Antimicrobial Effects of Ionizing Radiation on Artificially and Naturally Contaminated Cacao Beans

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With an initial microbial level of ca. 10 7 microorganisms per g of Ivory Coast cacao beans, 5 kGy of gamma radiation under an atmosphere of air reduced the microflora per g by 2.49 and 3.03 logs at temperatures of 35 and 50°C, respectively. Bahia cacao beans were artificially contaminated with dried spores of Aspergillus flavus and Penicillium citrinum, giving initial fungal levels of 1.9 × 10 4 and 1.4 × 10 4 spores per g of whole Bahia cacao beans, respectively. The average D 10 values for A. flavus and P. citrinum on Bahia cacao beans were 0.65 and 0.88 kGy, respectively.

The evaluation of organoleptic and antimicrobial effects of ionizing radiation on cocoa has been limited. In 1969, the Netherlands cleared for human consumption chocolate products processed from cacao beans that were irradiated to an average maximum dose of 0.70 kGy for insect disinestation (3). Amuh (2) showed that moldiness of unroasted cacao beans (less than 2 months old) can be prevented in typical storage conditions (80 to 85% relative humidity at a temperature of 27°C [11]) for at least a year when the beans are irradiated at 5 kGy. A combination of heat and ionizing radiation increases the potential antifungal effects of irradiation on cacao beans (1), thus increasing the shelf life during storage. The purpose of this investigation was to further extend the antimicrobial effects of ionizing radiation on naturally contaminated cacao beans and beans artificially contaminated with two mycotoxin-producing species (Aspergillus flavus and Penicillium citrinum) which represent two genera frequently isolated from dried cacao beans during storage (6, 10, 12).

P. citrinum (NRRL 5452) and A. flavus, isolated from cacao beans, were used in this investigation to artificially contaminate Bahia cacao beans. Single lots of unroasted Ivory Coast (natural microbiota) inoculated Bahia cacao beans were equilibrated to 6.19 to 6.42% moisture.

Spore suspensions (0.5 ml) of A. flavus and P. citrinum in 0.3 mM phosphate buffer (pH 7) were pipetted onto Difco potato glucose agar and incubated at 25°C for 3 weeks (A. flavus) or 10 weeks (P. citrinum). The fungal spores for each strain were harvested in sterile 0.005% Triton X-100 and aseptically transferred into a sterile petri dish containing five layers of sterile Whatman no. 3 filter paper. The plates were placed in an incubator at 35°C for 18 to 24 h and subsequently stored in a desiccator at room temperature. Bahia cacao beans were inoculated in 30-lb (ca. 13.6-kg) lots by manually tumbling them for 30 min with the dried spore preparation. The contents in the bag were subsequently placed into a clean Stokes vector tumbling machine and mixed for 24 h at 30 rpm. After separation from the filter paper and plates, the inoculated beans were incubated at 22°C under 70% to 75% relative humidity. The initial fungal spore counts on the Bahia cacao beans were 1.0 × 10 4 and 4.0 × 10 3 spores per g of whole beans for A. flavus and P. citrinum, respectively.

All cacao bean irradiations were performed with an experimental Co-60 gamma ray source located under water. The cacao beans were irradiated in 1/8-in. (0.32-cm)-thick high-density polyethylene tubes. Each tube contained ca. 120 g of beans which were irradiated under atmospheric conditions. The polyethylene tubes were wrapped in Thermyl-insulated heating tapes with electrical leads passing through a port at the top of the stainless steel container. Temperatures were monitored via thermocouples. Dosimetry of cacao bean samples in the 30-kGy range involved monitoring 12 prepared Perspex dosimeters (8) taped to the inner surface of the polyethylene sample tube as well as 8 dosimeters placed along the center line of the tube. The difference in rates between centrally and side-located dosimeters was 1.74 ± 0.06 kGy/h. Once the dose rate was determined, absorbed doses were set by exposure times. The equation for calculating corrected optical density change was: [postirradiation optical density × fade factor] – preradiation optical density) divided by the thickness (millimeters). The standard deviations for the dose points were between 0.5 and 1.1%. Control samples were held at a “no radiation” site in the pool at the appropriate temperature for a period equal to the sample receiving the longest irradiation time for a particular sample set.

Within 48 h post irradiation, the cacao beans were sampled for microbiological and moisture analyses. Three 25-g samples of cacao beans (two ends and middle of the tube) were separately blended for 5 min under stainless steel Waring blenders with 2.25 ml of 0.3 mM phosphate buffer (pH 7.0) as the diluent. Difco plate count agar was used to enumerate the total microbial load on the naturally contaminated Ivory Coast cacao beans, whereas potato glucose agar containing 40 μg of filter-sterilized chlorotetracycline and chloramphenicol per ml (added after the medium cooled to 50°C) was used to enumerate A. flavus and P. citrinum from the artificially contaminated Bahia cacao beans. Triplicate plates for each sample were incubated at 25°C for 2 days (plate count again) or 5 days (potato glucose agar). The remaining cacao beans were poured into a Whirl-pak bag for moisture analysis (7).

