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Contributed Paper

Effect of γ-irradiation on Microbiological, Biochemical and Sensory Qualities of Commercial Powdered Cocoa Beverage Premix

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ABSTRACT

Low-dose gamma irradiation has been considered effective for improving the microbiological safety of fresh and processed foods. In the present study, γ-rays in the dose-range of 0-15 kGy were used for microbial decontamination of commercially available, ready-to-use powdered cocoa beverage premix. Premix was artificially inoculated with *Aspergillus oryzae*, *Saccharomyces cerevisiae* and *Bacillus cereus*. A complete inactivation of the tested microbes was observed after treatment with 10-15 kGy in inoculated and with 2.5-5 kGy in non-inoculated commercial cocoa beverage premixes. Thiobarbituric acid (TBA) values increased linearly with the magnitude of radiation dose. The peroxide value, amino nitrogen content and protein solubility were not significantly affected by the γ-irradiation. The overall sensory quality of irradiated premix was not significantly affected with the radiation doses less than 10 kGy. The results indicated that low-dose γ-radiation (up to 10 kGy) can be used to improve the microbiological quality of powdered cocoa beverage premixes before being loaded into vending machines.

Keywords: cocoa beverage premix, gamma-irradiation, microbial inactivation, physicochemical property, sensory quality

1. INTRODUCTION

Irradiation has been considered as an effective sterilization method to extend food products shelf life without compromising their nutritional properties [1, 2]. One of the distinct advantages of irradiation of food is that product temperature does not increase with irradiation [3, 4]. Also, it is a safe, cost-effective method having capacity to reduce waste and improve productivity. Irradiation of food does not leave harmful substances and original flavor and biochemical quality of the food are not affected [5, 6]. Moreover, the product is available for use immediately after irradiation. However, certain disadvantages include: detrimental effects of irradiation on odor, flavor and color have been major roadblocks for effective use of this technology to extend shelf life and reduce pathogen loads of fresh meat [7].

The gamma radiation can non-invasively penetrate the product packaging and pass through the product itself, even if the product has a complex physical shape. Gamma rays have a large penetration depth of about

80 cm for radiation processing. Exposure to gamma radiation doses below 10 kGy is effective in enhancing food safety through the inactivation of pathogenic microorganisms such as *Salmonella* and *Campylobacter;* and in extending the shelf-life of food by eliminating the microorganisms responsible for food spoilage [8, 9].

Ionizing power of gamma rays is the basis for sterilization, because it disrupts the chemical bonds in DNA molecules and prevents cellular division. As a result, the cells of a microorganism exposed to a large enough dose of gamma radiation will not be able to produce critical proteins or enzymes, will not be able to propagate and eventually die. Gamma radiation processing is, therefore, generally characterized by its deep penetration, low dose rates, and effective destruction with minimal temperature effect of microorganisms and bacteria in products already sealed inside their final packaging. The irradiated product then remains sterile until the packaging is removed for final use. According to the *Codex Alimentarius* Commission, foods irradiated below 10 kGy present no toxicological hazard, and treatment levels below this level are commonly used throughout the world [10].

Ideally, dry food storage area should have a humidity level of 15% or less, and the temperature should be between 50°F (10 °C) and 70°F (21 °C). Warm and humid climates shorten the shelf life of foods by encouraging microbial growth. In hot drinks vending machines, the average temperature inside the cabinet is generally around 74.3-76.0 °C [11]. And, the presence of a variety of microorganisms (either as vegetative cells or spores) in the vended drinks and the powders has been reported [11]. Bacterial food poisoning, especially with *Bacillus cereus*, from a hotdrinks vending machine has also been reported [12, 13]. Nelms et al. [12] have shown that spores in powdered drink mix were the source of bacterial contamination.

Using a variety of advanced oxidation processes, including gamma irradiation, microbial vegetative cells and spores can be inactivated. However, for decontamination of dried food ingredients, treatment with ionizing radiation has been found more suitable than others [14]. Therefore, in the present study, we propose that prior exposure of powdered beverage premixes to gamma radiation before being loaded into vending machine could be effective in controlling microbial contamination. Accordingly, ready-to-use powdered cocoa beverage premix samples were chosen and artificially inoculated with different microbial strains, and subsequently treated with gamma radiation. Experiments were also conducted to determine the effect of gamma radiation on the physicochemical and sensory qualities of the product.

