Inactivation of a non-pathogenic strain of *E. coli* by ionising radiation


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Abstract

Ionising radiation is an effective method to reduce pathogenic *E. coli* O157:H7 in meat and poultry products. Radiation sensitivity of bacteria, however, depends on several factors. After applying an irradiation dose of 1 kGy to cultures of the non-pathogenic strain of *E. coli*, DSM 498, grown and irradiated in nutrient broth, reductions of 3–4 decimal units were achieved (*D* = 0.27 kGy). If grown on minced turkey meat, however, reduction rates were lower (*D* = 0.47 kGy). Even lower reduction rates were obtained during irradiation of frozen meat (*D* = 0.72 kGy) compared to treatments at cooling temperatures (*D* = 0.48 kGy). For data evaluation, both, first order reduction kinetics and the Weibull model were compared. The results emphasise the necessity to determine inactivation kinetics in food matrices of target extrinsic factors (e.g. temperature).

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Keywords: *E. coli* DSM 498; Turkey meat; Irradiation; Reduction kinetics

1. Introduction and fundamentals

Food is one of the indispensable necessities in life. Fortunately, many advanced and several developing countries have abundant supplies of fresh, safe and nutritious foods. Yet, despite many precautions and processes to ensure a safe food supply, microbial contamination is still a concern, even in advanced countries (ICGFI, 1999; RKI, 2003). There are numerous of food processing tools available that provide food protection. One promising tool is processing of food by irradiation especially if conventional heating may not be applied.

Irradiation is a physical treatment of exposing food to radiant energy in order to reduce or eliminate bacteria, as making it safer and retarding spoilage. The US Department of Agriculture through Food and Drug Administration (FDA) approved the treatment with ionising radiation as a procedure to reduce the level of microorganisms. According to FDA, 60% of the largest US meat plants failed to meet federal food safety regulations for preventing contamination of their products with *E. coli* O157:H7. This pathogenic bacterium causes an estimated 73,000 infections and 61 deaths in the United States each year. About 15 million pounds of beef were processed in 2002, with increasing tendency as expected by the US irradiation facility manufacturers (Biesz, 2003). In the European Community, for example, the irradiation of e.g. egg is authorised in France, Belgium and the Netherlands, of poultry in France, of chicken in the Netherlands, and of dried spices and herbs in all Member States, according to European Directive 1999/2/EC (EUD, 1999a, 1999b).

As ionising radiation is a suitable method to control pathogenic bacteria in food, a large number of *D* values have yet been published (e.g. Buchanan & Doyle, 1997; Olson, 1998). The impact of preservation processes on the microbial safety of the product is estimated from inactivation data. Radiation sensitivity of bacteria, however, depends on several factors (Mayer-Miebach,
In order to improve treatment the parameters influencing microbial behaviour have to be known. Traditionally, inactivation of vegetative microbial cells exposed to heat have been assumed to be governed by first order reaction kinetics ($D_{10}$-values) due to identical heat sensitivities of all the cells in a population. In contrast, non-linear survival curves with upward or downward concavity are often determined in semi-logarithmic coordinates (Peleg & Cole, 1998; van Boekel, 2002). Genetic repair, for example, is one mechanism leading to a “shoulder” on irradiation survival curves at initial doses (Molins, 2001). As an alternative approach, the Weibull model has been proposed to describe inactivation kinetics (Peleg & Cole, 1998). This model is based on the assumption that the resistance of a given microbial population towards inactivation treatment (e.g. thermal, irradiation) is due to the overall individual resistances of single organisms and should be characterised by a distribution of resistances, therefore. In case of irradiation, the survival curve $S(d)$ is a function of the irradiation dose $d$ and of the fraction of organisms $N$ with the same lethality (lethal dose $d_t$) and can be written as:

$$S(d) = \int_{d_t}^{1} f[d, d_t(N)] \, dN$$

Assuming that $d_t$ has a Weibull distribution, i.e.

$$dN/\, d_d = bnd_d^{n-1} \exp(-bd_d^n)$$

(2) 

(where $b$ and $n$ are constant) the survival curve results in

$$S(d) = \exp(-bd^n)$$

(3) 

and, if presented as a semi-logarithmic relationship

$$\log S(d) = -bd^n$$

(4) 

