A Research Note

Efficiency of Treatments Involving Ultraviolet Irradiation for Decontaminating Packaging Board of Different Surface Compositions

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ABSTRACT

Reflectivity, smoothness and geometry of several types of food packaging board were studied in relation to the effectiveness of decontamination treatments involving ultraviolet (UV-C, 254 nm) irradiation. Surfaces containing aluminum in the laminate reflected more light in the 325 to 550 nm range and showed a lower lethal effect when Bacillus subtilis spores were irradiated. Visible light of wavelengths between 325 and 550 nm is known to cause photoreactivation of UV damage in vegetative cells. It was suggested that a similar phenomenon might occur in spores on reflective surfaces. Smoothness of the board surface was not an important factor in the extent or the variability of the lethal effect. The geometry of the irradiated surface was shown to be important for aluminum/polyethylene laminate-lined surfaces only, as more spores were killed on board normal to incident UV-C irradiation than in cartons with reflective angles. Spores on the inner sides of this type of carton may have received more reflected light of photoreactivation wavelengths.

A combined decontamination treatment, using low concentrations of hydrogen peroxide and ultraviolet (UV-C) irradiation, has been described for deep, preformed food-packaging cartons (11). During that study, a difference in lethal effect was observed depending on the type of inner surface of the carton. The lethal effect was 1 to 1.5 decimal reductions greater for Bacillus subtilis spores on polyethylene-lined cartons than on aluminum/polyethylene laminate-lined cartons. This 1 to 1.5 decimal reduction difference in lethal effect was also noted when the decontamination effect of UV-C irradiation only was tested in both types of carton. We suggested that two types of lethal effect might be involved. First, there may be a synergistic effect, approximately the same for both types of carton, possibly caused by hydroxyl radical formation in the peroxide by the UV-C (2). The second effect was attributed to UV-C alone, and probably is caused by production of ‘‘spore photoproduct’’ in the DNA of the spore (9). The latter effect is dependent on the type of carton lining, and it was postulated that, in cartons with an aluminum layer, UV-C light may be reflected at a wavelength suitable for the operation of a system similar to photoreactivation in vegetative cells (4).

The objective of the present study was to determine whether UV-C light was reflected at photoreactivating wavelengths, and to investigate the effects of other properties of packaging surfaces on the extent of the lethal effect.

MATERIALS AND METHODS

Food packaging materials

Cartons. Aluminum/polyethylene laminate-lined and polyethylene-lined cartons, as described previously (11), were used.

Boards. Five types of board (supplied by Pakcentre Ltd., London) were used during this study: (a) standard polyethylene laminate milk board cut from a carton as above, (b) shiny polyethylene, (c) matt polyethylene, (d) standard aluminum/polyethylene laminate cut from a carton as above and (e) aluminum/Surlyn/polyethylene laminate. Pieces of board 70 × 70 mm were sterilized before inoculation by ethylene oxide, with Anprolene sterilizing gas ampules according to manufacturer’s instructions (H. W. Anderson Products, New York).

Determination of relative smoothness of packaging boards

The surface of pieces of the various types of board were sputter-coated with gold and examined with a scanning electron microscope (Cambridge Stereoscan Mk IIA) operated at 20 kV. The pieces of board were angled at 45° to the electron source, and the secondary electron emission mode was used.

Determination of wavelengths of light emitted by various packaging boards irradiated with UV light (258 nm)

An Amino-Bowman spectrophotofluorometer (American Instrument Co.) was used with the excitation wavelength set at 258 nm. Strips of board (17 × 45 mm) were placed in the...
cuvette holder at a 45° angle to the UV light source and irradiated. The relative fluorescence at wavelengths between 258 and 610 nm was recorded.

Inoculation of packaging surfaces
A spore suspension of B. subtilis NCTC 3610 (strain Marburg; N. R. Smith) was prepared as described previously (11). Cartons were inoculated for challenge experiments with 10⁷ spores as described previously. Boards were spray-inoculated in a similar manner, but a tenfold more concentrated suspension of spores was used for spraying to achieve a 10⁷ inoculum of spores/board.

Microbicidal treatments
Cartons were treated with UV-C (254 nm) radiation in the presence or absence of 1% (wt/vol) hydrogen peroxide, as described previously (11). Usually, pieces of board were positioned parallel to and 10 cm from the quartz screen of the UV-C lamp. This was accomplished by using a holder made from the same material as the board being treated, with the holder simulating the sides of a carton. On some occasions, the holder was designed so that incident UV-C light fell on the board at different angles, with the furthest edge of the board being 20 cm from the quartz screen of the lamp and against the wall of the holder. To achieve a 0° angle of incidence, boards were attached to the side of the holder with double-sided tape. For a 90° angle of incidence, boards rested on the base of the holder. For the 30° and 60° angles of incidence, the base of the holder was at an angle of 30° and 60°, respectively, from the side of the holder. Three replicate boards or cartons were treated on a single or on two occasions; on each occasion, three inoculated but untreated boards or cartons were included as controls.

