to account for inter-strain variation in UV-C resistance.

Working cultures were prepared by obtaining loops of inoculum from nutrient agar (NA, Hi-Media, Mumbai, India) stock cultures, and activating by transferring into 10 mL nutrient broth (NB, Hi-Media) and incubating at 37 °C for 24 h. Each of the activated cultures was separately enriched by transferring loops of inoculum to a new set of NB tubes and incubating for another 24 h. Working culture slants were finally prepared by transferring loops of inoculum from each enriched cultures into NA slants, and incubating for another 24 h. The working culture slants were subjected to refrigerated storage until use.

2.3. Salmonella propagation and artificial inoculation of desiccated coconut

Prior to inoculation, individual *Salmonella* working cultures were activated and enriched, following previously described protocols (Gabriel, 2015). A cocktail of inoculum consisted of the test seven strains was prepared by combining equal volumes of each enriched culture in a sterile glass tube. One ml aliquot of the cocktail was then subjected to centrifugation at 6000 rpm for 20 min to harvest the cells from the growth medium. After centrifugation, the supernate was decanted, and the pelleted cells were suspended in 1 mL sterile 0.85% NaCl (RCI Labscan, Bangkok, Thailand).

The spray inoculation method used in the study was adapted from Lang (2003) and Musgrove et al. (2010) with slight modifications. One ml of the saline cell suspension was transferred into a sterile manual piston atomizer and sprayed onto the surface of 100 g desiccated coconut sample, spread on a sterile aluminum tray. The atomizer was used at approximately 5–8 cm above the surface of the sample. In between sprays, the desiccated coconut was manually mixed with a sterile spatula. Inoculated samples were dried for 30 min in a laminar flow hood. By the end of the drying process, a final population of 5.5–6.0 log CFU/g was inoculated on the desiccated coconut flakes.

2.4. Ultraviolet-C exposure and survivor enumeration

The inoculated desiccated coconut was distributed into sterile Petri dishes (Phoenix Biomedical, Ontario, Canada), with 2 g of sample per dish, and a thickness of approximately 3 mm. The inoculated samples were then exposed to UV-C radiation using a fabricated UV-C box with a mounted UV light source (Toshiba Lighting and Technology Corp., Tokyo, Japan), characterized by a predominant emission of 254 nm radiation at a lamp-to-sample distance of 15 cm. The UV-C source was previously subjected to optical emission spectroscopy that confirmed predominant radiation emission of 254 nm at the treatment distance applied to desiccated coconut (Fig. 1). Measurements were done using a Spectrometer (Ocean Optics, Inc., FL., USA) with a dispersion of 0.2467 nm per pixel and an optical resolution of 1.0855 nm in the range of 200–1100 nm.

The 2.0 g samples in individual Petri Dishes were exposed to UV-C radiation for 0–40 min. After exposure to the predetermined time, 18 mL of sterile 0.1% peptone water (PW, Hi-Media) was added to the sample in the Petri dish, and transferred into a stomacher bag with side filter, and manually pummeled by hand for 30 s. The suspension was then subjected to serial 10-fold dilution with peptone water, after which 0.1 mL was obtained from appropriate