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Presensitization of microorganisms by acid treatments to low dose gamma irradiation with special reference to *Bacillus cereus*

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Abstract

Effect of acid presensitization to low dose gamma irradiation on *Bacillus cereus* in sheep/goat meat stored at refrigeration temperature was assessed. Food grade organic acids (viz. propionic, lactic and acetic acids) were used as presensitizers followed by treatment with 1, 2 and 3 kGy irradiation doses (Co^{60}). Two percent acetic acid plus 3 kGy irradiation elicited most effective to lower the total viable count and *B. cereus* count. Combination of treatments availed to reduce the dose required for elimination of radioresistant *B. cereus* and also masked the irradiation odour developed due to radiolysis. The study also revealed substantial increase in shelf life of mutton after combination treatment than single treatment without any adverse effect on acceptability of meat.

Keywords: Meat; Presensitization; Acid; Irradiation; *Bacillus cereus*

1. Introduction

Food-borne infections and intoxications are major health problems in developing countries. Most of the incidences of meat-borne illnesses are attributed to *Staphylococcus aureus*, *Clostridium* spp., *Bacillus cereus*, *E. coli*, *Salmonella* spp., etc. which are commonly found in meat. Sinha and Mandal (1977) isolated different microorganisms from fresh market meat and observed that *B. cereus* contributed 9% of total load. As this bacterium can grow in a wide range of temperature (10–45°C) and pH (6–9°C), it poses a great threat to public health. Various attempts have been made to eliminate or reduce the incidence of these pathogens by using various treatments like use of food grade chemicals, salts, antibiotics, heat, low temperature and recently irradiation (Jay, 1983). Gamma irradiation destroys Gram negative microflora completely, but it is not very effective against Gram positive sporeforming bacteria like *B. cereus, Clostridium* spp., and spores are found to be comparatively resistant. Thayer and Boyd (1994) required 7.5 kGy dose (5°C) to eliminate a challenge of 4.6×10^3 *B. cereus* from mechanically deboned chicken meat. Similarly, Shay, Egan and Wills (1988) required 2–5 kGy dose of gamma irradiation to destroy vegetative bacteria and 45 kGy to destroy the spores. High doses of irradiation can eliminate the Gram positive microflora without altering the texture and structure of meat substantially, but meat tends to develop 'burnt' odour due to radiolysis (Lefebvre, Thibault, Charbonneau, & Piette, 1994).

Food contains a myriad of scavenging substances, therefore, the radiosensitivity of a given microorganism may depend on the nature of food (Goldblith, 1971). Food often acts as radioprotective agent so that higher radiation doses are needed to inactivate or kill the microorganisms than that found in simple buffered medium. It is truly applicable for meat, which possess great scavenging activity.

Radiosensitizers are the physical processes or chemical substances, which enhance the effect of radiation and can also overcome the scavenging property of meat. These sensitizers can reduce bacterial levels and inactivate the enzymes. Consequently, the cellular integrity of

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food and its organoleptic properties may be retained (Schubert & Stegeman, 1981). In the field of food preservation, they can be used to lower the dose of irradiation required for reduction in bacterial population and also to avoid radiation odour, which may develop on application of high doses. Chemicals like salt, inorganic ions, nitrites, antibiotics, organic acids, etc. can be used to lower down the dose requirement to kill the radiation resistant organisms. Organic acids like lactic, propionic, acetic are commonly used in food industry for food preservation as they are categorized as Generally Recognized As Safe (GRAS). These acids are known to reduce the pH of meat and cause detrimental effect on bacterial cell.

In the present study, food grade organic acids (viz. propionic, lactic and acetic acid) were assessed for their radiosensitizing property against total viable count and *B. cereus* and their synergistic effect in combination with irradiation on shelf life of sheep meat stored at refrigeration temperature.