The constant parameter $n$ determines the shape of the distribution curve: If $n = 1$, the survival curve will appear linear in semi-logarithmic coordinates and hence first order kinetics may be applied. Using the Weibull model, it can be confirmed, if inactivation data follow first order kinetics, therefore (Peleg & Cole, 1998; van Boekel, 2002). This is important for irradiation inactivation in particular, because $D_{10}$-values calculated by first order kinetics without taking into account the “shoulder” curves (downward concavity) often found due to microbial genetic repair mechanisms may demand higher irradiation doses for reduction, than necessary for safe products. As a consequence, product damages and quality impairment by over-treatment are accepted. In contrast, product safety is not ensured in case of upward concavity of survival curves.

Our investigations concentrate on the effects of matrix and temperature on the resistance of a non-pathogenic $E. \ coli$ DSM 498 to ionising radiation. The strain DSM 498 was used as model organism before experiments with pathogenic $E. \ coli$ e.g. O157:H7 come into consideration. Minced turkey meat and a nutrient broth as a model medium were used as matrices in irradiation experiments. Inactivation kinetics were calculated using both, the conventional first order model ($D_{10}$-values) and the Weibull approach.

2. Materials and methods

$E. \ coli$ DSM 498 was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, 2002). Stock cultures grown aerobically on nutrient broth (NB) agar slants at 37 °C were maintained at 4 °C. Precultures containing $10^5$ CFU/ml, grown aerobically in 180 ml liquid NB-medium at 37 °C for 5 h, were used as inoculum for all irradiation experiments.

Irradiation of meat and model media samples was performed with electrons using a linear accelerator of the Institute (LINAC-CIRCE II, Linac Technologies S.A.; Orsay, France, 10 MeV, 10 kW); instantaneous dose rate was $10^7$ Gy/s. Nominal dose is surface dose; according to sample thickness the minimum dose is about 80% of nominal dose.

Turkey meat was purchased from a local slaughter-house. Directly after slaughtering, it was deboned and minced in a laboratory blender B400 (Büchi Labotechnik AG, Switzerland). Portions of 250 g were packaged and irradiated with a dose of 10 kGy to eliminate the original micro-flora, then stored at −85 °C until further use.

Meat samples for irradiation experiments were prepared by adding 20 ml aliquots of an $E. \ coli$ preculture to 200 g of minced turkey meat and homogenising the mixture in a stomacher 400 (Seward Medical, London, Great Britain). Portions of 25 g were filled into Petri dishes, incubated aerobically at 37 °C for 6 h to arrive at bacterial counts of $10^4$–$10^5$ CFU/g within the exponential growth phase, stored overnight at −85 °C until treatment. Sample irradiation was performed either directly at the frozen state or at cooling temperatures in the range of 0–2 °C prior to irradiation.

Liquid model media samples were produced by adding 20 ml aliquots of $E. \ coli$ preculture to 180 ml of NB followed by aerobic incubation at 37 °C for 5 h. Portions of 25 ml of NB with bacterial counts of about $10^5$ CFU/ml were filled into plastic flasks providing a maximum layer thickness of 1 cm, placed on ice and irradiated directly.

Doses in a range between 0.5 and 3.0 kGy were applied to both, meat and model media samples. Irradiation experiments were repeated twice. Surviving bacteria were quantified after homogenisation of each sample in 225 ml saline using a stomacher 400 (Seward
Medical, London, Great Britain), dilution in saline, plating on NB-agar and aerobic incubation for 48 h at 37 °C. Non-irradiated samples were used to determine initials counts.

3. Results and discussion

If irradiation is performed at cooling temperatures in nutrient broth (NB) as a model medium, a reduction rate of about 3–4 decimal units was achieved at the dose of about 1 kGy $D_{10}$ is 0.27 kGy. No bacteria were detectable after an irradiation dose of 2 kGy was applied to NB-cultures of *E. coli* DSM 498.

