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Antimicrobial Activity of Lactic acid on the Growth of Selective Foodborne Pathogens in Raw Chicken

Sudershan RV1*, Naveen Kumar R1, Kashinath L1, Bhaskar V2, Polasa K1

[1]*Food and Drug Toxicology Research Centre*
National Institute of Nutrition (Indian Council of Medical research), Hyderabad-500007, India
[2]*Statistical Division, National Institute of Nutrition (Indian Council of Medical Research), Hyderabad-500007, India.*

Abstract

The antimicrobial effect of lactic acid on the growth of selective foodborne pathogens such as Salmonella spp., Staphylococcus aureus and B.cereus in raw chicken, effect of lactic acid on mixed cultures of populations and to identify effective inhibitory concentration of lactic acid both in vitro and in vivo conditions were evaluated in this article. Turbidometric assay indicated that 1% lactic acid significantly reduced (P<0.01) the growth of salmonella and staphylococcus aureus. Agar diffusion method indicated that 2% lactic acid was (3 mm inhibition zone) effective against all the three foodborne pathogens. In vitro studies indicated that 1% lactic acid was effective in inhibiting the growth of selective food borne pathogens. This concentration was effective against all the three-foodborne pathogens at 10^2,10^3,10^4 cfu level in 3 min contact time. One percent lactic acid was also found to be effective against mixed bacterial population at 10^2,10^3,10^4 cfu level in 1 min contact time. In vivo studies with raw chicken indicated that 3% lactic acid has significantly reduced (P<0.01) the counts of Salmonella spp. and Staphylococcus aureus at 3 min contact time but this method was not effective against B.cereus. In in vivo studies the Salmonella spp. count reduced from 2.17 log cfu/g to not detectable level whereas in case of Staphylococcus aureus counts reduced from 3.08 log cfu/g to 1.79 log cfu/g (94.8%). Lactic acid was found to be effective up to 1 hour and 30 min in in vivo studies. These findings indicated that lactic acid could be used as an inhibitory agent to curtail the contamination of raw chicken by foodborne pathogens.

Keywords: Lactic acid · Antimicrobial activity · Foodborne pathogens · Raw Chicken

Introduction

Indian meat and poultry industry is one of the fastest growing segments of livestock sector. The poultry sector of modern India has transformed from backyard rearing to commercially organized, scientific and fastest growing sector among the livestock economy in the country (Bawa 2007).

In India chicken for consumption is processed in the unorganized sector and is not a prepared product as sold in western countries, but it is cut or sold as per the consumer requirements. The processing status is one of the major key issues of concern in the meat and poultry sector of India. Quality and hygiene levels are very low along with waste of meat contamination/deterioration. Lactic acid produced by lactic acid bacteria or itself in the form of additive to foods, acts as a natural antimicrobial agent. Lactic acid can inhibit the growth of many types of food spoilage bacteria, including gram-negative species of the families *Enterobacteriaceae* and *Pseudomonadaceae*. Among other organic acids, lactic acid is recognized as a biopreservative

*Corresponding author. mailing address: Dr. Sudershan RV
Scientist-D, Food and Drug Toxicology Research Center
National Institute of Nutrition (ICMR, Govt.of India)
Jamai-Osmania, Tarnaka Hyderabad-500007
Tel-+91-040-27197281, Fax- 040-27019074
Email: vemulasr@yahoo.com

agent in naturally fermented products (Kim et al. 1992, Sudeepa et al. 2007).

Organic acids are the most frequently used chemical decontaminants and are one of the generally recognized as safe (GRAS) compounds (Alakomi et al. 2000, Bolder 2001). Several authors have studied the effect of organic acids on bacterial populations as well as on certain specific pathogenic organisms (Anderson and Marshall 1990, Shelef 1994, Bajaj et al. 2003). Traditionally, lactic acid, a weak organic acid has been widely used to control growth of pathogenic bacteria in foods for several decades. The antimicrobial activity occurs through the diffusion of lactic molecules in to microbial cells until equilibrium is reached, in accordance with the pH gradient, causing membrane disruption, inhibition of essential metabolic reactions, stress on intracellular pH homeostasis and accumulation of toxic anions and ultimate death of microbial cells (Ibrahim et al. 2008)

Microbial loads in meat depend on the way the animal is slaughtered and eviscerated and the way by which the meat is generally handled and stored in terms of time and temperature. They also depend on hygienic conditions in the slaughterhouses (Bin-Jasses 2008). Contaminated food is the usual source of human infections, and poultry products are considered the major infection source for humans (Mead 1993, Stern et al. 2001). Poultry and poultry products have become a common food for humans in developing countries, and they are often sold in way side restaurants (Ekanem 1998). During 2006 red meat and poultry products were responsible in the European Union for 25% of reported foodborne outbreaks for which the vehicle involved was known (Alonso- Hernando et al. 2009). Street foods that are prepared from high risk foods like poultry chicken needs much more concern.