2. Materials and methods

Seven average-sized sheep carcasses slaughtered at Deonar Abattoir, Mumbai, India were selected randomly. Four carcasses were deboned and deglanded with hygienic precautions and meat was packed in various polyethylene packs (50 g each). Meat from three carcasses was exposed to 1, 2 and 3 kGy doses of irradiation (irradiation control) and one carcass was kept as control (no treatment). The remaining three carcasses were used for carcass washing by three acids viz. propionic acid, lactic acid and acetic acid (S.D. Fine Chemicals, Mumbai). Based on the results of a pilot study, the best suitable concentration of individual acid was fixed as 1% for propionic acid, 2% for lactic acid and 2% for acetic acid. The carcasses were sprayed with acid solutions at a fixed pressure of 3 kg/cm^2 for 2–4 min and allowed to drain. All acid washed carcasses were deboned, deglanded and packed in low density polyethylene (LDPE) pouches. Some packs from each acid treatment were maintained as acid control and remaining packs were exposed to 1, 2 and 3 kGy doses of gamma irradiation in package irradiator (designed by IRH, AECI, Canada presently MDS Nordion Int Inc.) with Co^{60} having a dose rate of 71 Gy/min at Food Technology Division, Bhabha Atomic Research Center, Mumbai, India.

Meat samples from each treatment along with the untreated control were stored at refrigeration (5–7°C) temperature. Analysis of the samples was carried out immediately after treatment and subsequently at regular intervals until the spoilage started.

Study was repeated on six different occasions and the nature of treatments, number of meat samples collected and analysis schedule is presented in Table 1.

The pH of samples was determined as per the method of Pippen, De Fremery, Lineweaver and Hanson (1965). Meat (15 g) was blended with 30 ml distilled water at 27–30°C and pH was noted with a glass electrode pH meter.

Sensory evaluation of meat was carried out by four member sensory panel using a six-point standardize scale for colour and a four-point scale for odour as per Woolthuis and Smulders (1985) and Acuff, Vanderzant, Savell, Jones, Griffin, and Ehlers (1987).

For total viable count, a 10-g sample was homogenized in 90 ml NSS and was serially diluted in 9 ml

Table 1
Nature of treatments, number of samples collected and analysis schedule for sheep/goat meat samples (study was repeated six times)[a]

Analysis interval	Control	Acid control			Irradiation control			Combination treatment									Total
		1% P.A.	2% L.A.	2% A.A.	1 KGy	2 KGy	3 KGy	1% Propionic acid +			2% Lactic acid +			2% Acetic acid +			
								1 KGy	2 KGy	3 KGy	1 KGy	2 KGy	3 KGy	1 KGy	2 KGy	3 KGy	
0 th day	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
3 rd day	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
6 th day	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
8 th day	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	16
9 th day	ND	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
12 th day	ND	ND	ND	ND	1	1	1	1	1	1	1	1	1	1	1	1	12
15 th day	ND	ND	ND	ND	1	1	1	1	1	1	1	1	1	1	1	1	12
18 th day	ND	ND	ND	ND	ND	1	1	1	1	1	1	1	1	1	1	1	11
21 th day	ND	ND	ND	ND	ND	1	1	ND	1	1	1	1	1	1	1	1	10
24 th day	ND	ND	ND	ND	ND	ND	1	ND	1	1	ND	1	1	ND	1	1	7
27 th day	ND	ND	ND	ND	ND	ND	ND	ND	ND	1	ND	1	1	ND	1	1	5
30 th day	ND	ND	ND	ND	ND	ND	ND	ND	ND	1	ND	ND	1	ND	ND	1	3
33 th day	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1	ND	ND	1	2
Total	4	5	5	5	7	9	10	8	10	12	9	11	13	9	11	13	141

[a] P.A., propionic acid. L.A., latic acid. A.A., acetic acid ND, not done (samples were not analyzed further due to spoilage.

NSS. Inoculum (0.1 ml) was seeded on plate count agar and incubated at 37°C for 24–48 h. For the *B. cereus* count, egg yolk agar and *B. cereus* selective agar (Hi-Media, Mumbai, India) were used followed by the confirmation with biochemical characterization as per Sneath, Peter, Nicolas, and Hold (1986) and Cowan and Steel (1993).

2.1. Statistical analysis

Data for total viable count (TVC), *B. cereus* count and pH for alone and combination treatments were analysed by analysis of variance for a completely randomized design with six replicates.