On the basis of such inactivation data, mathematical modelling based on the first order kinetics or the Weibull model (Peleg & Cole, 1998; Stumbo, 1973) is used to predict the remaining microbial content of food products after preservation processing. To ensure food safety, reliable microbial inactivation data are essential. In the present study, therefore, inactivation rate of *E. coli* DSM 498 was examined in a real food matrix (minced turkey meat), additionally. The results clearly demonstrate, that comparable inactivation rates of bacteria grown in minced turkey meat (Fig. 1) cannot be achieved with the same dose as in NB. In meat a dose of 1 kGy caused a reduction rate of only 2 decimal units with a corresponding $D_{10}$-value of 0.47 kGy, respectively.

The surrounding matrix clearly influences the resistance of *E. coli* to ionising radiation. In food, there is a competition between bacteria and food components, such as proteins, for interactions with radicals formed during the process (Patterson, 1989). A similar dependence of inactivation on the surrounding matrix during irradiation and more over on the growth matrix was estimated for *Salmonella typhimurium* DSM 554 (Mayer-Miebach & Spiess, 1999).

The results point up the importance of the demanding of the Scientific Committee on Food of the European Commission (SCF) to determine the critical limits for the doses applied for microbial destruction at realistic intrinsic and extrinsic conditions in food (SCF, 2003). The critical radiation target is the chromosomal DNA damage, which inactivates microorganisms mainly caused by hydroxyl radicals released from the hydration layer around the DNA molecule (Moseley, 1989). Low temperatures enhance bacterial resistance to ionising radiation by reducing the formation and the mobility of the hydroxyl radical as the principal agent of cell destruction (Billen, 1987; Taub, 1979; Thayer & Boyd, 1993, 2001). As a consequence, microbial sensitivity to irradiation is higher at ambient temperature than at freezing temperatures; e.g. the radiation resistance of vegetative bacteria is doubled in the frozen state (Clavero, Monk, Beuchat, Doyle, & Bracket, 1994). Minced turkey meat usually is stored and transported in the frozen state.

Inactivation rates of *E. coli* DSM 498 were measured in minced turkey meat both at cooling temperatures between 0 and 2 °C and in the frozen state (−85 °C), therefore. With $D_{10}$-values of 0.48 and 0.72, the results indicate a lower inactivation rate for the frozen meat product, as anticipated (Fig. 2). Comparable data are published for *E. coli* O157:H7 in ground beef, where a $D_{10}$ reduction of 0.21 kGy was required at a temperature of 4 °C, while a $D_{10}$ reduction of 0.31 kGy was necessary at −16 °C (Clavero et al., 1994).

Data obtained from irradiation inactivation of *E. coli* DSM 498 seem to comply with survivor curves fitted by first order kinetics (Figs. 1 and 2). Using the Weibull
model n-values close to 1 (n = 0.9–1.2) were found and the semi-logarithmic survivor curves (Figs. 1 and 2) did not show noticeable upward (n < 1) or downward concavity (n > 1). If the parameters b and n (Eq. (2)) are used to plot resistance curves (Fig. 3), only E. coli DSM 498 in minced turkey meat irradiated at cooling temperatures showed no maximum (n = 1), a criterion for the applicability of first order kinetics. However, as the maxima resulting from irradiation inactivation curves in frozen meat and model medium (Fig. 3) were not pronounced, first order kinetics may be applied in this cases, too.

4. Conclusions

The results presented indicate that inactivation kinetics for controlling pathogen inactivation in food systems have to be estimated on the basis of specific microorganisms and food matrices of concern and should include further extrinsic factors. In order to find the best compromise between quality and safety of commercially irradiated products, the Weibull model should be taken into consideration, as it may prevent product damages and quality impairment by over-treatment or ensure food safety by undertreatment, respectively. For E. coli DSM 498, however, conventional first order kinetics deliver $D_{10}$-values of sufficient accuracy.

Acknowledgments

This work was kindly supported by the Federal Ministry of Consumer Protection, Food and Agriculture, Germany. Special thanks to M. Knörr, A. Feuerstein, G. Moritz, C. Scheid, Institute for Process Engineering, Federal Research Centre for Nutrition, Germany, as well as K. Pardey (data processing in Fig. 3) and Professor H. Schubert, Institute of Food Process Engineering, University of Karlsruhe, Germany.

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