Effect of Electron Beam Irradiation on Color and Microbial Bioburden of Red Paprika

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ABSTRACT

The effect of irradiation with electron beams on the microbiological quality and color properties of red paprika was examined. The irradiation doses ranged from 0 to 12.5 kGy. The counts performed were total mesophilic aerobic microorganisms, Enterobacteriaceae, coliforms, sulfite-reducing clostridia, molds, and yeasts. It was concluded that molds, yeasts, and sulfite-reducing clostridia were the most resistant species, although a 10-kGy dose of irradiation leads to optimum sanitation. Extractable color and apparent color were analyzed to appraise the incidence of the irradiation treatments in the color properties of red paprika. Extractable color was determined according to the American Spice Trade Association method, and apparent color was analyzed by reflectance using the CIELab color space. Data showed no significant differences between the color properties of irradiated and nonirradiated samples. Irradiation was a suitable procedure to minimize the bioburden of red paprika with small modifications of its color properties.

The commercial application of irradiation in the control of foodborne parasites to ensure hygiene and to prolong freshness began in the early 1950s as a result of nonwarlike applications of nuclear energy (28). The first sources of ionizing radiation used were γ-rays from radionuclides such as 137Cs or 60Co of shorter half-life. Later, linear and circular electron accelerators were developed, which could produce electron beams of an energy level of 10 MeV, also called β-rays. Although electron beams are less penetrating than γ-rays, they present several advantages, such as generation from an electrical machine and no nuclear waste.

Irradiation is used to inactivate foodborne microorganisms, to reduce quality losses during storage, and to guarantee the hygienic quality of several foodstuffs, such as milk or egg products (13, 23), drinking water (39), poultry (26, 40), fresh or processed meats (5, 16), seafoods (11, 31), fruits and vegetables (20, 24), or grain, meals, and spices (10, 34, 38).

International codes for the safe use of irradiation have been established (6, 12, 27, 42), and reliable methods exist for the measurement of the dose applied and detection of irradiated foodstuffs (4, 7, 8, 29, 30, 33, 35, 36). Such safety precautions and the improved quality of irradiated foods are changin the attitudes of the consumers (3, 15, 32).

The microbial bioburden of spices can reach 108 CFU/g (18, 21), although the total mesophilic numbers are less. Because of their low moisture content, spices are microbiologically stable products; once in contact with water-rich foods, microorganisms can grow quickly. The ban in Europe on the use of ethylene oxide in the control of foodborne parasites, the difficulty of applying thermal treatments to powdered products such as spices, and the complexity and high cost of installations that use γ-rays have opened good perspectives for the use of electron beam irradiation in making spices safe for human consumption. In this article, we studied the effect of different doses of irradiation on microbiological survival in red paprika. Beside the microbiological analysis, the influence of irradiation on the color of paprika was evaluated.

MATERIALS AND METHODS

Samples. Two types of commercial red paprika obtained locally were used in this investigation. After arrival at the laboratory, the paprikas were immediately divided into samples of 500 g and introduced into polyethylene bags, which were thermosealed to avoid further microbiological contamination.

Irradiation treatment. The irradiation was carried out in a 10-MeV circular electron accelerator (Rhodotron). The treatment was performed in a single step, taking into consideration the volumetric density of the product, bag type, and characteristic of the installation. The programmed irradiation doses were 2, 5, 10, and 13 kGy.

Irradiation dosimetry was carried out by putting a band of cellulose triacetate on the surface of the bags. The cellulose triacetate bands were analyzed twice: by an aerial system, which performed the reading along the cellulose triacetate band with graphic integration of the results, and also by the Nissin system, officially calibrated according to the norm ANSI/AAMI/ISO 11137–1994. Differences between both systems were around 3% but always lower than 5%, which is the maximal difference admitted by law. The irradiation dosimetry revealed that the real doses applied were 2.4, 4.8, 10.0, and 12.5 kGy, respectively.

