

Original Research Article

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## Effect of Pediocin NCDC252 as Cell Free Supernatant Produced from *Pediococcus acidilactici* NCDC252 with EDTA on Total Viable Count and Sensory Evaluation of Chicken Carcasses Stored at Refrigeration Temperature

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### ABSTRACT

#### Keywords

Pediocin, *Pediococcus acidilactici*, Cell Free Supernatant, EDTA, TVC, Sensory Evaluation.

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Culture of *Pediococcus acidilactici* NCDC252 was grown in MRS broth and the crude pediocin NCDC252 in the form of cell free supernatant was prepared. A study on the effect of pediocin alone and with a chelator, disodium EDTA on total viable count and sensory evaluation of chicken carcasses stored at  $4 \pm 1^\circ\text{C}$  was carried out and compared with control without any treatment. The quality parameters like total viable count and sensory evaluation by colour score, appearance score and odour score were analyzed and discussed. Pediocin NCDC252 with disodium EDTA showed lower TVC than other two groups except on 6<sup>th</sup> day. On 5<sup>th</sup> day pediocin NCDC252 with disodium EDTA treated group showed significantly higher ( $P < 0.01$ ) colour score. Appearance score showed significantly higher ( $P < 0.01$ ) values for treated group than control on 3<sup>rd</sup> and 5<sup>th</sup> day. Odour score showed significantly higher ( $P < 0.01$ ) values for treated groups than control. The findings of this study revealed that

### Introduction

Poultry is one of the fastest growing sector in India with an average growth rate of 6 percent in egg production and 12 percent for broiler production per annum. In India, per capita consumption of poultry meat reached from 1.9 kg in 2010 to 2.2kg in 2013 (USDA, 2013). With this increase in production and consumption of poultry meat, demand for safety and quality of poultry meat has become a vital issue for poultry meat industry. Food safety is a top priority for authorities and

consumers worldwide. Poultry meat is a nutrient rich matrix that provides a suitable environment for proliferation of meat spoilage microorganisms and common food-borne pathogens. Hence considering the fact that poultry belongs to highly perishable foods providing an almost perfect medium for microbial growth the main concern of industries is the shelf-life extension of the poultry meat. To harmonize consumer demands without compromising safety, the

production of poultry meat is at a stage of innovative dynamics. Technologies such as bio preservation are alternative in which lactic acid bacteria (LAB) and/or their bacteriocins are used for preservation of meat. Bio preservation implies a novel scientific approach to improve the microbial safety of foods and to extend the shelf life.

Bio preservation is defined as the use of antagonistic microorganisms or their metabolic products to inhibit or destroy undesirable microorganisms in food (Jeevaratnam *et al.*, 2005). Lactic acid bacteria and their metabolites such as bacteriocins have the potential to be used as bio preservatives. Lactic acid bacteria (LAB) with GRAS (Generally Recognised as Safe) are used in fermented foods. Also bacteriocins produced from LAB degraded by gastrointestinal proteases considered safe as food bio preservative.

Bacteriocins are small, heat stable cationic ribosomally synthesized peptides or proteins of lactic acid bacteria, which display a wider spectrum of inhibition. Bacteriocins have been classified in following groups (Jeevaratnam *et al.*, 2005)

Class I – Lantibiotics e.g. Nisin

Class II – Non Lantibiotics which are small, heat-stable peptides e.g. Pediocin

Class III – Large heat labile protein e.g. Helveticin-J

Class IV – Complex proteins that require additional carbohydrate or lipid moieties to attain antimicrobial activity.

Pediocin is one of the Class II bacteriocin which can be applied in meat preservation though nisin is best known bacteriocin having GRAS status, but due to difficulties of using

nisin in raw meat applications, the use of pediocin like bacteriocin has been explored. It is produced by lactic acid bacteria strains mainly *Pediococcus acidilactici* which is commonly associated with fermentation of vegetables and meat-based products. Pediocin is effective against many strains of sub-lethally stressed gram negative, gram positive and pathogenic bacteria. Gram-negative spoilage organisms are resistant to bacteriocins because of the protective outer membrane. Treatment with chelators can alter the outer membrane permeability of gram-negative bacteria. Ethylene diamine tetra acetic acid (EDTA) is a food grade chelator used in present study.

A study was conducted to evaluate the effect of cell free supernatant of *Pediococcus acidilactici* culture with and without chelating agent disodium EDTA on total viable count and sensory evaluation of chicken carcasses at refrigeration temperature ( $4 \pm 1^\circ\text{C}$ ). The study was carried out in the Department of Meat Science and Technology, Madras Veterinary College, Chennai- 7.