2. Materials and Methods

2.1 Samples and Microorganisms

Ready-to-use powdered cocoa beverage premix (Dongil Food, Gimpo, Korea) was purchased from local suppliers. The standard cultures of *Aspergillus oryzae, Saccharomyces cerevisiae* and *Bacillus cereus* were purchased from Korean Culture Center of Microorganisms (KCCM).

2.2 Determination of Aerobic Counts in Premix

Total aerobic microorganisms in cocoa beverage premix were determined on Plate Count Agar (PCA) by the pour plate method.

2.3 Microbial Inoculation

Spiking study was conducted to determine the inactivation efficiency of different doses of γ-rays on vegetative microorganisms. Cocoa beverage powder was artificially inoculated using three different microbial species, namely *Aspergillus oryzae, Saccharomyces cerevisiae* and *Bacillus cereus.*

B. cereus and *S. cerevisiae* were cultured using nutrient broth under aerated (shaking) conditions at 37 °C for 48 h. Thereafter, cells were harvested by centrifugation at 2000 x g for 5 min, washed with sterile distilled water, and mixed with skim milk powder. Finally, the mixture was freeze-dried. *A. oryzae* was inoculated to steamed rice after cooling and incubated at 37 °C for 72 h. Thereafter, the obtained rice coated with mycelium was freeze-dried and ground to a fine powder and then used for irradiation experimentation. All the used microbial cells for irradiation purpose were in late vegetative state.

2.4 γ -Irradiation

Samples (10 g) were individually packed in polyethylene (PE) bags (25 μm), sealed and irradiated with 2.5, 5.0, 7.5, 10 and 15 kGy gamma rays separately at room temperature (25 °C) using the gamma-irradiation facility of Greenpia Technology Inc. (Yeoju, Gyeonggi-do, Korea). Cobalt-60 (^{60}Co) was used as gamma ray source for the irradiation. Samples were irradiated to achieve uniform target doses. Non-irradiated samples were served as control (0 kGy).

2.5 Kinetic Modeling of Microbial Inactivation

The inactivation pattern of microorganisms exposed to lethal agents can be explained by a first-order kinetic model.

$$\log \frac{N_0}{N} = \frac{k}{2.303} \cdot t$$

where N_0 is initial microbial population, N is microbial population at time t, t is exposure time (min), and k is inactivation rate constant.

2.6 D_m-value Calculation

Decimal reduction dose of gamma radiation D_m=Radiation Dose/ ($\log N_0 - \log N$). Where N_0 is the initial number of organisms, and N is the number of organisms surviving the radiation dose.

2.7 Enumeration of Viable Count

Viable counts of different microorganisms in all the tested samples (non-irradiated and irradiated) were determined by using standard plate culture method [15]. One gram of sample was added to 9 mL of sterile saline solution in a sterilized plastic bag and treated with a stomacher. An aliquot of 1 mL was transferred for making serial dilutions. One hundred microliter of each diluted sample was spread on selective media agar plates for each microorganism. For *B. cereus* enumeration, MYP agar was used. For *S. cerevisiae,* malt extract agar and for *A. oryzae,* PDA containing 0.005% rose bengal was used. Petri plates were incubated at 37 °C for 24 h in the case of *B. cereus* and 36 h in the case of *S. cerevisiae* and *A. oryzae.*

2.8 Biochemical Characterization

Nitrogen content of premix was determined by micro-Kjeldahl method [16]. Protein solubility was tested by adding 45 ml of distilled water to 0.5g sample [17]. Then, pH was adjusted to 7.0 with 0.1N NaOH and the total volume made up to 50 ml with distilled water. Thereafter, sample was well mixed and the supernatant was collected after centrifugation (20,000 x g for 15 min). Finally, it was diluted 10 times and optical density was measured at 280nm.

Acid value was determined according to a standard method of Korean Food Standards Codex [15]; peroxide value was determined according to AOAC method [18]; TBA analysis was carried out using a distillation method [19]. Total carbohydrate content was measured by Phenol-sulfuric acid method [20]; and reducing sugar content was measured by DNS method [21].

