INACTIVATION OF ESCHERICHIA COLI O157:H7 IN HAMBURGERS BY GAMMA IRRADIATION

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ABSTRACT

Escherichia coli O157:H7 causes bloody diarrhoea, haemorrhagic colitis and life-threatening complications like haemolytic uremic syndrome and thrombotic thrombocytopenic purpura. Among foods associated with outbreaks caused by this pathogen, hamburger is the most common one. The aim of this research was to determine the radiation dose to reduce the population of E. coli O157:H7 in hamburgers to non-detectable levels in order to render a safer product. Hamburgers, inoculated with Escherichia coli O157:H7, were exposed to gamma radiation (60Co) treatment, with doses ranging from 0 to 0.7 kGy. The average temperature during the process was 5.6ºC. Non-inoculated hamburgers were submitted to sensory evaluation after being exposed to irradiation doses of 0.8 kGy and 1.0 kGy. The D10 for the pathogen varied from 0.17 kGy to 0.27 kGy in hamburger. Considering the highest D10 value in hamburger, a dose of 1.08 kGy would be sufficient to reduce E. coli O157:H7 contamination in 4 log cycles, without affecting the sensory attributes of the product.

Key words: hamburger, patties, Escherichia coli O157:H7, food irradiation, gamma radiation

INTRODUCTION

Escherichia coli O157:H7 belongs to the enterohaemorrhagic class of Escherichia coli (EHEC). The disease caused by this bacterium is haemorrhagic colitis, and life-threatening complications like haemolytic uremic syndrome and thrombotic thrombocytopenic purpura may occur in haemorrhagic colitis patients (1). Various foods have already been associated with E. coli O157:H7 outbreaks, with hamburger being the most common. Processed food such as yoghurt, cheese and fermented sausage have also been involved in foodborne outbreaks caused by E. coli O157:H7 (16).

As cattle is the main reservoir of EHEC, with high rates of faecal carriage, the presence of this pathogen in raw meat is not surprising with contamination occurring during slaughter. Cooking meat thoroughly and pasteurizing milk are important measures of protection but are not sufficient by themselves.

Among food preservation methods, food irradiation is considered the most versatile treatment, nowadays. Microorganisms can be inactivated by impairment of important molecules or organelles, such as DNA and the cytoplasmic membrane (3). In 1980, the Joint Food and Agricultural Organization/International Atomic Energy Agency/World Health Organization committee on the Wholesomeness of Irradiated Food concluded that the irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard and introduces no special nutritional or
microbiological problems (22). Hence, gamma radiation became an important tool to be used by the food industry not only as a method of preserving food but also to improve food safety (12). Although the consumer resistance, these developments may lead towards more use of this technology in the future. In the United States, the US Food and Drug Administration approved irradiation of red meat in December 1997 (5) and, more recently, the United States Department of Agriculture (20) also approved its use for meat and poultry.

The aim of this research was to determine the gamma radiation dose to reduce the level of *Escherichia coli* O157:H7 in manufactured hamburger as well its effects on the sensory attributes.

**MATERIALS AND METHODS**

**Strain**

*Escherichia coli* O157:H7 EDL 933 (a hamburger isolate), obtained from the Departamento de Microbiologia, Imunologia e Parasitologia da Universidade Federal de São Paulo, Brazil, was used in this study. The isolate was grown in 5mL of nutrient broth (Difco, Detroit, MI) at 37ºC for 24h. After this period, the culture was spread plated onto Sorbitol MacConkey agar (Oxoid, United Kingdom) and incubated at the same conditions. Ten typical colonies (sorbitol negative) were, then, transferred to 100mL of tryptic soy broth (Difco, Detroit, MI) as diluent and at 37ºC, tubes showing growth were streaked onto Sorbitol MacConkey agar (Oxoid, England). The colonies were confirmed as *E. coli* O157:H7 using proper biochemical tests and the *E. coli* antiserum O157 assay (Probac do Brasil).

Microbiological data were transformed into log units of the Most Probable Number/g (log<sub>10</sub>MPN/g).

