AUTOMATED TURBIDIMETRY TO ESTIMATE INJURY OF *Salmonella newport* CELLS AFTER IRRADIATION IN FROZEN POULTRY

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Background

Irradiation treatment of poultry meat with low doses (1.5-3.0kGy) has been recommended to control potential risks of food-borne pathogens as *Salmonella* (CAST, 1996). Such recommendations are based on various studies, i.e. those from Thayer et al (1990), showing that the decimal reduction irradiation dose (ID_{10}) for *Salmonella* in foods could be estimated between 0.45-0.70 kGy. Therefore a minimum dose of 1.5 kGy would produce at least a 99% reduction of the risk. Like other bactericidal processes irradiation has also sublethal damaging effects on a fraction of the surviving cells. Injured cells loose their ability to grow on the presence of selective agents and have extended lag times due to repair processes Mackey and Derrick (1982). Rapid indirect enumeration methods as turbidimetry can not only give an estimation of the number of cells, but also of their growth ability, and thus be used to assess the sublethal effect of irradiation and estimate recovery times (Fielding et al. 1994).

Objective

To estimate the sublethal damage of *Salmonella newport* cells after irradiation of poultry meat by a turbidimetric method.

Material and Methods

Inoculum preparation: *Salmonella newport*, a meat isolate from the Instituto de Tecnología de Alimentos INTA, was aerobically grown in 40 ml of Brain Heart Infusion (BHI) for 18 h at 35°C. For inoculation the culture was prepared by centrifugation at 3000 g at 4°C for 30 min. and the pellet suspended in 10 ml of sterile physiological solution. Poultry samples: commercial frozen breast pieces were used to obtain samples for inoculation. After flaming and eliminating the surface layer, cylinders (area 4.95 cm^2, weight 6-10 g) were aseptically obtained. Inoculation procedure: meat cylinders were inoculated at the center with 100 µl of the microorganism suspension per 10 g of weight, and left to absorb for 15 min. Each sample was then placed into a sterile stomacher bag and between dry ice slabs for the irradiation procedure. Irradiation procedure: Frozen samples were gamma irradiated with a ^{60}Co source at the facilities of the Comisión Nacional de Energia Atómica, Argentina. Irradiation doses used for the injury assessment were: 1.62, 2.10, 2.60 and 3.12 kGy. Enumeration procedure: after irradiation 9 ml of peptone water (0.1%) per gram of material were added to the stomacher bags and homogenized for 2 min in a stomacher (Lab Blender 400, Seward Med, England). An aliquot of 0.02 ml of appropriate decimal dilutions in 0.1 % peptone were spread plated on a quarter of plate containing a non selective medium (BHI agar, Detroit, MI) or a selective medium Brilliant-green Phenol-red Lactose Sucrose agar (BPLS, Merck, Germany). Plates were incubated for 48 hours at 30±1 °C. Turbidimetric measurement of growth: Bioscreen C analyser system (Labsystem, Finland) with vertical light photometry detection was used to measure the optical density (OD) of multiple samples. From OD curves, the system provides the detection time (DT) parameter as the time to reach a threshold OD, where a significant OD change occur. DT can be considered as an apparent lag phase including the lag phase plus the time to reach the threshold OD after growth starts. Irradiated or non-irradiated samples homogenates (50 µl) were inoculated by triplicate, into honeycomb wells containing 250 µl of a non selective medium: tryptone soy broth (TSB, Difco, USA), or Rappaport Vassiliadis broth (RV, Merck, Germany) as a selective medium for *Salmonella*. The increase in turbidity was monitored automatically every 30 min at wide band (420-580 nm) at 37°C for 24 hours. Plates were shaken before measurements. Calibration curves: to estimate DT for a non-injured inoculum calibration curves were created using non-processed samples prepared by diluting a 18 h culture of the microorganism in either 0.1% peptone water or in a sterile 1/10 poultry homogenate in peptone water. Calibration curves for *Salmonella newport* cells were constructed by plotting the log CFU/ml enumerated in BHI agar against DT in either TSB or RV broth.

Lag extension measurement: lag extension was calculated as:

$$\text{Lag extension} = DT(n_0 \text{injured cells}) - DT(n_0 \text{ non-injured cells})$$

according to Mackey and Derrick (1984), where n_0 represents the number of cells. $DT(n_0 \text{injured cells})$ was experimentally measured, and $DT(n_0$ non-injured cells) estimated from the calibration curve for the same n_0 value.

Results and Discussion

Cell death during irradiation: the log number of surviving cells at each irradiation dose were plotted to obtain irradiation lethality curves. ID_{10} values obtained were 0.63 kGy when cells were plated on BHI agar, and 0.58 kGy on BPLS agar. The increasing fraction of injured cells with higher irradiation doses unable to recover in BPLS agar, would account for the lower ID_{10} value in that mediun.

Calibration curves:

OD curves of non-processed cells in peptone water showed longer DT values in RV broth than in TSB as could be expected from a selective media (Fig 1). More important RV broth was less suitable for DT quantification as OD curves were flatter, making DT determination less accurate. In poultry homogenates some OD curves in TSB presented shoulders at the start, nevertheless, DT values could be estimated. However, OD curves from these homogenates in RV broth could not be used to determine DT values as they did not present clear inflexion points. From these considerations, and the fact that poultry homogenates presented a more similar environment for comparison with the processed inoculum, this calibration curve was used for calculation of the lag extension. Regression parameters from calibration curves are shown in Table 1

Table 1. Slope and determination coefficient (R^2) from calibration curves (log CFU/ml *vs* DT) of *Salmonella newport*

Peptone water				Poultry homogenate			
TSB		RV broth		TSB		RV broth	
Slope: -0.0157	R^2: 0.999	Slope: -0.0101	R^2: 0.923	Slope: -0.020	R^2: 0.965	Slope:ND*	R^2: ND*

*ND: Not Determined as OD curves were not suitable

Lag extension and Injury

At the same inoculum DT values for processed samples were in all cases over the calibration curve for a non-processed inoculum (Fig. 2), from the figure it also appears that differences in DT increase for lower counts/higher irradiation doses. The mean lag extension in the range of dose irradiation tested was 45 ± 20 min. It must be noted at this point that the absolute values for lag extension observed would be the effect of the irradiation plus an amount of injury caused by the freezing-thawing process. When lag extension was plotted against the number of log reductions (Fig. 3) or the irradiation dose (Fig. 4) a tendency to increasing lag extension times for more severe treatments is seen, although it was not statistically significant ($p>0.05$). This result agrees with those from Mackey and Derrick (1982) who reported a slow lineal increase in lag extension for *S. typhimurium* cells irradiated in phosphate buffer. In our case the facts that only a limited range of low irradiation doses were tested, and the observed high variance between replicates make more difficult to detect significant differences between irradiation doses

Conclusion

In the range of recommended irradiation doses for this kind of product, injured cells needed a relatively short time to recover given favourable conditions. Automated turbidimetry prove to be a rapid and simple alternative to assess sublethal effects of the process, through the lag extension calculation.

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