2.9 Sensory Evaluation

Irradiated premix samples were evaluated by twelve trained tasters, males and females, 20-30 years of age (Students of Dept. of Food Science & Biotechnology, Gachon University, Korea). Tasters were asked to indicate how much they liked or disliked each sample on a 9-point hedonic scale (9 =like extremely; 1=dislike extremely) according to flavor, taste, mouth-feel and overall acceptability characteristics.

For the statistical analysis of sensory evaluation data, the one-way ANOVA test and Duncan's multiple range tests were performed with SAS software, version 9.2, (SAS Institute Inc., Cary, NC).

3. Results and Discussion

3.1 Pattern of Microbial Inactivation in Artificially Inoculated Samples

As shown in Figure 1, a significant, radiation dose-dependent reduction of counts of all the three types of tested microorganisms inoculated in powdered cocoa beverage premix was noted. The inactivation of microorganisms followed the first-order reaction kinetics. In general, microbial inactivation follows first-order kinetics. Therefore, it can be characterized by a single rate constant 'k' or its reciprocal, the *D*-value (decimal reduction time), which is considered a measure of resistance to an applied lethal agent [22].

Aspergillus oryzae seems to be more susceptible to gamma rays as it showed the lowest D_m-value (1.127 kGy) among the three microorganisms, followed by *Saccharomyces cerevisiae* (D_m-2.599 kGy) and *Bacillus cereus* (D_m-3.177 kGy). Especially, *B. cereus*, which is a common food poisoning microbe that has been detected in automatic beverage vending machines, showed the greatest resistance to the gamma irradiation among the inoculated microorganisms.

Radiation dose-dependent inactivation of inoculated microorganisms was observed, as shown in Figure 2. Total inactivation of *Bacillus* and S*accharomyces* spp., but not *Aspergillus* was noted at 10 kGy radiation dose. However, all the tested strains were completely inactivated at 15 kGy dose. It was found that gamma rays at the radiation dose of 2.5 kGy were sufficient enough for effective inactivation of aerobic microorganisms in cocoa beverage premix in original sample (without microbial inoculation) (data not shown). Aerobic count in original cocoa beverage premix was 4.0 log CFU/g. Thus, it can be concluded that low-dose (< 5 kGy) gamma irradiation would be sufficient to achieve complete inactivation of total aerobic count.

Radiation dose in the range of 1.5-5.0 kGy, under certain conditions, has been shown to be effective for control of many foodborne parasites [23]. In another study, irradiation

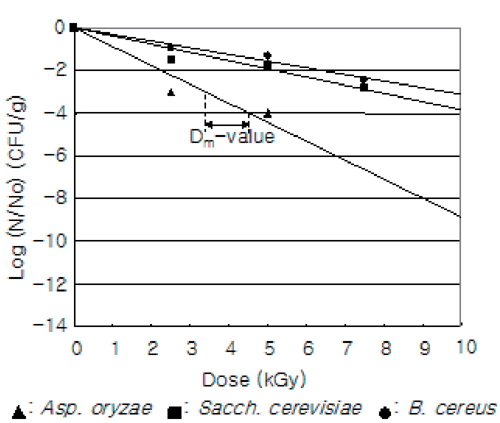

Figure 1. Inactivation kinetics of inoculated microorganisms in cocoa beverage premix upon irradiation.

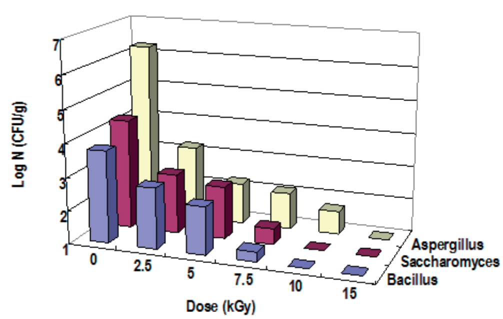

Figure 2. Dose-dependent effects of gamma radiation on inoculated microorganisms in powdered cocoa beverage premix.

at doses range between 2.0 and 3.0 kGy has been shown to reduce the total viable counts of pre-packaged vegetables, ready-to-eat meals and fruits, to levels below the detection limit of 100 cells/g [24].