Microbiological analysis. The survival of the most usual microorganisms found in paprika was analyzed using Oxoid culture medium (Unipath Ltd., Wade Road, Basingstoke, Hampshire, England) and sample dilution with buffered peptone water.
The total mesophilic aerobic count estimates the total microbial load without specifying the type of germ; it reflects the hygienic level of the raw material. It is a test to know the health conditions of the spices. For total mesophilic aerobic count, the pour plate technique was used with standard plate count agar medium incubated 48 to 72 h at 31°C, according to the standard American Public Health Association method (1).

Not only do molds and yeast deteriorate the foods, but they also can have pathogenic, allergic, or toxic action. Nontreated paprika presents a high count of these microorganisms, and these microorganisms, therefore, could contribute to the alteration of the foods. Molds and yeasts were determined after incubation for 5 days at 22°C in oxitetracycline gluten yeast extract agar (22).

Enterobacteriaceae are germs indicative of fecal pollution; in Europe they are an index widely used in foods to appraise their hygienic quality. The total Enterobacteriaceae count was performed after a previous enrichment at 37°C for 24 h in EE Mossel broth. The samples that showed growth were inoculated on violet red bile glucose agar and incubated for 24 h at 37°C. The colonies grown on this medium were identified according to the cytochrome-oxidase test and also on Kligler agar after incubation at 37°C for 24 h. Finally, the total Enterobacteriaceae count was made by obtaining the most probable number of three series of three tubes in those samples that showed growth in EE Mossel broth, since they produced negative results on the cytochrome-oxidase test and showed growth on Kligler agar (1).

In products slightly processed or not processed, the determination of coliforms as indicative of fecal contamination is preferable. Coliforms were determined with the most probable number technique using MacConkey broth (purple). Three series of three tubes were incubated at 35°C for 48 h. The tubes that showed yellow tint (acid production) and gas were considered positive (41).

Sulfite-reducing clostridia belong to the Clostridium genus, and because of their high capacity to sporulate, they are resistant to the sterilization treatments. Sulfite-reducing clostridia count was performed after incubation for 24 h at 37°C in anaerobic condition in agar for perfringens with Shahidi-Ferguson selective supplement (1).

Physicochemical determinations. Extractable color, moisture, lipid extract, ashes, and sand were analyzed according to the methods of the American Spice Trade Association (2).

For apparent color characterization, 50 g of each sample was put in a petri dish without covering and smoothed. Reflectance color was determined using a Minolta CM-5081 chromometer, and the result were expressed in the CIELab color space (25). Standard illuminant D_65 and 10° viewing angle were used. The values of \( L^* \) (lightness), \( a^* \) (red-green coordinate), \( b^* \) (blue-yellow coordinate), \( h \) (hue angle), and \( C^* \) (chroma) were registered. In the evaluation of \( h \), we used the most widely accepted international criterion of assigning the angle of 0° to the semiaxis +a* (redness), the angle of 90° to the semiaxis +b* (yellowness), the angle of 180° to the semiaxis –a* (greenness), and the angle of 270° to the semiaxis –b* (blueness). The color difference (\( \Delta E^* \)) was calculated as \((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\)^1/2. Data registered were the mean of five determinations in five different points of the product with three repetitions in each one.

Microbial count. Culture responses were expressed as log CFU/g. The \( D_{10} \) values (dose in kiloyrags resulting in a 90% reduction of viable CFU) were obtained as the reciprocals of the slopes of the linear regressions of the log survivor values, as determined by least-squares analysis.

Statistical calculations. Data were subjected to analysis of variance and means separations by Scheffe's test. Statistical studies were performed with Statgraphic software (Statistical Graphic Corp. and Graphic Software Systems Inc., Rockville, Md.).

RESULTS

Characterization of samples. Table 1 summarizes the moisture, lipid extract, ashes, sand, and extractable color of the paprika samples investigated. The main difference between both of the samples lay in the extractable color, which was 76.47 American Spice Trade Association units in sample A and 88.56 in sample B, which represents a difference of approximately 14%. The rest of the parameters were similar.