Recovery and enumeration of survivors
Organisms were recovered from flat board by washing the board in 100 ml of quarter-strength Ringers solution (Oxoid) + 0.5% (wt/vol) proteose peptone (Difco) containing catalase (150 Sigma units/ml) in a Colworth 400 Stomacher (A. J. Scoreward Ltd., London). Recovery of survivors from cartons and the enumeration procedure have been described previously (11). Mean and standard deviations were calculated for the data. Significant differences between treatments and surfaces were calculated using a multiple t-test (3,5).

RESULTS

Determination of the relative smoothness of packaging boards
The scanning electron micrographs revealed marked differences in the surface contours of the polyethylene film lining the four types of board studied. The matt polyethylene board had the roughest surface of the four, and the shiny polyethylene board was by far the smoothest. The standard aluminum/polyethylene laminate board was smoother than the board incorporating Surlyn, although the difference was not marked. The electron micrographs are not presented here.

Determination of the wavelengths of light reflected from various packaging boards irradiated with UV light (258 nm)
A broad spectrum of light was emitted by all types of board (Fig. 1), mostly between the wavelength of 300 and 600 nm. The peak emission was between ca. 370 and 410 nm. Boards containing aluminum in the laminate reflected considerably more light in this spectral range than those without, although the presence of Surlyn reduced reflectivity by approximately one-third. Of the three boards with no aluminum in the laminate, the matt polyethylene surface reflected the least light, i.e., about half that reflected by the other two boards at the peak.

Effect of surface on decontamination efficiency
The mean reduction in numbers of spores of B. subtilis was greater on boards with no aluminum in the laminate for both the UV-C only treatment and the combined treatment with 1% (wt/vol) hydrogen peroxide and UV-C (Table 1), although for the latter treatment the difference was not significant at the 0.97 protection level. There was no relationship between the smoothness of the board and the extent or the variability of the lethal effect.

Comparison between boards and cartons
For standard polyethylene-lined surfaces, there was very little difference in the lethal effect of UV-C irradiation obtained on cartons or on boards (not significant at the 0.97 protection level). However, for standard aluminium/polyethylene laminate-lined surfaces, the difference was considerably more marked (Table 2; significant at the 0.97 protection level).

Effect of angle of incidence of UV-C irradiation
There was no effect of angle of incidence on polyethylene-lined boards (Table 3; not significant at the 0.97 protection level). However, for aluminium/polyethylene laminate in Table 3. ANOVA was used to test for differences in the lethal effect.
polyethylene laminate-lined boards, significant differences between the lethal effect at various angles were observed. The lowest lethal effect was observed with board at 0° to the incident light, then, in increasing order of kill, 60°, 90° and 30°.

TABLE 1. The lethal effect of decontamination treatments on Bacillus subtilis spores on packaging boards.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Type of boarda</th>
<th>Decimal reduction in number of viable spores per boardb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>UV-C</td>
<td>Standard aluminium</td>
<td>1.89</td>
</tr>
<tr>
<td></td>
<td>Surlyn/aluminium</td>
<td>2.21</td>
</tr>
<tr>
<td></td>
<td>Matt polyethylene</td>
<td>2.63</td>
</tr>
<tr>
<td></td>
<td>Shiny polyethylene</td>
<td>2.66</td>
</tr>
<tr>
<td>H₂O₂ + UV-C</td>
<td>Standard aluminium</td>
<td>3.04</td>
</tr>
<tr>
<td></td>
<td>Surlyn/aluminium</td>
<td>2.89</td>
</tr>
<tr>
<td></td>
<td>Matt polyethylene</td>
<td>3.22</td>
</tr>
<tr>
<td></td>
<td>Shiny polyethylene</td>
<td>3.45</td>
</tr>
</tbody>
</table>

a10 cm from quartz screen of lamp.
bResults from three replicate tests done on each of two occasions.

H₂O₂ treatment was 0.1 ml of a 1% (wt/vol) solution.

TABLE 2. Influence of packaging surface on the lethal effect of UV-C irradiation on Bacillus subtilis spores.

<table>
<thead>
<tr>
<th>Type of board</th>
<th>Form of board</th>
<th>Decimal reduction in number of spores per carton or board</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Standard aluminium/polyethylene laminate-lined</td>
<td>Carton</td>
<td>1.21</td>
</tr>
<tr>
<td>Standard polyethylene-lined</td>
<td>Boardb</td>
<td>3.15</td>
</tr>
<tr>
<td></td>
<td>Carton</td>
<td>3.32</td>
</tr>
<tr>
<td></td>
<td>Boardb</td>
<td>3.19</td>
</tr>
</tbody>
</table>

aThree replicate tests done on one occasion.
b10 cm from quartz screen of lamp.