3. Results and discussion

3.1. Immediate effect of treatments

The initial total viable and *B. cereus* counts in control (untreated) mutton samples were 5.15 and 3.40 $\log_{10}$ cfu/g, respectively with initial pH 6.64 and acceptable colour and odour (1.00). These parameters were within normal limits (Vijaya Rao, Bhagirathi, & Sharma, 1983).

3.1.1. Acid treatment

Marginal antimicrobial effect was observed when carcasses were washed with organic acid solutions at fixed pressure of 3 kg/cm^2. Reductions in total viable counts were 4.80, 4.81 and 4.59 $\log_{10}$ cfu/g after spraying with 1% propionic, 2% lactic and 2% acetic acid, respectively (Fig. 1). Sensitization of *B. cereus* with acids caused reduction in counts to 3.12 (1% propionic acid), 3.10 (2% lactic acid) and 3.00 (2% acetic acid) $\log_{10}$ cfu/g (Fig. 2). Antimicrobial property of acetic acid was found comparatively greater than lactic and propionic acids. Levine and Fillers (1940) examined the action of acetic acid on microorganisms in meat. They concluded that acetic acid was effective not only because of hydrogen ion concentration but also due to function of undissociated acetic acid molecules. The results are in accordance with Woolford (1975) who stated that acetic acid was more effective against *Bacillus* spp. and other Gram-positive organisms than lactic acid. In another study, Bell and Lacy (1983) reported that the lowering of pH in meat to less than 5 prevented the growth of *Bacillus* spp.

pH of meat was reduced substantially after acid treatments. The values were in accordance with Levine and Fillers (1940) and Surve, Sherikar, Bhilegaonkar, and Karkare (1991) (Fig. 3). Colour and odour scores of meat treated with acids were quite acceptable (1.00).

In the experiment, concentrations of acids were fixed to 1% for propionic acid and 2% for lactic and acetic acids as further higher concentrations caused discolouration and lowered the acceptability of meat in the pilot study.

Higher concentrations of acid, however, reduced the microbial count or sensitized them in greater extent to ionizing radiations, it also lead to brown discolouration of meat. Mulder and Krol (1975) examined the effects of acids on microbiological and sensory attributes at various concentrations. They found maximum inhibitory effect by 5% lactic acid but lead to discolouration of meat.

3.1.2. Irradiation treatment

When samples were irradiated at three different doses viz. 1, 2 and 3 kGy, reduction in the TVC (3.89, 3.35 and 2.75 $\log_{10}$ cfu/g) and *B. cereus* count (3.12, 2.87 and 2.10 $\log_{10}$ cfu/g) was observed. A dose-dependent reduction pattern was also observed by Maxcy (1983). He further stated that the survival of bacterial flora was dependent on condition of application, microenvironment of the food as well as dose of irradiation. Lethal effect of ionizing radiation was attributed to inactivation of bacterial DNA (Ginoza, 1967).

Colour and odour of the irradiated samples were acceptable, except in samples treated with 3 kGy dose,

