# A Microbiological Method for Identifying Irradiated Frozen Chicken

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Investigations were carried out to identify irradiated frozen chicken using the synergistic killing effects of two types of combination treatments. In a model study, frozen chicken was contaminated with Lactobacillus plantarum, irradiated and heated in water bath. An irradiation dose of 3 kGy plus heating at 55°C for 15 minutes resulted in a 3 log reduction in the cfu/g in irradiated samples. Also, a dose of 4 kGy plus treatment with 8-8.5% NaCl in aqueous solution synergistically reduced the Lactobacilli load by about 4 log cycle. Finally, the result of 8% NaCl plus 4 kGy on the natural Lactobacilli load in frozen chicken was significant to differentiate between irradiated and unirradiated frozen chicken. The NaCl treatment method could therefore be further developed to become a control method for identifying irradiated meat products.

## INTRODUCTION

There is the need to control trade and imports in irradiated food items in view of differing food regulations existing from one country to another. Consequently, there is a demand for detection methods. Such methods will serve both to prevent illegal import of irradiated foods, to check on compliance with labelling regulations and also serve to prevent the multiple irradiation of foods. Many methods are being developed to identify irradiated foods. These include the electron spin resonance (ESR) technique (RAFFI et al., 1988), the thermoluminiscence and chemiluminiscence (HEIDE & BÖGL, 1988), measurement of o-tyrosine using chromatography (MEIER et al.,1988) and DNA changes (PLEGEAU & COPIN, 1988). Among the biological techniques, SJOBERG et.al., (1990) reported that the best methods for control purpose were the direct epifluorescent filter technique with aerobic plate counts in combination with chemiluminescence measurements. Other biological methods include microflora shift in irradiated foods (KAMPELMACHER, 1988). In general, the gram-positive bacteria including Pseudomonas, are more sensitive to irradiation than are the gram-positive organisms such as the lactic acid bacteria, Moraxella-Acinetobacter and Micrococcus species (FARKAS, 1989). Combination treatments help to maximise the effectiveness of mild irradiation treatments while minimizing other organoleptic quality changes (CAMPBELL-PLATT & GRANDISON, 1989). Microfloral in food items were found to be more sensitive to heat and other treatments after irradiation (KISS & FARKAS, 1981). Irradiation combined with heat act synergistically to exert their effects by energy absorption, leading to damage to cell membranes or DNA (MAXCY & ROWLEY, 1978). High salt treatment plus irradiation has also been reported as a combination treatment affecting the radiation resistance of even the most highly radiation-resistant bacteria (FARKAS & ROBERTS, 1976). Lactobacillus plantarum was reported by HASTINGS et al.,(1986) to have high radiation resistance in radurized meat. These facts dictated the choice of Lactobacilli for this study. This study used the synergistic effects of combined irradiation, heat and NaCl treatments to identify frozen chickens that have been

irradiated.

## 2. MATERIALS AND METHODS

Chilled chicken drum sticks were "cleaned" with irradiation at 5 kGy and stored until required. Pure culture Lactobacilli plantarum, (CIVO ATCC No 8014) preserved at -80°C, were thawed, inoculated on TSI slant in a test tube, and incubated at 37°C for 24 hours. Two loops from the TSI slant was inoculated into a selective medium, the "de MAN, ROGOSA SHARPE (MRS) (1960) broth" (Oxoid, U.K.) and incubated at 30°C for 24 hours in an orbiter shaker incubator (GALLENKAMP, U.K). Good growth was judged by the turbidity of the broth. The 200ml Lactobacilli culture was added into sterile 1800ml MRS broth and mixed thoroughly giving a population of $10^6$ cells per ml. Thawed, cleaned chicken, were dipped into the inoculation bath for 30 minutes. The contaminated chicken legs, vacuum sealed in stomacher bags, were packed in dry ice (-80°C) inside polysterene boxes and properly tape-sealed to ensure that the samples were well frozen during irradiation. The boxes were irradiated using a source of $7 \times 10^{14}$ Bq (18,900 Curies) $^{60}$Cobalt with a dose rate of 1.1264 kGy per hr. Samples from contaminated, irradiated and unirradiated (control) chicken were thawed blended in aqueous diluent containing 0.1% peptone + 0.85% NaCl. The extracts, $10^{-1}$ dilution, were poured into sterile screw-capped plastic tubes for further treatments. Extracts were divided into three groups, each group treated with irradiation plus heating; irradiation plus 8% NaCl solution; and the untreated control group respectively. Test samples (containing natural Lactobacilli contamination) were also analysed.