Microbiological analysis. Table 2 shows data on microorganism survival after each irradiation dose for paprika samples A and B. The two samples presented similar total mesophilic aerobic counts and microbial destruction. The \( D_{10} \) values (2.14 and 2.12 kGy, respectively) indicate that low levels of irradiation (2 to 3 kGy) substantially diminished (>90%) the numbers of total mesophilic aerobic microorganisms. For the 4.8-kGy dose, microbial destruction was higher than 99%. The 10-kGy dose of irradiated paprika reduced the total count to 2 to 2.4 log CFU/g, although the slope of the lethal curve decreased because the number of surviving cells was low for doses higher than 5 kGy and also because the surviving pathogens, mainly of the sporulated type, were the most resistant to irradiation. In the samples irradiated at a dose of 12.5 kGy, the microbiological load was completely destroyed.

Sample B showed a higher degree of contamination with Enterobacteriaceae bacteria. A low dose of irradiation (2.4 kGy) produced a substantial reduction of these microorganisms (97 to 99%). The \( D_{10} \) value was calculated for doses between 0 and 4.8 kGy, ranges for which the lethal curve was more linear. The results indicate that a dose of 3 kGy would eliminate 90% of the Enterobacteriaceae present in the paprika. An irradiation dose of 10 to 12 kGy completely destroyed this bacterial group, eliminating the

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture (%)</th>
<th>Extractable color (ASTA units)</th>
<th>Lipid extract (%)</th>
<th>Ashes (%)</th>
<th>Sand (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.15 ± 0.30</td>
<td>76.47 ± 0.13</td>
<td>11.09 ± 1.30</td>
<td>5.19 ± 0.50</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>B</td>
<td>5.78 ± 0.45</td>
<td>88.56 ± 0.16</td>
<td>13.85 ± 2.03</td>
<td>6.19 ± 0.73</td>
<td>0.97 ± 0.08</td>
</tr>
</tbody>
</table>

*Mean ± SD of triplicate samples. ASTA, American Spice Trade Association.*
TABLE 2. Log of microbiological count (log CFU/g) in irradiated paprika.

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>Enterobacteriaceae</th>
<th>Molds and yeasts</th>
<th>Sulphite-reducing clostridia</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB</td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>0</td>
<td>6.40 ± 3.18</td>
<td>3.65 ± 2.74</td>
<td>4.29 ± 3.18</td>
</tr>
<tr>
<td>2.4</td>
<td>5.30 ± 6.38</td>
<td>2.60 ± 1.85</td>
<td>3.15 ± 2.70</td>
</tr>
<tr>
<td>4.8</td>
<td>4.68 ± 4.68</td>
<td>2.40 ± 1.70</td>
<td>2.30 ± 1.70</td>
</tr>
<tr>
<td>10.0</td>
<td>4.04 ± 4.18</td>
<td>2.40 ± 1.70</td>
<td>1.80 ± 0.70</td>
</tr>
<tr>
<td>12.5</td>
<td>3.30 ± 3.97</td>
<td>2.74 ± 2.43</td>
<td>2.18 ± 0.70</td>
</tr>
</tbody>
</table>

SD of triplicate samples. ASTA, American Spice Trade Association.

The apparent color of the two paprika samples was unacceptable, bearing in mind the pathogen-free state of the samples irradiated with this dose.

Irradiation doses lower than 5 kGy only caused losses of 1 to 2% in extractable color, percentages that are far less than those produced during elaboration processes. The highest irradiation dose tested (12.5 kGy) only produced a loss of around 6 to 7%, which could be considered as acceptable, bearing in mind the pathogen-free state of the samples irradiated with this dose.