Microscopic examination of colonies from naturally contaminated cacao beans exposed to gamma radiation ranging from 0 to 5 kGy revealed that the majority of the cultures were sporeforming gram-positive rods belonging to the genus Bacillus. Further specification of the Bacillus cultures was according to the methods outlined by Gordon et al. (5).

Fungal and bacterial counts were converted to log 10 microorganisms per gram of whole beans. All data were

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TABLE 1. Antimicrobial effects of gamma radiation on naturally contaminated cacao beans

<table>
<thead>
<tr>
<th>Irradiation rate (kGy)</th>
<th>Log death (per g)</th>
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<tbody>
<tr>
<td></td>
<td>35°C</td>
</tr>
<tr>
<td>2</td>
<td>1.37 (6.19%)*</td>
</tr>
<tr>
<td>3</td>
<td>1.74 (6.27%)*</td>
</tr>
<tr>
<td>3.22</td>
<td>2.09</td>
</tr>
<tr>
<td>4</td>
<td>2.49 (6.71%)*</td>
</tr>
</tbody>
</table>

* The initial microbial level ranged from 9.3 × 10⁶ to 9.7 × 10⁶ microorganisms per g of whole cacao beans.

** Microorganisms surviving irradiation at 2 to 4 kGy were mainly Bacillus spp.

† Equals the initial log total microorganism count per gram minus the log total microorganism count per gram after a certain irradiation dose.

‡ Irradiation temperature.

§ Moisture percentage of whole cacao beans.

analyzed statistically comparing the geometric means. Each geometric mean was calculated from data of three 25-g samples plated in triplicate. Least-squares linear regression lines were determined from data involving A. flavus and P. citrinum spores exposed to various doses of gamma radiation D₁₀ values, which equaled the number of kiloGrays required to kill 1 log of fungal spores on the cacao beans, for A. flavus and P. citrinum were calculated from the linear regression lines. Student’s t test was used to calculate the significant differences between the slopes of the linear regression lines for the fungal spores of a species exposed to gamma radiation at ambient temperature (31°C) and 46°C.

The antimicrobial effects of gamma radiation at 35 and 50°C on naturally contaminated Ivory Coast cacao beans are presented in Table 1. The initial microbial level was ca. 10⁷ microorganisms per g of whole cacao bean, in which the predominate microflora was catalase-positive, gram-positive rods. After 5 kGy of gamma radiation, microbial death was 2.49 and 3.03 logs per g of whole beans at temperatures of 35 and 50°C, respectively. The microorganisms surviving 2- and 4-kGy radiation doses were mainly Bacillus spp., e.g., Bacillus brevis and Bacillus megaterium, which are normal contaminants of dried, unroasted cacao beans (9).

The antifungal effects of gamma radiation on A. flavus and P. citrinum spores on artificially contaminated Bahia cacao beans were evaluated (data not presented). No significant (P > 0.05) differences in the death of A. flavus exposed to increasing doses of gamma radiation were calculated at ambient temperature (30°C) versus 46°C under atmospheric conditions. The D₁₀ values for A. flavus spores inoculated on cacao beans and exposed to gamma radiation at 31 and 46°C were 0.66 and 0.65 kGy, respectively. P. citrinum spores on cacao beans were more resistant to gamma radiation than A. flavus spores (D₁₀ at 31°C = 0.91 kGy; D₁₀ at 46°C = 0.84 kGy). No significant (P > 0.005) differences between the linear regression line slopes were calculated for P. citrinum exposed to increasing levels of gamma radiation at 31 and 46°C under atmospheric conditions.

The predominant microflora of mechanically or sun-dried fermented cacao beans is aerobic sporeforming bacteria belonging to the genus Bacillus which can reach a concentration of 10⁷/g of whole cacao beans (4, 9). Our study confirms these data in which the major genus isolated from unroasted Ivory Coast beans was Bacillus, e.g., B. brevis and B. megaterium. At 5 kGy of radiation, ca. 99.9% of the bacteria present on whole cacao beans can be killed (Table 1). Irradiation at 50°C compared with 35°C under atmospheric conditions did not substantially increase the antimicrobial effectiveness of gamma radiation.

This communication demonstrates the feasibility of using gamma irradiation to drastically reduce the levels of two xerophilic fungi commonly isolated from cacao beans during storage. With a 4.0- to 5.0-kGy dose level, A. flavus and P. citrinum spores on cacao beans would be reduced by ca. 7 and 5 logs per g, respectively, which could drastically reduce, if not virtually eliminate, these two fungi or genera from cacao beans. With a minimum of fungal contamination during storage, the shelf life of irradiated cacao beans could be significantly extended in cocoa-producing countries or areas with high relative humidities (>80.0%), or both. Storage of cacao beans in a high relative humidity (>85.0%) environment before irradiation will substantially decrease the benefit of radiation for shelf life extension (1, 2). Therefore, gamma radiation (4.0 to 5.0 kGy) should be applied to cacao beans either immediately after drying or to beans stored at relative humidities < 72.0% (4) to ensure that the xerotolerant fungi do not proliferate during storage to a level that exceeds the number of D values obtained by irradiation. The organoleptic ramifications of irradiated cacao beans must be further investigated; in particular, whether irradiated cacao beans affect the sensory qualities of chocolate products.

LITERATURE CITED