In the present study, an attempt was made to develop a suitable and practical method to minimize and control the pathogens contamination in raw chicken and feasibility of using lactic acid which has antimicrobial activity as preservative agent. Therefore the objectives of this study was to investigate antimicrobial effect of lactic acid on the growth of selective foodborne pathogens such as *Salmonella spp., Staphylococcus aureus* and *B.cereus* in raw chicken and the effect of lactic acid on mixed cultures of populations and to identify effective inhibitory concentration of lactic acid both *in vitro* and *in vivo* conditions.

Materials and Methods

Raw chicken samples to be tested were collected from the market and were packed in sterile zipped polythene bags, these were transported in insulated, iced containers to the laboratory for analysis. Strains of Staphylococcus aurues (NCIM 5021), B.cereus (NCIM 2458) were procured from National collection of Industrial Microorganisms (NCIM), Pune, India and Salmonella enteritidis (MTCC 3219) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. These bacterial strains were maintained in 15% Glycerol at -80℃. Strains were transferred to Nutrient Broth (HIMEDIA) before use. Each strain was grown on Nutrient Broth (HIMEDIA) at 37℃.

Overnight cultures of the strains were diluted appropriately to get the required bacterial load before inoculation in to nutrient broth and the raw chicken. The overnight cultures of the test strains were diluted to get the initial inoculum level (102,103,104 cfu) and 1 ml of this known bacterial load was transferred to the control tube as well as acidified broths (eg. 1%= 9.9 ml broth + 0.1 ml lactic acid). After adding the inoculum the exact time was noted. After each specified duration i.e. 1 minute, 3 min and 5 min aliquots of 100 µl were taken from the appropriate concentration of lactic acid at the time specified and surface plated on the surface dried nutrient agar media. The inoculated plates were incubated at 37℃ for 24 hours

The raw chicken samples were allowed to thaw to come down to room temperature, then the samples were divided in to 4 parts i.e. 25 g approximately each. Three of the weighed samples were treated in the lactic acid (1%, 2% and 3%) solution and each of them was maintained for 1, 3 and 5 min respectively. The remaining one sample was dipped in sterile water to maintain as control. After the required time of acid treatment is over the chicken samples were immersed in buffered peptone water. Aliquot of 100 µl of them were then taken and surface plated on the surface dried selective media by using sterile glass spreader (Spread plate method). Incubation was done at 370C for 24 hours and observed for any significant reduction in the colonies by comparing with their respective controls.

From the overnight culture a standardized inoculum level of 107 cells was inoculated in to the nutrient broth. One flask was kept as control and the other flasks were added with lactic acid (V/V) to get different concentrations such as 0.5%, 1%, 1.5%, 2% and 3%. The control and lactic acid containing medium were inoculated with the same bacterial load. The optical density of the broths was measured at 610 nm for 0 hour value. Subsequent readings of the absorbance were taken at every two hours time interval. Each respective broth was incubated at 370C. The absorbance values were tabulated and further compared to study any significant decrease in the bacterial population of lactic acid treated samples.

The test organisms were cultured in nutrient broth at 370C for 24 hours. Using sterile cotton swab the cultures were dipped and gently swabbed on the surface dried nutrient agar plates. Then using a sterile gel puncture, wells are made in the swabbed culture plates. In the well appropriate amount of lactic acid of different concentration (1%, 2% and 3%) was added using sterile micro dispenser. Results were noted down after the plates were incubated at 370C for 24 hours. For organoleptic evaluation chicken dry curry was prepared by taking 250 gm of two raw chicken

samples and one sample was maintained as control by washing it with sterile distilled water. The other chicken sample was treated with 3% lactic acid for 2 min contact time. After 2 min of incubation at room temperature the excess lactic acid was drained off. Till the completion of the incubation period, the untreated chicken sample was kept in refrigerator. After the completion of the incubation period a common recipe was prepared by using both the treated and control chicken samples in two different bowls. The prepared dish was served to panel members for organoleptic evaluation.