## Materials and Methods

Freeze dried culture of *Pediococcus acidilactici* NCDC252 was obtained from National Collection of Dairy Culture (NCDC), Karnal, Haryana. MRS broth and MRS agar was used for cultivation of *Pediococcus acidilactici*. Dehydrated form of media was obtained from M/s Himedia Laboratories, Mumbai. It was used as per the manufacture's instruction.

Freeze dried culture of *Pediococcus acidilactici* NCDC252 was propagated in MRS broth at  $37^\circ\text{C}$  for 24 hr. Broth was supplemented with L- cysteine hydrochloride (0.3 g/lit) (Klare *et al.*, 2005). Cultures were preserved in MRS glycerol broth (15 per cent glycerol) and stored at  $-20^\circ\text{C}$ . Non lactic

indicators were propagated in Nutrient broth and were preserved in Nutrient glycerol broth at -20°C. The cultures were activated before use by successive transfer at 24 h in Nutrient broth.

### **Preparation of Cell Free Supernatant (CFS)**

The culture was propagated in 100 ml of sterile MRS broth supplemented with L-cysteine hydrochloride. The broth culture was incubated at 37°C for 16 -18 hrs. Cell free Supernatant was obtained as per the modified method of Piard *et al.*, (1992) by centrifugation at 12000xg for 20 min at 4°C. The supernatant was separated. The pH of CFS was adjusted to 6.5 using 1N NaOH. The inhibitory effect of hydrogen peroxide was eliminated by addition of catalase (5mg/ml) (Tufail *et al.*, 2011). The CFS was then filter sterilised by passing through a 0.22µm syringe filter.

Mass production of anti-bacterial substance was done as per the modified method of Barefoot and Klaenhammer (1984) s. The chelator used was disodium Ethylene Diamine Tetra Acetic acid obtained from M/s Himedia laboratories, Mumbai. Fifty milli molar solution of disodium EDTA was prepared and sterilized by filtration through a 0.22 µm filter. The solution was stored no longer than 4 h at room temperature before use (Economou *et al.*, 2009). Fresh chicken carcasses were purchased from local meat market, Chennai and were transported under hygienic conditions to the department of Meat Science and Technology, Madras Veterinary College, Chennai. The carcasses were washed with clean potable water.

### **Spraying chicken carcasses with cell free supernatant and disodium EDTA**

Plastic trays were initially washed with potable water and kept dry. Two separate

sprayers were used for different treatments. First carcass was sprayed with only cell free supernatant (Plate 1), the second carcass was sprayed with cell free supernatant and disodium EDTA (Plate 2) and third carcass was kept as control. After treatment breast pieces were separated from each carcass and packed in pre labelled zip lock pouches and stored at 4±1°C. All parameters were analysed on 0, 3<sup>rd</sup>, 5<sup>th</sup> and 6<sup>th</sup> day for all the three groups.

Total viable count of the meat samples were determined by the method described by APHA (1984). Dehydrated media from Himedia, Mumbai were utilized for the microbial assessment. The organoleptic qualities of stored meat was assessed by subjecting the meat sample to sensory evaluation for colour, odour and appearance by trained and semi – trained panel drawn from the Department of Meat Science and Technology, Madras Veterinary College, Chennai- 600 007 on a nine point hedonic scale as given in the score card. The members were provided with a score card having a nine point descending scale to assess the colour, odour and appearance of meat. The data obtained in this study was analysed statistically in SPS software (version 20.0) as per the methods outlined by Snedecor and Cochran (1994).

## **Results and Discussion**

### **Total Viable Count (TVC) (log/g)**

The mean ± SE values of TVC of chicken carcasses treated with pediocin, pediocin with disodium EDTA and control stored at 4 ± 1°C on 0, 3<sup>rd</sup>, 5<sup>th</sup> and 6<sup>th</sup> day are presented in table 1. The mean ± SE values of TVC of fresh chicken carcasses on 0 day was 4.40 ± 0.03. The analysis of variance revealed a significant (P<0.05) difference between the different treatments and highly significant (P<0.01) between the storage periods.

## Sensory evaluation

### Colour score

The mean  $\pm$  SE values of colour score of chicken carcasses treated with pediocin, pediocin with disodium EDTA and control stored at  $4 \pm 1^{\circ}\text{C}$  on 0, 3<sup>rd</sup>, 5<sup>th</sup> and 6<sup>th</sup> day are presented in table 2.

The mean  $\pm$  SE value of colour score of fresh chicken carcasses on 0 day was  $8.88 \pm 0.04$ . The analysis of variance revealed a no significant ( $P>0.05$ ) difference between the different treatments but highly significant ( $P<0.01$ ) difference between the storage periods.