Gamma irradiation causes oxidative stress and affects biomolecules by causing conformational changes, oxidation, rupture of covalent bonds, and formation of free radicals [25]. The hydroxyl (HO•) and superoxide anion ($O_2^{\bullet -}$) radicals that are generated by radiation could modify the molecular properties of the proteins and lipids causing oxidative modifications of the proteins and lipid peroxidation. Chemical changes of the proteins caused by gamma irradiation are fragmentation, cross-linking, aggregation and oxidation caused by oxygen radicals which are generated by water radiolysis [26].

3.2 Biochemical Composition of Cocoa Beverage Premix

As shown in Table 1, the powdered cocoa beverage premix samples (non-irradiated) are rich in sugars. Among the total sugars, reducing sugars comprise nearly 50 percent. However, the powdered cocoa beverage premix samples have low protein and fat contents. Thiobarbituric acid (TBA) values, the extent of lipid oxidation, of these mixes are relatively low. Free acid and peroxide values (POV) - the former is a shelf life indicator test for hydrolytic rancidity and the latter an indicator for autoxidation - are in the normal expected range.

Table 1. List of ingredients and biochemical composition of cocoa beverage premix.

Major ingredients	Glucose (39.39%) Coffee cream (13.13%) Cocoa powder (7.88%)
Moisture (%)	5.09
Ash (%)	2.36
Protein (%)	1.75
Fat (%)	1.59
Total sugar (%)	80.49
Reducing sugar (%)	48.61
Total dietary fiber (%)	12.17
Amino nitrogen (mg %)	16.83
TBA value (mg malondialdehyde/kg)	0.210

3.3 Biochemical Changes Upon Irradiation

3.3.1 pH and TBA values

Powdered cocoa beverage premix samples were treated by gamma radiation at doses up to 15 kGy. The initial pH values of these samples were within the range 7.0 to 7.5. A slight but insignificant decrease of initial pH was observed in the tested samples upon exposure to gamma rays, as shown in Figure 3.

In contrast to the pH values, thiobarbituric acid (TBA) values of the beverage premix were increased upon gamma irradiation in a radiation dose-dependent manner, as shown in Figure 3. The initial TBA value of the premix was around 0.5 mg malondialdehyde/kg. A linear increase of TBA values was observed upon exposure to radiation doses of 5, 10 and 15 kGy. It clearly indicates that lipid oxidation in the samples was increased with increasing radiation dose. And it seems that TBA value and radiation dose is in proportional relationship. The increased lipid oxidation could be due to very intense alterations in the structure of

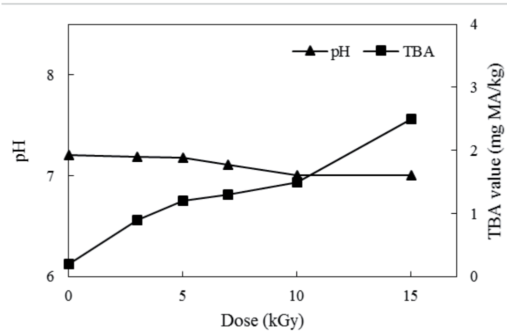

Figure 3. pH and TBA values of the cocoa beverage premix upon gamma irradiation.

the lipids from gamma irradiation. Earlier, it was found that the alterations in the molecular structure of the milk fat undergoing irradiation in the doses from 1 to 2 kGy were small and that in the doses of 3 and 4 kGy destruction of the lipids took place [27].

3.3.2 Peroxide value (POV) and acid value

There was an insignificant increase in lipid peroxide value of cocoa beverage premix upon irradiation, as shown in Figure 4. Oxidation of an unsaturated oil or fat takes place via the formation of hydroperoxides. And these are subsequently decompose into volatile secondary oxidation products, the majority of which have unpleasant odours. Hydroperoxides are an important aspect of rancidity development though they have no off-flavors [28].