The effect of lactic acid treatments on appearance, color, odour, texture, taste and overall palatability of raw chicken were assessed 45 min after the treatments by an evaluation panel comprising 7 staff members of the institute. The 5 point differential scale for sensory evaluation from very bad to very good was used for scoring the quality of chicken. Descriptive analysis (Mean, Standard deviation, Standard Error, Minimum, and Maximum) was done for each category of the groups. The scores were tested for any difference between the means in the groups by ANOVA test. As there was heterogeneity in the data for each group non parametric Kruskal – Wallis test was performed. (SPSS 15.0 windows version was used).

Results and Discussion

Turbidometric assay indicated that 1% lactic acid significantly reduced ($P<0.01$) the growth of *salmonella*, *staphylococcus aureus and B.cereus* (Fig 1&2). The growth curves of *Salmonella* grown in nutrient broth acidified with 2% and 10% lactic acid are presented in Fig 1. The effect of different concentration of lactic acid (0.5%, 1%, 2% and 3%) against all the three foodborne pathogens (Cocktail) indicated that 1% lactic acid significantly reduced the growth of all the three foodborne pathogens. OD values for the growth of all the 3 organisms grown in nutrient broth acidified with different % of lactic acid are presented in Fig 2(c). Agar diffusion method indicated that 2% lactic acid was (3mm inhibition zone) effective against all the three foodborne pathogens (Fig 3). In this method 1% lactic acid did not show any effect on these three foodborne pathogens.

In vitro studies indicated that 1% lactic acid effectively inhibited the growth of all the three food borne pathogens. This concentration was effective against all the three-foodborne pathogens at 10^2,10^3,10^4 cfu level in 3 min contact time. *In vivo* studies with raw chicken indicated that 3% lactic acid significantly reduced ($P<0.01$) the counts of *Salmonella enteri*. and *Staphylococcus aureus* at 3 min contact time but this method was not effective against *B.cereus*. *In vivo* studies demonstrated reduction in *Salmonella* spp. count from 2.17 log cfu/g to not detectable level where as in case of *Staphylococcus aureus* counts reduced from 3.08 log cfu/g to 1.79 log cfu/g (94.8%). Population of foodborne pathogens on the raw chicken sample treated with 3% LA are presented in Fig 4.

Effects of lactic acid on sensory characteristics of raw chicken have been presented in Table 1. Statistical analysis of sensory scores on sensory characteristics indicated that there is no significant difference ($P>0.05$) in the between the two groups i.e., treated and control. Overall treatment of raw chicken with the lactic acid has no effect on the sensory scores. *In vivo* study showed that 3% lactic acid was more effective than 2% and 1% lactic acid. It may be due to the reason that acid exhibits effective antimicrobial action, only when appropriate amounts of undissociated molecules of that acid penetrate bacterial cell by means of diffusion and interfere with intracellular enzymes (Selvan et al. 2007). In *in vitro* assay, initially 2% and 10% lactic acid on nutrient broth (V/V) was tested for *Salmonella*. From the results (Fig 1), it is clearly indicated that 10% is too high for the bacteria to survive and hence the bacteriostatic effect was observed. The pKa of lactic acid is 3.08, so the pH below this will make the acid to remain undissociated. Such undissociated acids are 100-600 times effective (Eklund 1985). Hence the concentration of the lactic acid was reduced to below 3% in the subsequent experiments.

No statistical difference was observed in sensory scores of lactic acid treated and control raw chicken samples. Woolthuis and Smolders (1985) used lactic acid (2%) to decontaminate calf carcasses and reported that there was no effect on the flavour of treated carcasses whereas slight to traces of chemical odour was observed. Delmore et al. (2000) found that immersion treatments of variety meats had greatest effect on their color and also reported that the color of beef liver was lighter after immersion in 2% lactic acid for 10 seconds. Selvan et al. (2007) also observed similar discoloration in buffalo meat streaks treated with 4% lactic acid for 10 seconds. The effect of lactic acid on the growth of *Salmonella* spp. in carrot juice (Ibrahim et al. 2007), catfish skin (Kim and Marshall 2000), chicken breast (Anang et al. 2007) was reported and 2% lactic acid reduced *Salmonella* spp. count from 5.9 log 10 cfu/skin to below limit of detection of 2.0 log 10 cfu/skin by 5 minutes exposure, and in case of chicken breast the count was decreased by 2.90 log cfu/g with 2% lactic acid. The kinetics of the bactericidal effect of lactic acid decontamination on meat- borne pathogens in an in-vitro model indicated that, 2% lactic acid decontamination at 37 degree C for 30-90 seconds is suitable for elimination of *Salmonella* on meat but not for *L.monocytogenes* (Nettern et al. 1994), but in the present study antimicrobial effect of lactic acid both by *in vitro* and *in vivo* indicated that 3% lactic acid was found to be more effective in reducing the count of selective foodborne pathogens. In conclusion, the data from the present study suggest that lactic acid (3%) can be used as an anti microbial agent to reduce the growth of

these selective foodborne pathogens i.e. *Bacillus cereus, Staphylococcus aureus and Salmonella* spp.