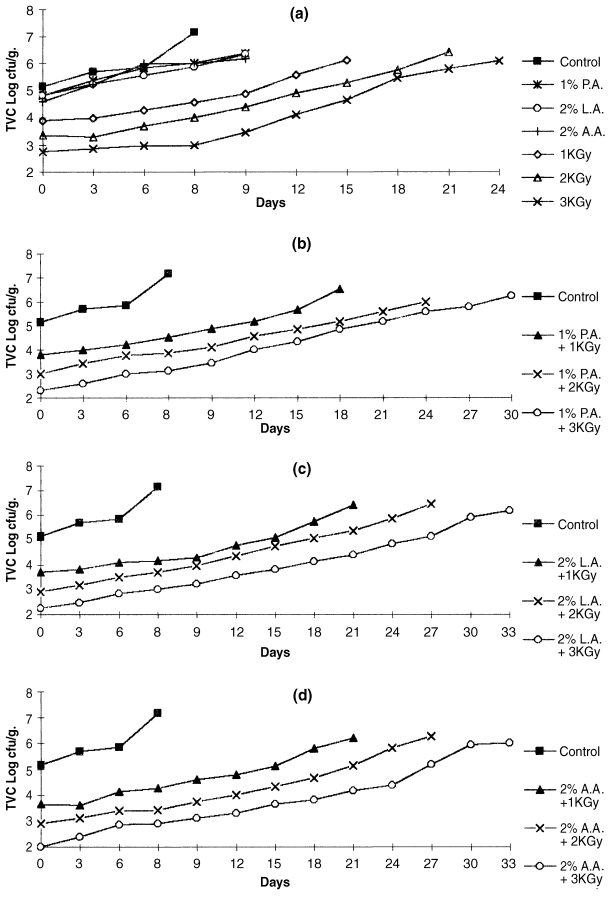

Fig. 1. (a) Effect of acid and irradiation alone on total viable count (TVC). (b) Effect of propionic acid (P.A.) plus irradiation. (c) Effect of latic acid (L.A.) plus irradiation. (d) Effect of acetic acid (A.A.) plus irradiation.

which showed slight burnt odour. Batzer and Doty (1995) stated that undesirable odour produced by irradiation may be due to sulpher compounds whereas, according to Wick (1966) odour may be due to production of methional and phenyl actaldehyde.

After irradiation with 1, 2 and 3 kGy doses the pH of meat samples was not altered significantly ($P > 0.01$; Fig. 3). Dempster (1985) and Lefebvre et al. (1994) made the same observations.

3.1.3. Combination treatment

Presensitization with acid treatment followed by low dose gamma irradiation reduced the total viable count and *B. cereus* population more than irradiation alone. TVC and *B. cereus* counts were reduced to 3.77 and 3.00, and 3.00 and 2.71 $\log_{10}$ cfu/g when sensitized with 1% propionic acid and irradiated with 1 and 2 kGy doses, respectively. Propionic acid plus 3 kGy treatment reduced the TVC to 2.30 $\log_{10}$ cfu/g of meat, whereas, *B. cereus* could not be recovered from the same treatment.

Lactic acid also showed a comparable effect as sensitizer. Samples treated with 2% lactic acid plus 1, 2 and 3 kGy doses showed corresponding reduction in TVC by 1.45, 2.24 and 2.90 log units whereas, *B. cereus* count reduced by 0.63, 0.86 and 1.03 log units, respectively. Total viable counts after 2% acetic acid plus 1, 2 and 3 kGy irradiation treatment were 3.64, 2.90 and 2.00, respectively. *B. cereus* after 2% acetic acid plus 1 and 2 kGy dose reduced to 2.42 and 2.15, respectively, whereas, *B. cereus* could not be detected when acetic acid was used as sensitizer in combiation with 3 kGy irradiation dose. Thayer, Boyd, Lakritz, and Hampson (1995), from their study, concluded that gamma irradiation produced greater lethality for microorganisms at reduced pH. However, no significant difference in pH values of the meat was noted either before or after irradiation. Results are in accordance with Naik, Paul, Chawala, Sherikar, and Nair (1993) who reported that acetic acid pretreatment and irradiation had added effect on microbial quality of meat without serious effect on sensory attributes.

pH values of meat samples were reduced substantially due to pre-acid treatment; but no added effect of irradiation on pH was observed. Combination treatments did not alter the sensory attributes of meat, except 2% lactic acid plus 3 kGy treatment, in which a slight bleached colour developed. However, burnt odour, which developed due to 3 kGy irradiation alone treatment, was