## RESULT

Bacterial loads from the treatment groups are shown in Figures 1-4.

## DISCUSSION

For these methods to be useful, the synergistic effects of the treatments must be highly significant to at least 2 log reduction in bacterial load. The heat treatment of 55°C for 15 minutes would only be beneficial if the frozen chicken has been irradiated at doses above 3.0 kGy. For the NaCl treatment, an optimum salt solution of 8% is adequate to express the synergistic effects desired (figures 3 & 4). In the bland determination, no organisms developed in frozen chickens irradiated at 4 kGy and treated with 8% NaCl. Whereas a count of more than 4 log cfu/g was recorded in the unirradiated 8% salt treated samples.

## CONCLUSION

It is concluded that frozen chickens irradiated above 3 kGy could be identified by these methods. As a trade control method, and like all biological methods, There is the disadvantage of time. It takes 7-10 days to arrive at a conclusion and this may affect trading schedules.

## REFERENCES

CAMPBELL-PLATT, G; A.S. GRANDISON (1989): Food irradiation and combination process. Proc. 7th I.M.R.P. meeting, 23-28 April, 1989 Noordwijkerhout, the Netherlands.

Fig. 1: Survival of Lactobacilli in Irradiated Chicken at diff. Doses & Temp

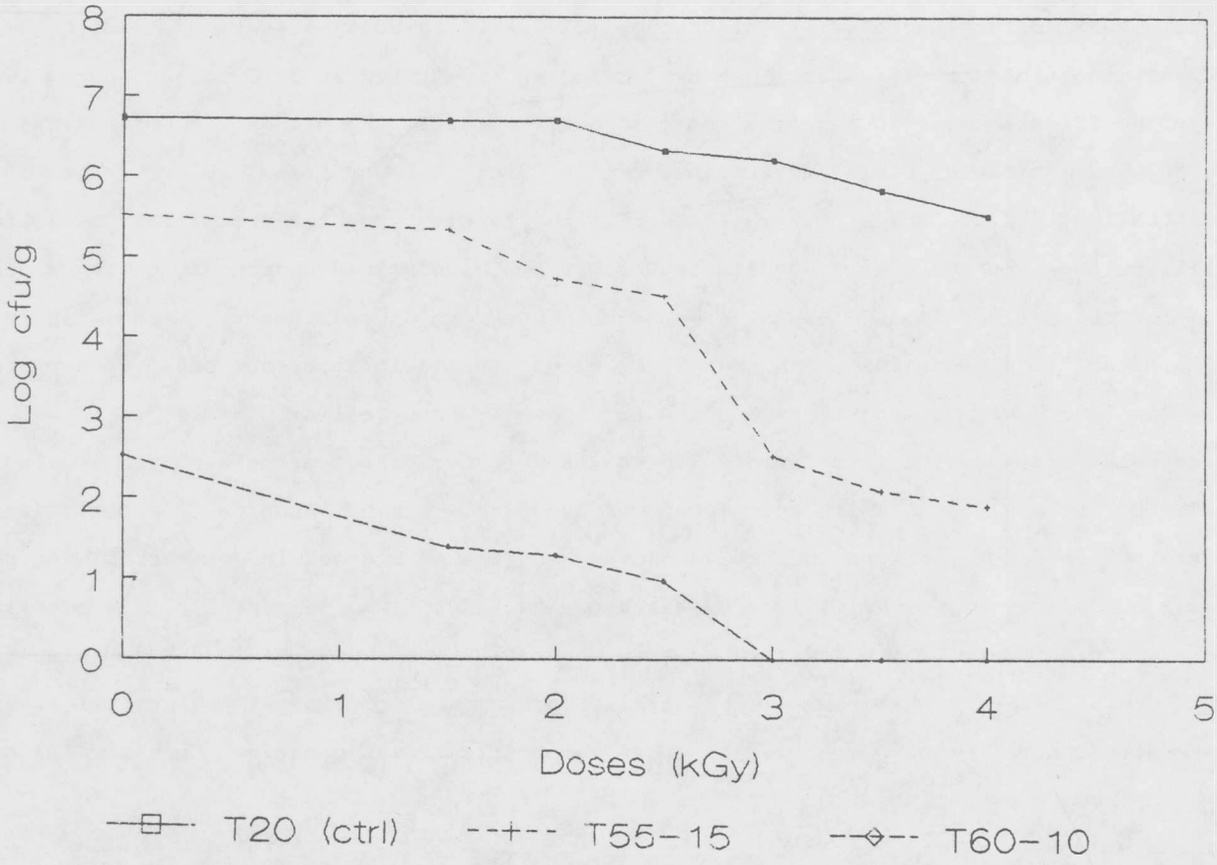



Fig. 2: Survival of Lactobacilli in Irradiated Chicken at diff. Temp/Time

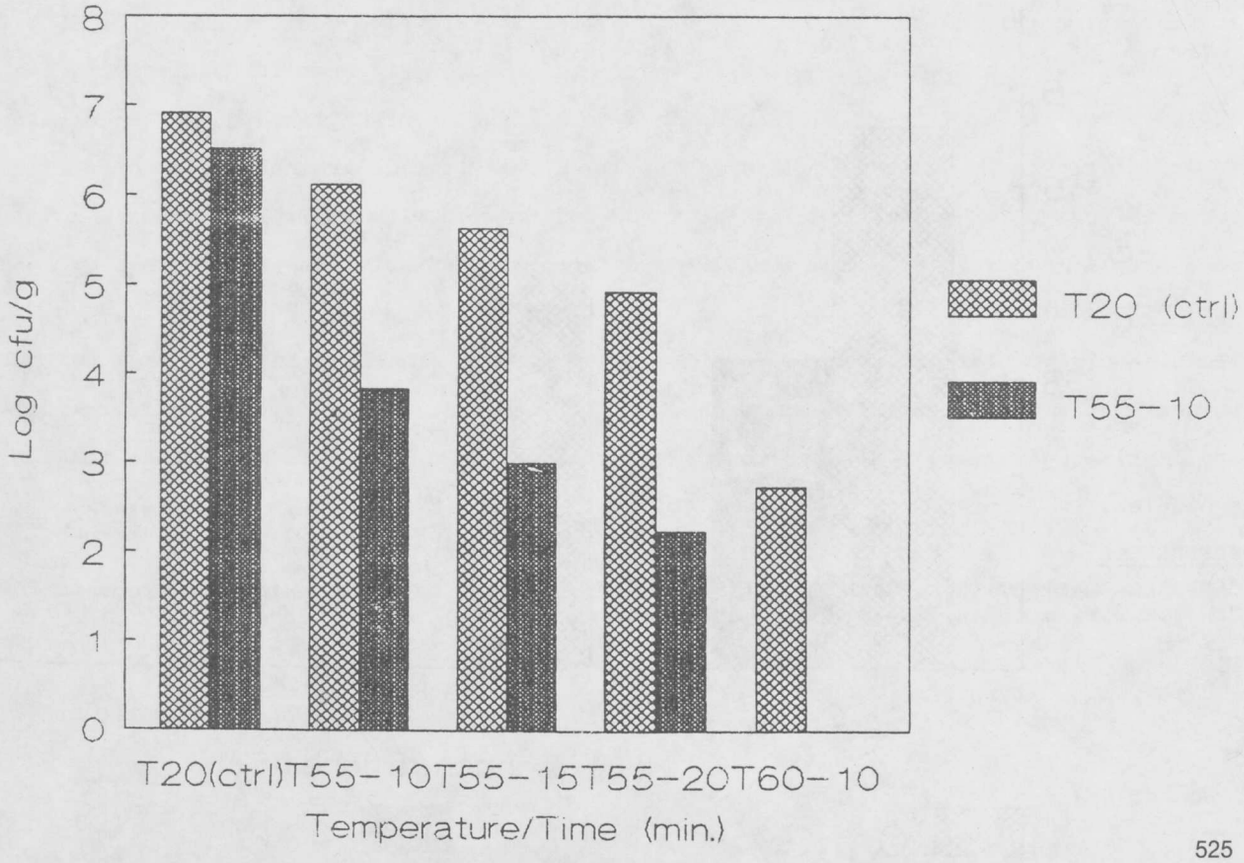


Fig.3: Survival of Lactobacilli in Irradiated Chicken at diff. NaCl Concns

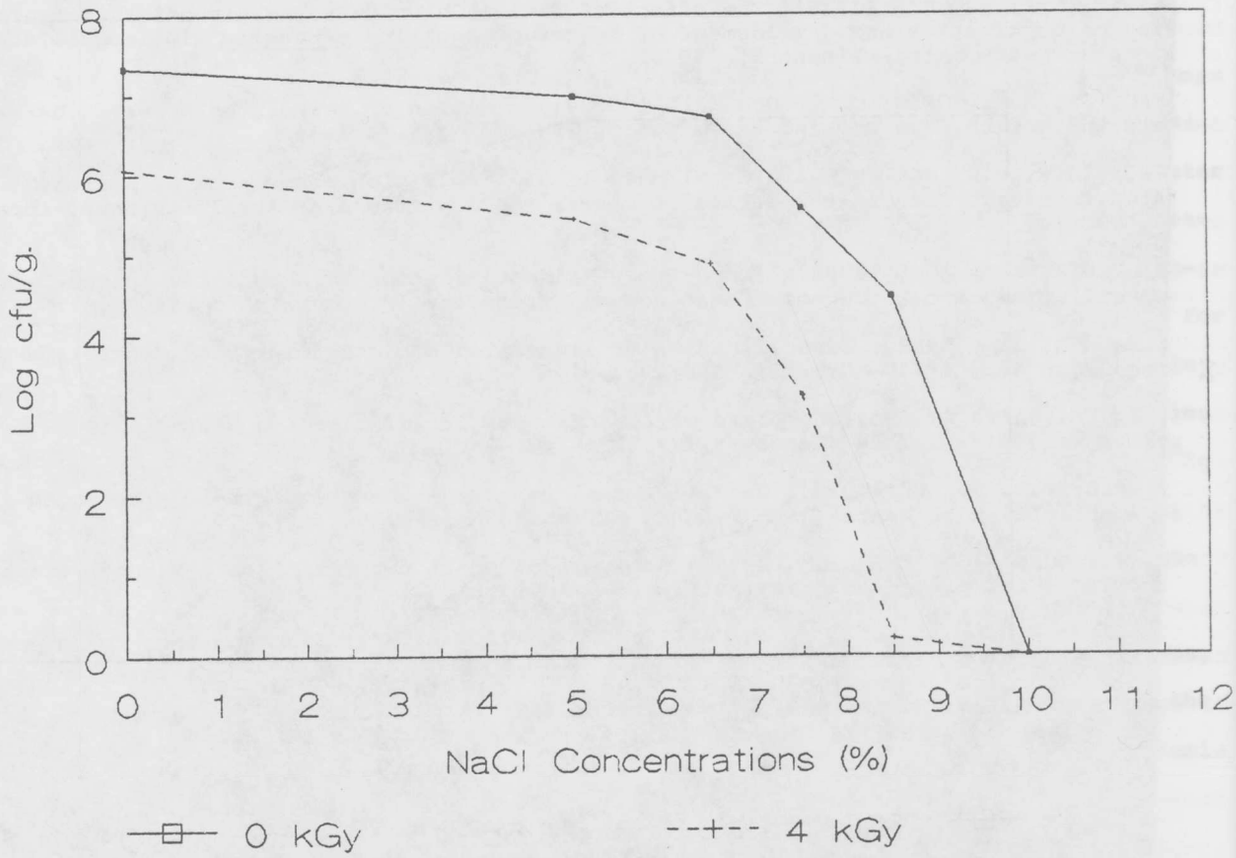
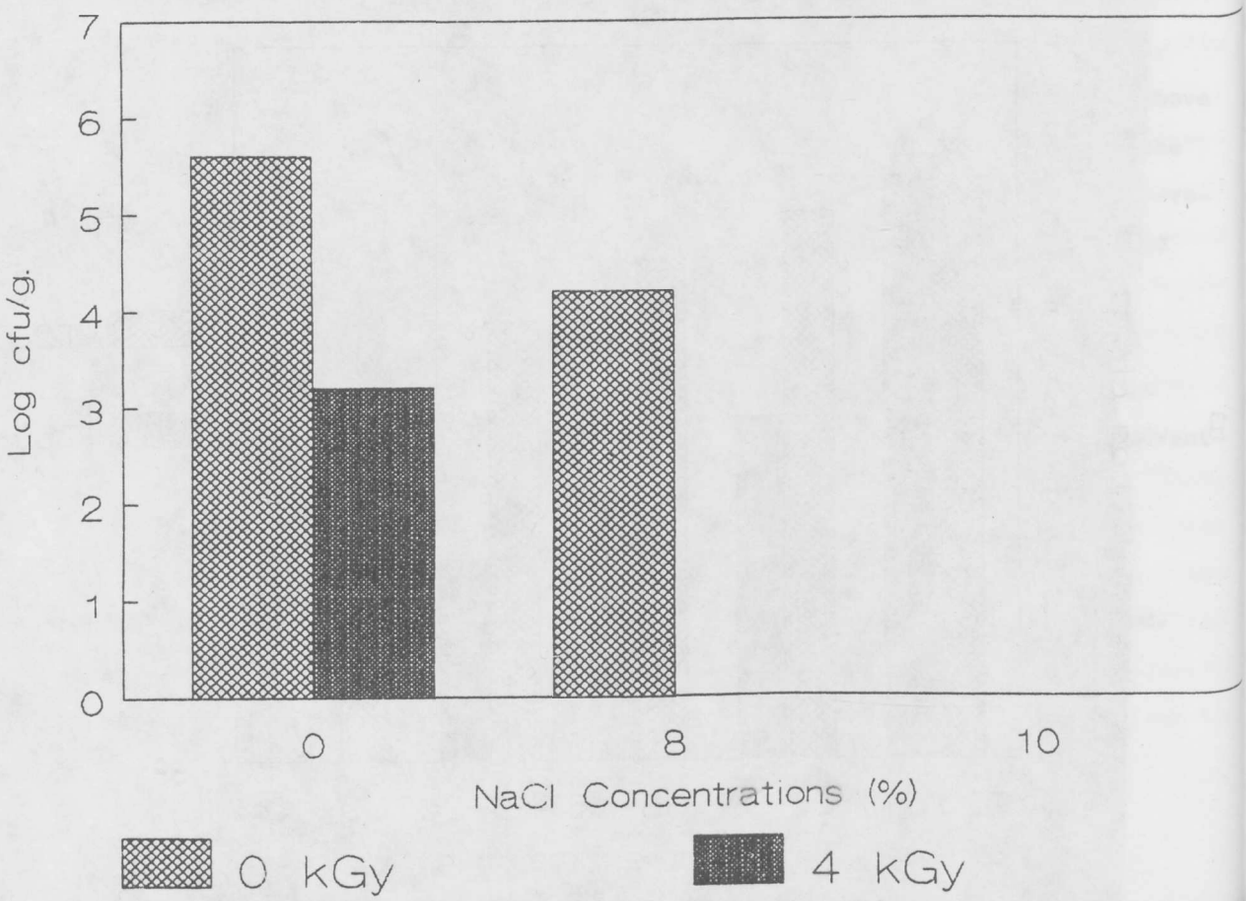