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Tables

Sensory attributes	Treated Sample (2% LA)	Control
Appearance	**3.9±0.38[a]**	**3.2±0.76[a]**
Color	**3.7±0.95[a]**	**3.0±0.58[a]**
Odour	**3.4±0.79[a]**	**2.7±0.76[a]**
Texture	**4.0±0.58[a]**	**3.2±1.11[a]**
Taste	**3.8±0.69[a]**	**3.7±0.49[a]**
Overall palatability	**4.0±0.58[a]**	**3.6±0.53[a]**

(n=7)

Table 1. Effect of lactic acid (LA) on sensory quality of raw chicken

[a]Means between the two products sharing the same letter did not differ significantly (P<0.

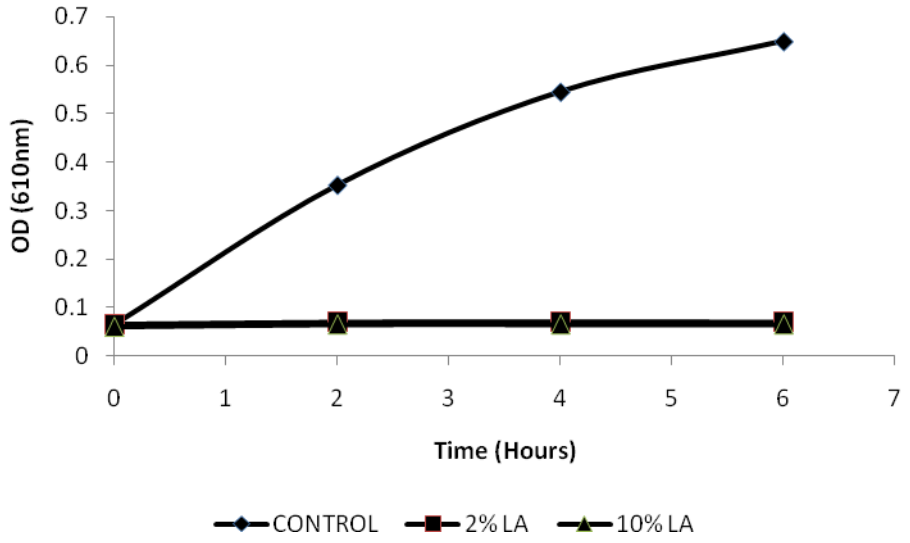
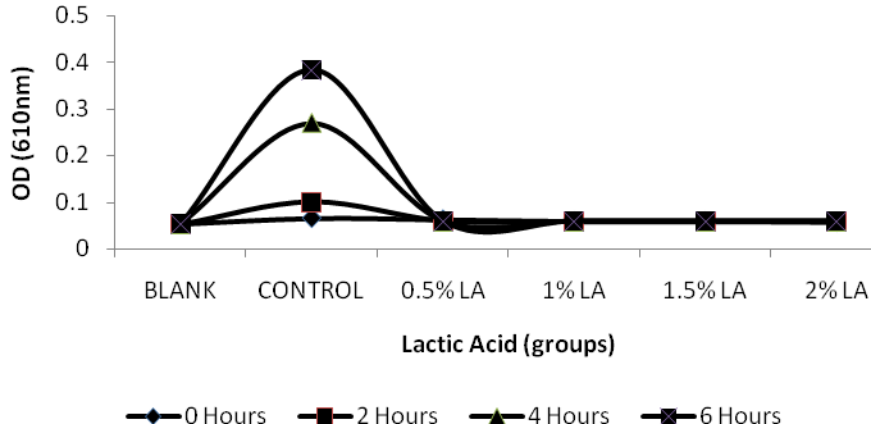


