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Assessment of Antibacterial Activity of Pediocin NCDC252 Produced from *Pediococus acidilactici* NCDC252 and Study of Its Effect on Physico-chemical Properties of Chicken Carcasses Stored at Refrigeration Temperature

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A B S T R A C T

The crude pediocin NCDC252 in the form of cell free supernatant was prepared from *Pediococcus acidilactici* NCDC252. Culture was grown in MRS broth for 16-18 hrs and centrifuged at 12000xg for 20 minutes at 4˚C. The pH of the supernatant was adjusted to 6.5 and catalase was added (5mg/ml). Antibacterial activity of pediocin produced by *Pediococcus acidilactici* was assessed against *Staph. Aureus* and *E. coli* by agar well diffusion. It showed antibacterial activity against *Staph aureus* but not against *E. coli.* A study on the effect of pediocin alone and with a chelator, disodium EDTA on physico-chemical properties of chicken carcasses stored at 4 ± 1˚C was carried out and compared with control without any treatment. The quality parameters like pH, water holding capacity, extract release volume, thiobarbituric acid value, tyrosine value, Hunter color LAB analysis were analyzed and discussed, pH showed non-significant difference during storage period except on 5th day, pediocin NCDC252 with disodium EDTA treated carcasses showed lower pH than other two groups. Water holding capacity was significantly higher for treated groups compared to control group on 3rd and 5th day. On 5th and 6th day pediocin NCDC252 with disodium EDTA treated group showed significantly higher ($P<0.01$) ERV values compared to other two groups. Pediocin NCDC252 alone and pediocin NCDC252 with disodium EDTA treated groups showed significantly lower ($P<0.01$) thiobarbituric acid values than control group. On 6th day highly significant difference ($P<0.01$) in tyrosine value was noticed in all three groups. There was no significant difference in Hunter color LAB- L* and a* value in between treatments and during storage period. There was no significant difference in b* values in between treatments but a significant increase were noticed on 6th day in pediocin NCDC252 with disodium EDTA treated and control group.

Introduction

Poultry meat is a very popular food commodity around the world and its consumption has increased over the last decades in many countries. India as we know is the third largest egg producer and fifth largest poultry meat producer in the world. There has been a rapid rise in the demand for livestock products in India. It is of utmost importance for the poultry industry to develop new and effective methods of preservation to

extend shelf life of meat. In order to control spoilage of meat i.e. enzymatic, oxidative and microbial spoilage, low temperature storage and chemical techniques are the commonly used methods of preservation. During refrigeration, microorganisms are not destroyed but their growth is retarded. Sometimes, these microorganisms can also multiply at relatively low temperature, the result of their metabolic activity is manifested as meat spoilage, and consequently, they are the most important factors of shelf life of meat. The current need is to develop and implement alternative technologies such as bio preservation, which includes biological antimicrobial systems - lactic acid bacteria (LAB) and/or their bacteriocins.

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) status have a long history of application in fermented foods.

Hence, attention has been focused on LAB from different sources that produce bacteriocins that are considered safe as food bio preservative which can be degraded by gastrointestinal proteases.

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. Helviticin-J

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

Among the Class I bacteriocins nisin is one of the best known bacteriocin having GRAS status. There are difficulties using nisin in raw meat applications, the use of other bacteriocins has been explored. Pediocin is one among them, which can be applied in meat preservation. It is a class II bacteriocin 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. Such injured bacteria present in meat have been stored at refrigeration temperature. In preservation of meat, gram-negative spoilage organisms and pathogens are especially problematic due to their inherent resistance to bacteriocins because of the protective outer membrane of gram-negative bacteria, which covers the cytoplasmic membrane and peptidoglycan layer of the cells. Treatment with chelators can alter the outer membrane permeability of gram-negative bacteria. Ethylene diamine tetra acetic acid (EDTA) is a chelator and food grade permeabilizer having low cost and it is commercially available.

A study was conducted to evaluate the effect of cell free supernatant of *Pediococcus acidilactici* culture with and without chelating agent disodium EDTA on physico-chemical properties of chicken carcasses at refrigeration temperature (4 ±1˚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. *S. aureus* and *E. coli* culture was obtained from Department of Veterinary Public Health and Epidemiology, Madras Veterinary College, Chennai. MRS broth and MRS agar was used for cultivation of *Pediococcus acidilactici*. Nutrient broth and nutrient agar was used for cultivation of indicator organism. 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˚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˚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.