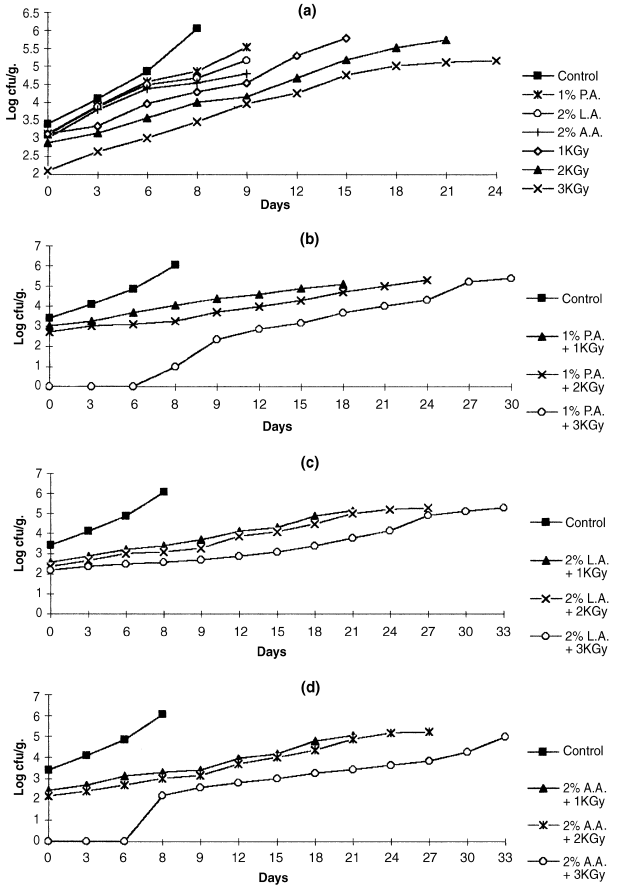

Fig. 2. (a) Effect of acid and irradiation alone on *Bacillus cereus*. (b) Effect of propionic acid (P.A.) plus irradiation. (c) Effect of lactic acid (L.A.) plus irradiation. (d) Effect of acetic acid (A.A.) plus irradiation.

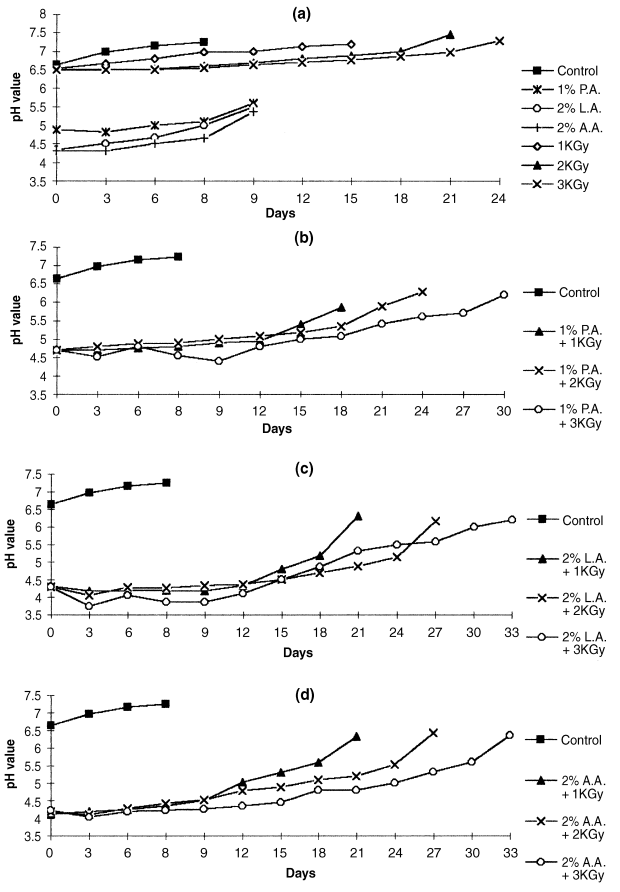

Fig. 3. (a) Effect of acid and irradiation alone on pH of meat. (b) Effect of propionic acid (P.A.) plus irradiation. (c) Effect of lactic acid (L.A.) plus irradiation. (d) Effect of acetic acid (A.A.) plus irradiation.

abolished in combination treatments. Discolouration due to lactic acid in combination with irradiation was also noted by Niemand, Van der Linde, and Holzapfel (1983).

Thus, irradiation, in combination with food grade chemicals like acids (sensitizers), was proved beneficial not only to lower the dose required to kill microbes (*B.cereus*) but also to eliminate the immediate effects of ionizing radiations on sensory attributes of meat.

3.2. Storage at refrigeration temperature

On the 8th day of refrigeration storage, control samples showed spoilage changes. Total viable count and *B. cereus* load were increased up to 7.15 and 6.05 $\log_{10}$ cfu/g. respectively with gradual increase in pH. Further analysis of the samples was not done as samples were totally spoiled.