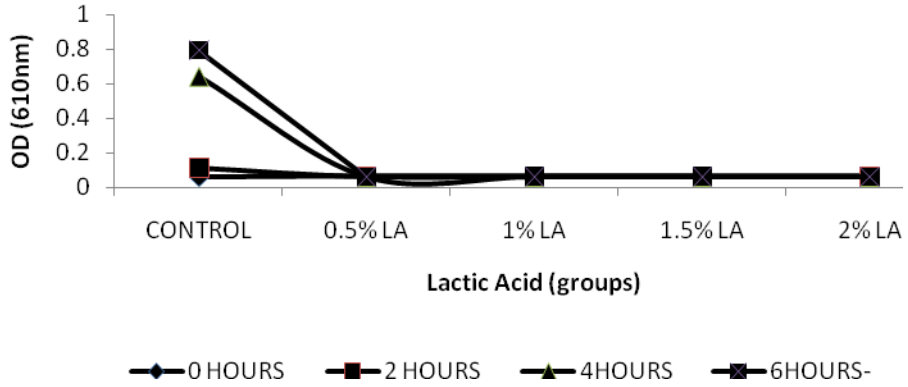
FIG.1 OD values for the growth of Salmonella in nutrient broth acidified with 2% and 10% lactic acid (turbidometric assay)

(n=3)

(a)



(b)



(c)

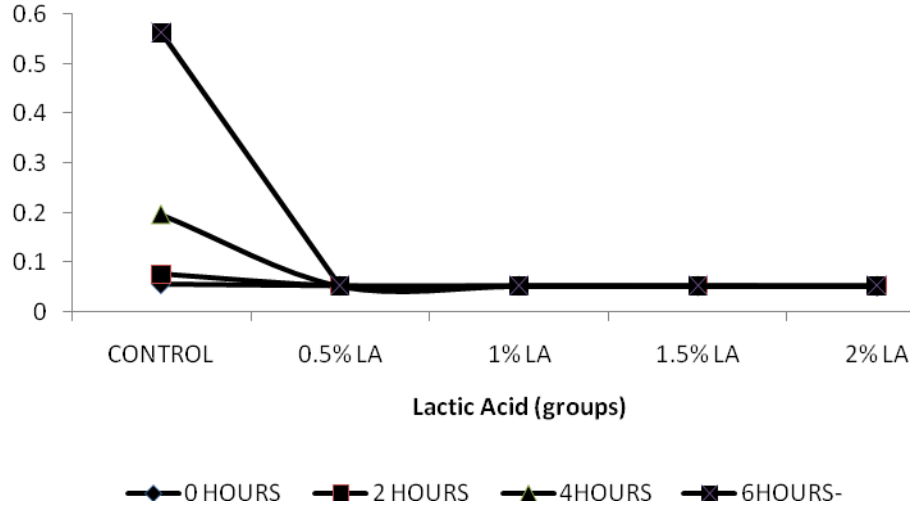


Fig.2 OD values for the growth of (a) *Staphylococcus aureus*, (b) *Bacillus cereus* and (c) *S.aureus, B .cereus* and *Salmonella* spp. in nutrient broth acidified with different % lactic acid (turbidometric assay)

(n=3)

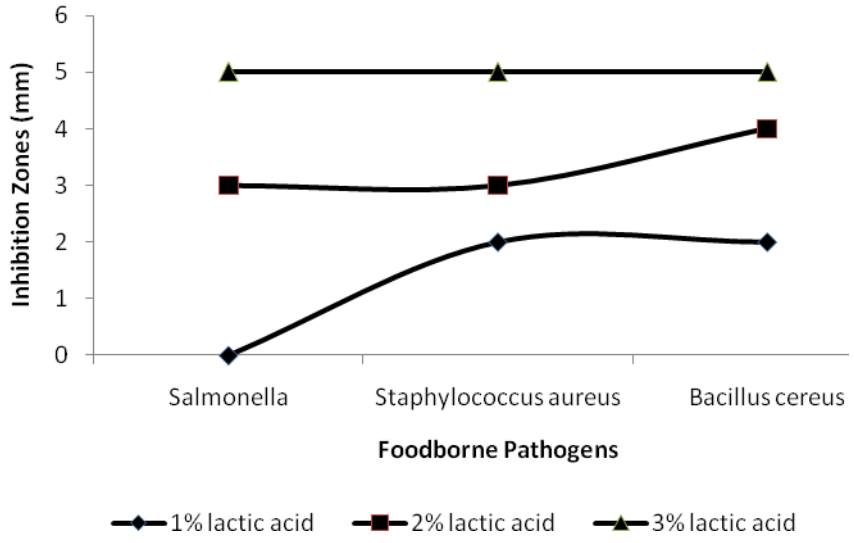


Fig.3 Inhibition zones of *Salmonella* spp., *S.aureus and B. cereus* formed due to 1%, 2% and 3%LA (n=3)