### Appearance Score

The mean  $\pm$  SE values of appearance score of chicken carcasses treated with pediocin, pediocin with disodium EDTA and control stored at  $4 \pm 1^{\circ}\text{C}$  on 0, 3<sup>rd</sup>, 5<sup>th</sup> and 6<sup>th</sup> day are presented in table 3.

The mean  $\pm$  SE value of appearance score of fresh chicken carcasses on 0 day was  $8.93 \pm 0.04$ . The analysis of variance revealed a no significant ( $P>0.05$ ) difference between the different treatments but highly significant ( $P<0.01$ ) difference between the storage periods.

### Odour score

The mean  $\pm$  SE values of odour score of chicken carcasses treated with pediocin, pediocin with disodium EDTA and control stored at  $4 \pm 1^{\circ}\text{C}$  on 0, 3<sup>rd</sup>, 5<sup>th</sup> and 6<sup>th</sup> day are presented in table 4.

The mean  $\pm$  SE value of odour score of fresh chicken carcasses on 0 day was  $8.81 \pm 0.07$ . The analysis of variance revealed a no significant ( $P>0.05$ ) difference between the

different treatments but highly significant ( $P<0.01$ ) difference between the storage periods.

### Total viable count

The mean value of total viable count on 0 day was  $4.40 \pm 0.03$ . The same total viable count in fresh broiler meat was observed by Chouliara *et al.*, (2007), Patsias *et al.*, (2008) and Petrou *et al.*, (2012). The results indicated an increase in TVC in all the three groups as storage period increased.

A highly significant difference was noticed in total viable count of treated and control group during entire storage period. Pediocin NCDC252 with disodium EDTA showed significantly lesser TVC compared to other two groups except on 6<sup>th</sup> day where it shows insignificantly lower TVC value than pediocin NCDC252 alone treated group. Control showed highest total viable count on 3<sup>rd</sup>, 5<sup>th</sup> and 6<sup>th</sup> day indicating lower microbial quality of meat compared to treated carcasses.

At the end of storage life TVC all three groups was below 7 log cfu/g. At the end of storage life Cannarsi *et al.*, (2008) noted higher TVC value ( $8.19 - 8.89$  log cfu/g) in chilled buffalo meat treated with different concentration of nisin alone, stored upto 8 days. Mehar *et al.*, (2005) used 6 log<sub>10</sub> cfu/g value of TVC as indicator of the shelf life end-point. On 3<sup>rd</sup> day TVC for control group was almost 6 log cfu/g.

Same value was achieved by pediocin NCDC252 treated group on 5<sup>th</sup> day. For pediocin NCDC252 with disodium EDTA treated sample on 6<sup>th</sup> day TVC was above 6 log cfu/g. It indicates that pediocin NCDC252 alone and pediocin NCDC252 + disodium EDTA treated groups have longer shelf life than control group.

**Table.1** Mean ± SE values of Total Viable Count of control and Treated chicken carcasses stored at 4±1°C

| Days                | Pediocin                  | Pediocin +EDTA            | Control                   | Overall mean             |
|---------------------|---------------------------|---------------------------|---------------------------|--------------------------|
| 0 <sup>th</sup> Day | 4.40 ± 0.06 <sup>A</sup>  | 4.40 ± 0.06 <sup>A</sup>  | 4.40 ± 0.06 <sup>A</sup>  | 4.40 ± 0.03 <sup>W</sup> |
| 3 <sup>rd</sup> Day | 4.89 ± 0.12 <sup>Bb</sup> | 4.61 ± 0.10 <sup>Aa</sup> | 5.87 ± 0.02 <sup>Bc</sup> | 5.12 ± 0.14 <sup>X</sup> |
| 5 <sup>th</sup> Day | 5.93 ± 0.13 <sup>Cb</sup> | 5.38 ± 0.15 <sup>Ba</sup> | 6.68 ± 0.10 <sup>Cc</sup> | 5.99 ± 0.15 <sup>Y</sup> |
| 6 <sup>th</sup> Day | 6.41 ± 0.12 <sup>Da</sup> | 6.30 ± 0.05 <sup>Ca</sup> | 6.87 ± 0.08 <sup>Cb</sup> | 6.53 ± 0.08 <sup>Z</sup> |
| Over all mean       | 5.41 ± 0.17 <sup>X</sup>  | 5.17 ± 0.16 <sup>X</sup>  | 5.96 ± 0.20 <sup>Y</sup>  | —                        |