The apparent color of the two paprika samples was
DISCUSSION

The paprika samples analyzed showed a substantial degree of microbiological contamination. Electron beam irradiation was effective in reducing microorganism numbers. The results obtained would confirm that a 10-kGy dose of irradiation leads to optimum sanitation, not forgetting that the destruction of Enterobacteriaceae would ensure the absence of other pathogenic gram-negative bacteria (21). Although a lower dose might be adequate to destroy most of the bioburden, the different resistance to irradiation of the microorganisms could result in a dramatic change in the dominant microflora of paprika after low-dose treatment. We, therefore, think that a dose of 10 kGy is optimum. These levels of irradiation are below the limit of 30 kGy admitted by the Food and Drug Administration in the treatment of spices (27).

Molds, yeasts, and, above all, sulfite-reducing clostridia were the most resistant species. This behavior is related to their capacity to form spores that are much more resistant to the irradiation than vegetative cells (14). On the other hand, the lower water activity of dehydrated foods reduces the capacity of microbiological regeneration during storage (28). The results obtained after low-dose irradiation with electron beams were similar to those reported by other authors who irradiated paprika or other spices with γ-rays (9, 17, 19, 34, 37).

Color is the most important sensorial characteristic of paprika. Any alteration as a consequence of the irradiation is related to the formation of free radicals that oxidize the pigments. In our experiment, the alteration in color in response to irradiation could be evaluated with accuracy by means of the ΔE* parameter. The values of ΔE* between the control and the irradiated samples were small, always lower than 4.04. It is, therefore, practically impossible to distinguish the irradiated paprika from their corresponding reference. The moisture or lipid content affects the apparent color more strongly than electron beam irradiation.

In conclusion, low-dose irradiation with electron beams is demonstrated to be a suitable procedure for the inactivation of foodborne microorganisms in paprika. It hardly modifies its color characteristics and is extremely effective in reducing the viable population of microorganisms that may contribute to the spoilage of food products in which it is used as a natural colorant.

REFERENCES

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**TABLE 4. Effect of irradiation treatments on the apparent color (CIELab parameters) of red paprika**

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>ΔE*</th>
<th>C*</th>
<th>h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>0</td>
<td>47.20 A*</td>
<td>88.56 A</td>
<td>39.83 A</td>
<td>32.85 A</td>
<td>54.27 A</td>
<td>43.75 A</td>
</tr>
<tr>
<td>2.4</td>
<td>47.08 A</td>
<td>88.09 A</td>
<td>39.64 A</td>
<td>32.93 A</td>
<td>54.34 A</td>
<td>44.16 A</td>
</tr>
<tr>
<td>4.8</td>
<td>47.28 A</td>
<td>87.78 A</td>
<td>39.65 A</td>
<td>32.76 A</td>
<td>54.67 A</td>
<td>43.56 A</td>
</tr>
<tr>
<td>10.0</td>
<td>47.38 A</td>
<td>83.86 B</td>
<td>39.22 A</td>
<td>32.39 A</td>
<td>53.09 A</td>
<td>40.81 B</td>
</tr>
<tr>
<td>12.5</td>
<td>47.42 A</td>
<td>82.92 B</td>
<td>39.22 A</td>
<td>32.25 A</td>
<td>53.36 A</td>
<td>40.63 B</td>
</tr>
</tbody>
</table>

*Values are the mean of 25 measurements; means separation was carried out by the Scheffe’s test.

b Means within a column followed by a different letter are significantly different at P < 0.05 level of significance.

markedly different, because they were elaborated with different pepper varieties. Thus, sample A showed lower lightness (L*), slightly more redness (a*), and higher yellowness (b*). Nevertheless, its content of carotenoid pigments (American Spice Trade Association color) was lower than of sample B. A low irradiation dose had no appreciable effect on the apparent color of paprika, and only for doses of 10 to 12.5 kGy did the coordinates a* and b* diminish slightly, whereas L* remained unchanged. From the C* and h, it was seen that there were no appreciable variations between the irradiated samples and control.