**Irradiation treatment**

Cardboard packages containing 12 frozen hamburgers were acquired at the retail level at São Paulo city, Brazil. After the temperature reached 2ºC in the center of each hamburger, as measured with a thermometer, individual hamburgers were asseptically transferred to styrofoam trays and 1mL (10<sup>5</sup>CFU/mL) of *E. coli* O157:H7 was evenly distributed inside and on the surface of the hamburgers, using a syringe. After 15 minutes, each tray was wrapped with PVC (polyvinyl chloride) film and transported to the irradiation plant in insulated boxes. Non-inoculated samples were used as controls. In each experiment, 21 trays containing one hamburger each were submitted to the irradiation treatment. The experiment was repeated 3 times.

Hamburgers were irradiated at EMBRARAD S.A., with a commercial-size 60Co Gamma Beam JS7500 Irradiator (Nordion International Inc., Kanata, Ontario, Canada). The dose rate provided by the 60Co source ranged from 3.5 kGy/h to 5.0 kGy/h.

Sets of 3 trays were exposed to each of the following irradiation doses: 0.0 kGy; 0.1±3% kGy; 0.2±3% kGy; 0.3±3% kGy; 0.4±3% kGy; 0.5±3% kGy and 0.7±3% kGy. These doses were established using National Physical Laboratory (Middlesex, United Kingdom) dosimeters. After irradiation, hamburgers were maintained refrigerated until the beginning of microbiological analyses. Temperature of hamburgers was monitored before and after irradiation.

**Microbiological analyses**

The enumeration of *E. coli* O157:H7 in irradiated hamburgers was done using the Most Probable Number (MPN) method, with tryptic soy broth (Difco, Detroit, MI) as diluent and recovery medium (14). After 24h incubation at 37ºC, tubes showing growth were streaked onto Sorbitol MacConkey agar (Oxoid, England). The colonies were confirmed as *E. coli* O157:H7 using proper biochemical tests and the *E. coli* antiserum O157 assay (Probac do Brasil).

**RESULTS AND DISCUSSION**

D<sub>10</sub> values for *E. coli* O157:H7 in hamburger ranged from 0.17 kGy to 0.27 kGy (Fig. 1). D<sub>10</sub> values in solid food were higher than in liquid medium (data not shown). These results were already expected since compounds like proteins, carbohydrates, lipids, condiments and other components present in hamburgers will compete with the bacterium for interaction with free radicals produced during radiolysis (21). The broad variation in D<sub>10</sub> values for hamburgers can be due to differences in composition of the hamburgers belonging to different brands. According to Monk et al. (11), some food preservatives also affect the growth or death of microorganisms when food is submitted to irradiation treatment. Therefore, the presence of these compounds could also have influenced the values obtained in this research.

The D<sub>10</sub> values of *E. coli* O157:H7 reported here are very similar to those obtained by other authors. Patterson (13) reported D<sub>10</sub> values of 0.27 kGy and 0.39 kGy for non-pathogenic *E. coli* in chicken meat packed in vacuum and in air, respectively. Thayer and Boyd (17) found a D value of 0.28...
of bacteria, among them, *Yersinia enterocolitica*, *Vibrio parahaemolyticus*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Campylobacter jejuni*, found D values varying from 0.03 to 0.06 kGy for the less radioresistant, such as *E. coli* O157:H7. On the other hand, *S. typhimurium* and *L. monocytogenes* showed the highest resistance.

Our results do not agree with those of Dion et al. (4) and Clavero et al. (2) probably due to differences in the temperature during irradiation treatment, presence or absence of oxygen and substrate. Besides, different species or strains of the same species may require different doses to achieve the same degree of inactivation (3). Thayer and Boyd (18) concluded that the radioresistance of different serotypes of *E. coli* (2 non-pathogenic strains and 7 pathogenic ones) in red meat depended on the serotype and also on the temperature during irradiation (5ºC and -5ºC).