Means bearing different superscript between rows (A, B, C, D), between columns (a, b, c) and between overall mean (W, X, Y, Z) differ significantly (p<0.05)

**Table.2** Mean ± SE values of Colour score of control and Treated chicken carcasses stored at 4±1°C

| Days                | Pediocin                  | Pediocin +EDTA            | Control                   | Overall mean             |
|---------------------|---------------------------|---------------------------|---------------------------|--------------------------|
| 0 <sup>th</sup> Day | 8.88 ± 0.08 <sup>D</sup>  | 8.88 ± 0.08 <sup>C</sup>  | 8.88 ± 0.08 <sup>C</sup>  | 8.88 ± 0.04 <sup>Z</sup> |
| 3 <sup>rd</sup> Day | 6.73 ± 0.26 <sup>Cb</sup> | 7.45 ± 0.15 <sup>Bb</sup> | 5.67 ± 0.35 <sup>Ba</sup> | 6.62 ± 0.23 <sup>Y</sup> |
| 5 <sup>th</sup> Day | 5.76 ± 0.11 <sup>Ba</sup> | 6.74 ± 0.17 <sup>Bb</sup> | 5.27 ± 0.24 <sup>Ba</sup> | 5.92 ± 0.18 <sup>X</sup> |
| 6 <sup>th</sup> Day | 4.68 ± 0.58 <sup>A</sup>  | 5.03 ± 0.47 <sup>A</sup>  | 4.30 ± 0.49 <sup>A</sup>  | 4.67 ± 0.29 <sup>W</sup> |
| Over all mean       | 6.52 ± 0.36               | 7.03 ± 0.31               | 6.03 ± 0.39               | —                        |

Means bearing different superscript between rows (A, B, C, D) between columns (a, b, c) and between overall mean (W, X, Y, Z) differ significantly (p<0.05)

**Table.3** Mean ± SE values of appearance score of control and Treated chicken carcasses stored at 4±1°C

| Days                | Pediocin                  | Pediocin + EDTA           | Control                   | Overall mean             |
|---------------------|---------------------------|---------------------------|---------------------------|--------------------------|
| 0 <sup>th</sup> Day | 8.93 ± 0.07 <sup>D</sup>  | 8.93 ± 0.07 <sup>D</sup>  | 8.93 ± 0.07 <sup>D</sup>  | 8.93 ± 0.04 <sup>Z</sup> |
| 3 <sup>rd</sup> Day | 7.41 ± 0.21 <sup>Cb</sup> | 7.62 ± 0.18 <sup>Cb</sup> | 5.90 ± 0.36 <sup>Ca</sup> | 6.98 ± 0.23 <sup>Y</sup> |
| 5 <sup>th</sup> Day | 5.63 ± 0.40 <sup>Bb</sup> | 6.37 ± 0.48 <sup>Bb</sup> | 4.48 ± 0.16 <sup>Ba</sup> | 5.50 ± 0.28 <sup>X</sup> |
| 6 <sup>th</sup> Day | 4.23 ± 0.36 <sup>A</sup>  | 4.56 ± 0.45 <sup>A</sup>  | 3.62 ± 0.24 <sup>A</sup>  | 4.13 ± 0.22 <sup>W</sup> |
| Over all mean       | 6.55 ± 0.39               | 6.87 ± 0.37               | 5.73 ± 0.43               | —                        |

Means bearing different superscript between rows (A, B, C, D) between columns (a, b, c) and between overall mean (W, X, Y, Z) differ significantly (p<0.05)

**Table.4** Mean ± SE values of Odour score of control and Treated chicken carcasses stored at 4±1°C

| Days                | Pediocin                   | Pediocin +EDTA            | Control                   | Overall mean             |
|---------------------|----------------------------|---------------------------|---------------------------|--------------------------|
| 0 <sup>th</sup> Day | 8.81 ± 0.13 <sup>D</sup>   | 8.81 ± 0.13 <sup>D</sup>  | 8.81 ± 0.13 <sup>D</sup>  | 8.81 ± 0.07 <sup>Z</sup> |
| 3 <sup>rd</sup> Day | 6.73 ± 0.20 <sup>Cb</sup>  | 7.29 ± 0.15 <sup>Cb</sup> | 5.89 ± 0.36 <sup>Ca</sup> | 6.64 ± 0.19 <sup>Y</sup> |
| 5 <sup>th</sup> Day | 5.77 ± 0.23 <sup>Bb</sup>  | 6.62 ± 0.17 <sup>Bc</sup> | 4.78 ± 0.13 <sup>Ba</sup> | 5.72 ± 0.21 <sup>X</sup> |
| 6 <sup>th</sup> Day | 4.45 ± 0.18 <sup>Aab</sup> | 5.07 ± 0.29 <sup>Ab</sup> | 3.82 ± 0.21 <sup>Aa</sup> | 4.45 ± 0.17 <sup>W</sup> |
| Over all mean       | 6.44 ± 0.34                | 6.95 ± 0.29               | 5.83 ± 0.40               | —                        |