As shown in Figure 4, compared to non-irradiated samples, insignificant decrease in the acid value of cocoa beverage premix was observed after 5 kGy dose irradiation. At radiation dosage of 7.5 and 10 kGy, acid values of the cocoa beverage premix were increased, compared to non-irradiated sample. Acid values (mg KOH/g) of 9.5 and 10.3 were observed for at 7.5 and 10 kGy dose irradiation, respectively. However at 15 kGy a slightly low acid value was noted, compared to 10 kGy irradiation. Overall, acid value variations were not significant at all the tested radiation dosages, compared to non-irradiated sample.

Radiation dose-dependent increase of free amino nitrogen contents was observed in the cocoa beverage premix, as shown in Figure 5. The observed increase in free amino nitrogen content due to ionizing radiation exposure is in agreement with earlier reports [29, 30]), wherein dose-dependent increases of amino acid content have been observed in germinating seeds.

Compared to non-irradiated samples, a slight but insignificant changes were observed in the protein solubility of gamma-irradiated samples, as shown in Figure 5. In contrast, however, a decrease of protein solubility due to proteins aggregation was observed in irradiated soybean protein [31].

3.4 Sensory Properties

Flavor quality of cocoa beverage premix was decreased significantly ($p < 0.05$) with increasing the radiation dose, as shown in Table 2. Up to 5.0 kGy, changes in flavor characteristics were mild. However, taste and overall acceptability were significantly negatively affected only over 10 kGy dose. However, mouthfeel of premix powder did not alter significantly ($p > 0.05$) upon irradiation over the tested doses (0-15 kGy). Therefore, gamma rays at a dose of 10.0 kGy

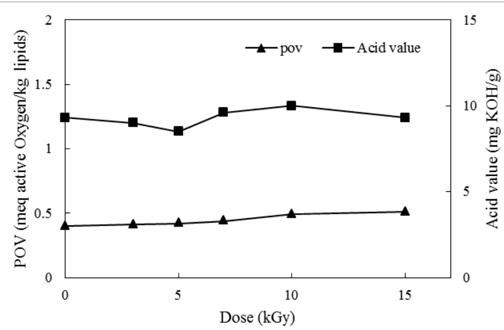

Figure 4. Effect of gamma irradiation on peroxide value (POV) and acid value of the cocoa beverage premix.

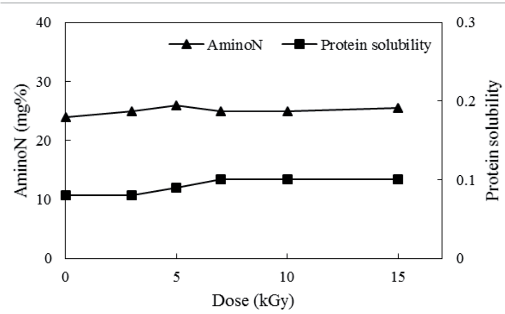

Figure 5. Effect of gamma irradiation on amino nitrogen content and protein solubility of the cocoa beverage premix.

Table 2. Sensory scores* of irradiated cocoa beverage premix.

Dose (kGy)	Flavor	Taste	Mouthfeel	Overall acceptability
0	5.59[a]	4.33[a]	4.22[a]	4.11[ab]
2.5	4.70[abc]	4.48[a]	4.22[a]	4.37[a]
5	5.19[ab]	4.56[a]	4.67[a]	4.78[a]
7.5	4.48[bc]	4.00[a]	4.04[a]	4.07[ab]
10	4.11[bc]	4.22[a]	4.04[a]	4.30[a]
15	4.04[c]	2.89[b]	3.63[a]	3.11[b]

*Means followed by the same letter within a column are not significantly different at $\alpha = 0.05$ as determined by Duncan's multiple range test.

can be used for improving the microbiological quality of cocoa beverage premix without compromising its sensory properties.

4. Conclusion

It can be concluded that gamma irradiation dosage of 15kGy was effective in 100% inactivation of artificially inoculated microorganisms; however, radiation doses up to 10 kGy can be used for decontamination of cocoa beverage premix without altering its biochemical and sensory characteristics. As gamma rays have the capacity to penetrate great depths in materials, they can readily be used for in-package powdered cocoa beverage premix sterilization.

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