3.2.1. Acid treatment

All acid-treated samples had a shelf life up to the 9th day of refrigeration storage where, samples showed spoilage changes (off colour and odour) with TVCs 6.35, 6.34 and 6.15 $\log_{10}$ cfu/g following acid treatments with 1% propionic, 2% lactic or 2% acetic acid, respectively and *B. cereus* count was to 5.52, 5.15 and 4.78 $\log_{10}$ cfu/g. Results were in accordance with Surve et al. (1991). Throughout the storage period, TVC and *B. cereus* count were increased gradually whereas, pH values of acid treated samples did not increased beyond 6.5.

3.2.2. Irradiation treatment

Treatment with gamma irradiation was found to extend the shelf life of meat. Samples could be stored up to 15, 21 and 24 days after treatment with 1, 2 and 3 kGy, respectively. At spoilage, TVCs were 6.12, 6.40 and 6.08 $\log_{10}$ cfu/g and *B. cereus* counts were 5.78, 5.73 and 5.16 $\log_{10}$ cfu/g, respectively. The pH of spoiled meat was shifted toward alkalinity with unacceptable sensory characters. Klinger, Fuchs, Basker, Juven, and Lapidot (1986) investigated the effect of irradiation on meat stored at 1–2°C. They found that the sensory quality of irradiated meat decreased to unacceptable level after 3–4 weeks, whereas, Paul, Venugopal, and Nair (1990) observed that, after gamma irradiation by 1 and 2.5 kGy, lamb meat chunks remained acceptable for 3 and 5 weeks, respectively. Low dose gamma irradiation can initially hamper *B. cereus* in meat but it can recover in storage period. A similar finding was made by Grant, Mixon, and Patterson (1993) who could detect toxin produced by *B. cereus* even in irradiated (2 kGy) beef roast and gravy when samples were spoiled; however, the toxin production was delayed by irradiation.

3.2.3. Combination treatment

Acid pretreatment and irradiation showed an added effect on the microbiological quality of meat, which ultimately resulted in the lengthening of microbial spoilage. Samples treated with 2% lactic acid plus 3 kGy and 2% acetic acid plus 3 kGy showed maximum shelf life i.e. up to 33 days and the recovery of *B. cereus* in corresponding samples was up to 5.28 and 4.97 $\log_{10}$ cfu/g, whereas, TVC of respective samples was reached to 6.18 and 6.00 $\log_{10}$ cfu/g. The sensitizing effect of propionic acid in combination with 3 kGy on *B. cereus* was found effective on 0th day but, TVC and *B. cereus* count were reached up to 6.25 and 5.39 $\log_{10}$ cfu/g. on the 30th day. Though TVC and *B. cereus* were recovered at refrigeration storage, the time required was markedly increased by the combination treatments. Radiation treatment and antimicrobial factors such as reduction in pH can extend not only the shelf life of the combination treated refrigerated product, but can also improve their microbiological safety (Vas, 1981). Synergistic effect of both acid and irradiation was continued in storage period. These sensitizers, when combined with 1 and 2 kGy ionizing radiations also showed better results on shelf life, total viable count and *B. cereus* at refrigeration storage than single irradiation treatment (Figs. 1 and 2).

Though the lactic acid, in combination with gamma irradiation, showed better antimicrobial effect on TVC and against *B. cereus*, discolouration (bleached appearance) of mutton samples was observed even in refrigeration storage. However, for sensitization with propionic and acetic acid, no disagreeable colour of mutton samples was noticed. Burnt odour to mutton samples due to 3 kGy ionizing radiation dose was abolished by acid pretreatment.

The study revealed that sensitization with acid treatment is advantageous against radioresistant organisms as well as to extend the shelf life of meat. Acetic acid (2%) in combination with 3 kGy dose was found to be most antibacterial without any adverse effect on sensory attributes. Combination treatments are useful to lower the dose required for destroying radioresistant organisms in meat and ultimately help to eliminate the ill effects developed due to the higher dosage or concentration of treatments.

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