Detection of antibacterial activity of CFS using indicator organisms

Cell free supernatant of *Pediococcus acidilactici* NCDC252 were screened for antibacterial activity by the agar well diffusion assay advocated by Schillinger and Lucke (1989) with some modification. Nutrient agar plates were prepared and the plates were overlaid with 10 ml of nutrient soft agar (containing 0.7% agar) respectively and inoculated with 0.3 ml of an overnight culture of the indicator organisms (approximately 5×10^7 cfu/ml). After solidification, three wells of 6mm diameter were cut using a sterile cork borer. The bottom of the wells were sealed using a drop of agar. 100 µl of the cell free supernatant was placed in each well. The supernatant was allowed to diffuse through the agar. The Nutrient agar plates were then incubated aerobically for 24-48 hours at 37˚C and subsequently examined for zones of inhibition.

Mass production of antibacterial substance

Mass production of anti-bacterial substance was done as per the modified method of Barefoot and Klaenhammer (1984). Three litres of MRS broth containing L- cysteine hydrochloride was prepared in 3 glass flasks each capacity of one litre. The broth was sterilized by autoclaving at 121˚C at 15 lbs pressure for 15 minutes. Thirty ml of an overnight culture of *Pediococcus acidilactici* NCDC252 bacteria producing pediocin NCDC252 was inoculated into the sterilized 3 litre MRS broth. MRS broth containing culture was incubated aerobically at 37˚C for 16 to 18 hrs. Cells were removed by continuous centrifugation at 12000xg for 20 minutes at 4˚C. To eliminate growth inhibition caused by organic acids and hydrogen peroxide, the pH of the cell free supernatant was adjusted to pH 6.5 using 1N

NaOH and catalase (5mg/ml) was added. The supernatant was then filter sterilized through 0.22µm pore size syringe filter to remove the cellular debris. This material designated as pediocin NCDC252 in crude form and was frozen at -20˚C.

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 (Figure 1), the second carcass was sprayed with cell free supernatant and disodium EDTA (Figure 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 quality parameters were analysed on 0, 3rd, 5th and 6th day for all the three groups.

The pH of the chicken meat samples was measured using a digital pH meter (Digisun Electronic System, Model: 2001). Water holding capacity of the chicken meat samples was assessed by adopting the filter paper press method recommended by Grau and Hamm (1957) with certain modifications. The extract release volume of chicken meat samples were determined by the method outlined by Pearson (1967). Thiobarbituric acid (TBA) number was measured by the modified method outlined by Strange *et al.,* (1977). Tyrosine value was determined by the modified method of Strange *et al.,* (1977). Colour of meat sample was measured using Hunter lab Mini scan XE plus Spectro-colorimeter (Model No. 45/O-L, Reston Virgenia, USA) with geometry of diffuse/80 (sphere - 8mm view) and an illuminant of D65/10 deg. 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).

On MRS agar, *Pediococcus acidilactici* NCDC252 it shows circular milky white colonies after incubation for 24 - 48 hrs (Figure 3). On Grams staining, gram-positive spherical organism was observed. The organism was arranged in pairs and tetrads (Figure 4).

The cell free supernatant of *Pediococcus acidilactici* NCDC252 organism was screened for the antibacterial activity against indicator organism i.e. *Staphylococcus aureus* by agar well diffusion method. A clear zone of inhibition of 2mm (Osmanagaoglu *et al.,* 2001) was noticed around the wells after 24 hours of incubation (Figure 5). However, the cell free supernatant has not shown antibacterial activity against *E. coli.* There was no zone of inhibition observed around the wells (Figure 6).

Results and Discussion

pH

The mean ± SE values of pH of chicken carcasses treated with pediocin, pediocin with disodium EDTA and control stored at 4 ± 1^{0}C on 0, 3rd,5th and 6th day are presented in table

1 and figure 1. The mean ± SE value of pH of fresh chicken carcasses on 0 day was 6.21 ± 0.04. The analysis of variance revealed a no significant (P>0.05) difference between the different treatments but a significant (P<0.05) difference between the storage period.

Water Holding Capacity (WHC) (cm^2)

The mean ± SE values of WHC of chicken carcasses treated with pediocin, pediocin with disodium EDTA and control stored at 4 ± 1^0C on 0, 3^{rd}, 5^{th} and 6^{th} day are presented in table 2 and figure 2. The mean ± SE value of WHC of fresh chicken carcasses on 0 day was 1.58 ± 0.05. The analysis of variance revealed a highly significant (P<0.01) difference between the different treatments and between the storage periods.