TABLE 3. Influence of angle of incidence on the lethal effect of UV-C irradiation on Bacillus subtilis spores.

<table>
<thead>
<tr>
<th>Angle of incidence of UV-C lightb</th>
<th>Aluminium/polyethylene laminate-lined</th>
<th>Polyethylene-lined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Estimated SD</td>
</tr>
<tr>
<td>0°</td>
<td>1.87</td>
<td>0.16</td>
</tr>
<tr>
<td>30°</td>
<td>2.85</td>
<td>0.16</td>
</tr>
<tr>
<td>60°</td>
<td>2.19</td>
<td>0.12</td>
</tr>
<tr>
<td>90°</td>
<td>2.43</td>
<td>0.16</td>
</tr>
</tbody>
</table>

aResults from duplicate tests done on each of two occasions.
bFarthest edge of board was 20 cm from quartz screen of lamp.

DISCUSSION

Previous work (11) established that an effective decontamination of deep, preformed food-packaging cartons can be achieved using a combined treatment of low concentrations of hydrogen peroxide and UV-C irradiation. However, the extent of the lethal effect was dependent on the type of inner surface of the carton, because more B. subtilis spores were killed on polyethylene-lined cartons than aluminium/polyethylene laminate-lined ones. The objective of the present study was to investigate certain characteristics of several types of board that might influence the lethal effect. Squares of board were used for most experiments because they were easier to handle than cartons. However, some tests on cartons were included as a comparison.

As shown previously for cartons, the lethal effect against B. subtilis on boards with no aluminum was greater than on boards with aluminium in the laminate, both for UV-C irradiation alone and in combination with 1% hydrogen peroxide (although not significantly so in the latter case; Table 1). ‘‘Within occasion’’ variation in lethal effect was very low; however, from comparison between Tables 1 and 2 it would seem that ‘‘between occasion’’ replication was prone to more variability, particularly for aluminium-containing boards. No estimate of ‘‘between occasion’’ variability was calculated because too few data were collected.

It was suggested (11) that UV-C may reflect from the aluminium layer at a wavelength capable of causing a photoreactivation effect. The present study on the reflectivity of several types of board showed that boards containing aluminium reflected considerably more light between wavelengths of 325 and 550 nm than did boards without aluminium. These wavelengths are known to be implicated in the photoreactivation of vegetative cells previously exposed to UV irradiation (6-8,10).

The smoothness of packaging board, as determined by electron microscopy, was not an important factor in either the magnitude or the variability of the lethal effect. Therefore, board smoothness does not appear to affect reflectivity at photoreactivating wavelengths.

It has been stated that UV irradiation must be more intense for cartons than for flat surfaces (1). In the present study, this was found to be true only for aluminium-containing boards.
containing cartons and boards, where the lethal effect was greater on the board (a significant difference at the 0.97 protection level; Table 2). There are three possible explanations for this. Firstly, the board was only 10 cm from the UV-C lamp. Secondly, spores on the side of the carton may be exposed to less intense UV-C irradiation than those at the bottom. These two explanations would seem unlikely in view of the fact that there was no difference in magnitude of the lethal effect obtained on polyethylene cartons and boards. The third possible explanation is that spores on the sides of an aluminium/polyethylene laminate carton are subject to more reflected light of photoreactivation wavelengths. This was tested by irradiating boards at various angles to the incident UV-C irradiation. No significant differences were observed between lethal effects on polyethylene-lined boards irradiated at different angles (Table 3). However, for aluminium/polyethylene laminate-lined boards, there was a progression in lethal effect, increasing with the angle of incidence as follows: 0°, 60°, 90° and 30°. Because the board was held in a holder made from the same type of packaging material, light of photoreactivation wavelengths may have reflected from it, causing variable numbers of spores to recover according to the angle of the board. This may well occur inside a carton made from this material, in which case a greater lethal effect on spores on the base than on those on the sides of the carton might be expected.

In conclusion, three properties of packaging material were studied in relation to the effectiveness of decontamination treatments involving UV-C. The smoothness of the board was not found to be an important factor in the magnitude or the variability of the lethal effect. The reflectivity of the surface was found to alter the magnitude of the lethal effect. In the case of aluminium/polyethylene laminate-lined surfaces, the geometry of the surface to be irradiated was important. The last two factors should be considered carefully when choosing the optimum packaging material for aseptic food packaging were UV-C is part of the decontamination process.

ACKNOWLEDGMENTS

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REFERENCES