Another aspect to be considered is the methodology used in the recovery of cells after irradiation. The MPN methodology probably provided a better recovery of stressed cells than other methodologies where a recovery step is not included before the isolation step. Though culture media and incubation conditions for growth and estimation of pathogen populations following irradiation treatment are secondary factors, they must be considered. Stressed cells take two or more hours to recover after irradiation. Consequently, the medium and the incubation conditions can interfere in the recovery of the cells (11,19).

The effect of irradiation on sensory quality of beef is of concern due to the formation of free radicals. Fresh meat has a high moisture content and the reaction of ionizing radiation with water leads to the formation of free radicals that react with molecules in the food, resulting in compounds with undesirable odor and taste (7).

Table 1 shows the results for the sensory evaluation. In our experiment, a dose of 1.2 kGy, capable of reducing the counts of *E. coli* O157:H7 in more than 4 log cycles, imparted an unfavourable odor and taste to the hamburgers. Lefebvre et al. (9) observed the same phenomenon, but Rodriguez et al. (15) reported no changes in sensory attributes of beef treated with 2 kGy.

In relation to colour, the trained panel detected a slightly different colour in the hamburgers treated with 1.0 kGy (Table 1). Similar results were found by Ginger et al. (6) and Huber et al. (8).

Therefore, considering the highest D_{10} value (0.27 kGy), a dose of 1.08 kGy would cause a 4 log cycle reduction in the population of *E. coli* O157:H7 in hamburgers, without substantially changing their sensory attributes. Considering that hamburgers are usually ingested with mayonnaise, tomato sauce and/or mustard sauce, the slight rancidity taste detected by the trained panel can be overcome.

**Figure 1.** Reduction of *Escherichia coli* O157:H7 inoculated in hamburger exposed to gamma radiation.
Table 1. Sensory attributes of hamburgers exposed to radiation doses of 0.0kGy, 0.8kGy and 1.0 kGy.

<table>
<thead>
<tr>
<th>Sensory attributes</th>
<th>Standard (0.0kGy)</th>
<th>Test 1 (0.8kGy)</th>
<th>Test 2 (1.0kGy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odour</td>
<td>strong seasoning odour; light meat odour.</td>
<td>strong seasoning odour; light meat odour.</td>
<td>strong meat and seasoning odour.</td>
</tr>
<tr>
<td>Flavour</td>
<td>strong seasoning, light meat flavour; little to moderate salt.</td>
<td>strong seasoning, light meat flavour; little salt.</td>
<td>strong meat and moderate seasoning; light tsaal; leaves rancid fat taste.</td>
</tr>
<tr>
<td>Texture</td>
<td>tender, slightly “springy” to touch and juicy.</td>
<td>tender, slightly “springy” to touch and juicy.</td>
<td>slightly tender but juicy.</td>
</tr>
</tbody>
</table>

RESUMO

Inativação de Escherichia coli O157:H7 em hambúrgueres submetidos à irradiação gama

Escherichia coli O157:H7 causa colite hemorrágica que pode evoluir para síndrome urêmica hemolítica e síndrome púrpura trombótica trombocitopenica. Entre os alimentos mais envolvidos nos surtos causados por essa bactéria destacam-se os hambúrgueres de carne bovina. Esta pesquisa teve como objetivo determinar o valor D10 de E. coli O157:H7 em hambúrgueres e sugerir uma dose que torne o alimento seguro em relação à essa bactéria e sem alterar suas características sensoriais. Amostras de hambúrgueres industrializados foram inoculadas com Escherichia coli O157:H7 e submetidas à irradiação com raios gama (60Co). A temperatura média durante o processo foi de 5.6ºC. O valor D10 no hambúrguer variou de 0,17 a 0,27 kGy. Considerando o maior valor D10 obtido, a dose de 1,08 kGy seria suficiente para reduzir a população de Escherichia coli O157:H7 em 4 ciclos logarítmicos, sem que houvesse rejeição do produto pelo painel treinado de analistas.

Palavras-chaves: Escherichia coli O157:H7, hambúrguer, radiação ionizante, irradiação gama, irradiação de alimentos

REFERENCES