Means bearing different superscript between rows (A, B, C, D) between columns (a, b, c) and between overall mean (W, X, Y, Z) differ significantly (p<0.05)

## Sensory evaluation

### Colour score

The colour score for fresh chicken meat on 0 day was  $8.88 \pm 0.08$ . Rio *et al.*, (2006) also recorded colour score of  $8.20 \pm 0.56$  of chicken legs stored at  $3 \pm 1^\circ\text{C}$ . The results revealed that colour score showed significant decrease as storage period increased. This is in agreement with Jayesh (1999) and Sekar (2003). The overall mean of colour score showed no significant difference in between treatments but a highly significant difference in between storage period. On 3<sup>rd</sup> day both treated group had significantly higher colour score than control group. On 5<sup>th</sup> day pediocin NCDC252 with disodium EDTA treated group had significantly higher colour score compared to other two groups. On 6<sup>th</sup> day no significant difference was found in colour score of all the groups. Control group showed colour score below 6 on 3<sup>rd</sup> day whereas same value was achieved by pediocin NCDC alone treated group on 5<sup>th</sup> day. The pediocin NCDC252 with disodium EDTA treated group showed a colour score below the 6 on 6<sup>th</sup> day. It indicates that pediocin NCDC252 alone and pediocin NCDC252 with disodium EDTA both affects colour score on sensory evaluation of chicken carcasses stored at  $4 \pm 1^\circ\text{C}$ .

### Appearance score

Appearance of foods refers to their desirability. The mean appearance score for fresh chicken meat on 0 day was  $8.93 \pm 0.07$ . The overall mean values of appearance score showed no significant difference in between treatments but a highly significant was noticed in between storage periods. Appearance score for all three groups decreases as storage period increases. This was in agreement with Jayesh (1999) and Jayanthi (2003). On 3<sup>rd</sup> and 5<sup>th</sup> day pediocin

NCDC252 alone and pediocin NCDC252 with disodium EDTA treated group had significantly higher appearance score than control group. On 6<sup>th</sup> day no significant difference was found in appearance score of all groups. Control group showed an appearance score below 6 on 3<sup>rd</sup> day whereas same value was achieved by pediocin NCDC252 alone treated group on 5<sup>th</sup> day. For pediocin NCDC252 with disodium EDTA treated group showed a colour score below the 6 on 6<sup>th</sup> day. The results revealed that appearance score showed a significant decrease as storage period increased. Same results was obtained by Economou *et al.*, (2009) for nisin EDTA treated chicken breast stored at  $4 \pm 0.5^\circ\text{C}$  under MAP.

### Odour Score

The odour score for fresh chicken meat on 0 day was  $8.81 \pm 0.13$ . Patsias *et al.*, (2008) also noted odour score in range of 8.4 -8.8 for chicken breast stored at  $4^\circ\text{C}$  during initial period of storage. The results revealed that odour score showed a significant decrease as storage period increased. This is in agreement with Rio *et al.*, (2006) and Balamatsia *et al.*, (2007). The overall mean values of odour score showed no significant difference in between treatments but a highly significant difference in between storage periods.

Economou *et al.*, (2009) stated that score of 6 is the limit of odour acceptability of meat. On 3<sup>rd</sup> day of refrigerated storage, pediocin NCDC252 alone and pediocin NCDC252 with disodium EDTA treated group have significantly higher odour score than control group. On 5<sup>th</sup> day pediocin NCDC252 with disodium EDTA treated group showed significantly higher odour score that other two groups. The results of the study indicated that the chicken carcasses treated with pediocin NCDC252 with disodium EDTA had better quality characteristics than the other two

groups upto 5<sup>th</sup> day of storage life. Culture of *Pediococcus acidilactici* NCDC252 was grown in MRS broth and the crude pediocin NCDC252 in the form of cell free supernatant was prepared. Cell free supernatant was used for spraying of chicken carcasses along with and without disodium EDTA. Carcasses were stored at refrigeration temperature and their meat quality parameters like, total viable count and sensory evaluation assessed. The results of the study indicated that the chicken carcasses treated with pediocin NCDC252 with disodium EDTA had better microbial and sensory quality characteristics than the other two groups upto 5<sup>th</sup> day of storage life.

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