Fig. 4: Survival of Lactobacilli in Irradiated Chicken at 8 % NaCl Concn



De MAN, J.C.;M.ROGOSA: M.E. SHARPE (1960): A medium for the cultivation of Lactobacilli, J. Appl. Bacteriol. 23,130-135

FARKAS, J. (1989): Microbiological safety of irradiated foods. IAEA-TECDOC 490, 53-75

FARKAS, J.,T.A. ROBERTS (1976): The effect of Sodium Chloride, gamma irradiation and/or heating on germination and development of spores of Bacillus cereus in single germinants and complex media, Acta Aliment 5, 289.

PLEGEAU, J.; M.P. COPIN; C.M. BOURGEOIS (1988): Detection of irradiated Norway Lobsters by DNA elution method. WHO Working Group Report ISH-HEFT 125, 453-460

HASTINGS, J.W.;W.H. HOLZAPFEL: J.G. NIEMAND (1986): Radiation resistance of Lactobacilli isolated from radurized meat relative to growth and environment. Appl. Environm.Microbiol. 52(4), 898-901.

HEIDE, LYDIA; K.W. BÖGL (1988): Improving the identification of irradiated spices by measuring chemiluminescence, thermoluminescence and viscosity. Fleischwirtschaft 68, 1559-1564.

KAMPELMACHER, E.H.(1988): Identification of irradiated foods by microbial control. WHO Working Group Report ISH-HEFT 125, 190-206.

KISS, I.; J. FARKAS (1981): Combined effect of gamma irradiation and heat treatment on microflora of spices. IAEA Vienna, STI/PUB/568 107-359

MAXCY, R.B; D.B. ROWLEY (1978): Radiation-resistant vegetable bacteria in a proposed system of radapertization of meats. IAEA Vienna, STI/PUB/470,347-359.

MEIER, W.; BURGIN; D. FROHLICH (1988): Analysis of small o-tyrosine as a method for the identification of irradiated chicken meat. Beta-gamma 1, 34-36

RAFFI, J.J.; J.P.L. AGNEL: L.A. BUSCARLET: C.C. MARTIN (1988). Electron spin resonance identification of irradiated strawberries. J.Chem.Soc. Faraday Trans. 1, 84, pp.3359

SJÖBERG, ANNA-MAIJA: M.MANNINEN., PIA HARMALA: SINIKKA PINNIOJA (1990): Methods for detection of irradiation of spices, Z. Lebensm, Unters. Forsch, 190, 99-103