Extract Release Volume (ERV) (ml)

The mean ± SE values of ERV of chicken carcasses treated with pediocin, pediocin with disodium EDTA and control stored at 4 ± 1^0C on 0, 3^{rd}, 5^{th} and 6^{th} day are presented in table 3 and figure 3. The mean ± SE value of ERV of fresh chicken carcasses on 0 day was 20.67 ± 0.23ml. The analysis of variance revealed a significant (P<0.05) difference between the different treatments and a highly significant (P<0.01) difference between the storage periods.

Thiobarbituric Acid Number (TBA) (mg/kg)

The mean ± SE values of TBA (mg malonaldehyde/kg) of chicken carcasses treated with pediocin, pediocin with disodium EDTA and control stored at 4 ± 1^0C on 0, 3^{rd}, 5^{th} and 6^{th} day are presented in table 4 and figure 4. The mean ± SE value of TBA (mg malonaldehyde/kg) of fresh chicken carcasses on 0 day was 0.023 ± 0.001. The analysis of variance revealed a significant (P<0.05) difference between the different treatments and highly significant (P<0.01) difference between the storage periods.

Tyrosine Value (TV) (mg/100g)

The mean ± SE tyrosine values of chicken carcasses treated with pediocin, pediocin with disodium EDTA and control stored at 4 ± 1^0C on 0, 3^{rd}, 5^{th} and 6^{th} day are presented in table 5 and figure 5. The mean ± SE tyrosine value of fresh chicken carcasses on 0 day was 11.78 ± 0.47. 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.

Hunter color lab analysis

Lightness (L^*)

The mean ± SE values of L^* of chicken carcasses treated with pediocin, pediocin with disodium EDTA and control stored at 4 ±1^0C on 0, 3^{rd}, 5^{th} and 6^{th} day are presented in table 6 and figure 6. The overall mean ± SE value of L^* of fresh chicken carcasses on 0 day was 52.89 ± 0.55. The analysis of variance revealed a no significant (P>0.05) difference between the different treatments and between the storage periods.

Redness (a^*)

The mean ± SE values of a^* of chicken carcasses treated with pediocin, pediocin with disodium EDTA and control stored at 4 ± 1^0C on 0, 3^{rd},5^{th} and 6^{th} day are presented in table 7 and figure 7. The mean ± SE value of L^* of fresh chicken carcasses on 0 day was 4.09 ± 0.13.

The analysis of variance revealed a no significant (P>0.05) difference between the different treatments but a significant (P<0.05) difference between the storage periods.

Yellowness (b^*)

The mean ± SE values of b^* of chicken carcasses treated with pediocin, pediocin with disodium EDTA and control stored at 4 ± 1^{0}C on 0, 3rd,5th and 6th day are presented in table 8. The mean ± SE value of b^* of fresh chicken carcasses on 0 day was 12.09 ± 0.13. The analysis of variance revealed a no significant (P>0.05) difference between the different treatments and between the storage periods.

Preservation or extension of shelf life of meat is very vital for marketing of meat and meat products. Now-a-days many methods including chemical preservation are adopted leading to hazardous residues and consumers resistance to such techniques. Hence, attempt has been made to extend physic-chemical quality of chicken meat using pediocin.

Table.1 Mean ± SE values of pH of control and treated chicken carcasses stored at 4±1ºC

Days	Pediocin	Pediocin +EDTA	Control	Overall mean
0th Day	6.21 ± 0.08	6.21 ± 0.08	6.21 ± 0.08	6.21 ±0.04^{XY}
3rd Day	6.11 ± 0.04	6.15 ± 0.10	6.14 ± 0.07	6.13 ± 0.04^{X}
5th Day	6.25 ± 0.02^{b}	6.17 ±0.02^{a}	6.28 ± 0.01^{b}	6.23 ± 0.01^{Y}
6th Day	6.27 ± 0.02	6.25 ± 0.03	6.34 ± 0.05	6.29 ± 0.02^{Y}
Over all mean	6.21 ± 0.02	6.20 ± 0.03	6.24 ± 0.03	_

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

Table.2 Mean ± SE values of WHC (cm^2) of control and Treated chicken carcasses stored at 4±1ºC

Days	Pediocin	Pediocin +EDTA	Control	Overall mean
0th Day	1.58 ± 0.09	1.58 ± 0.09^{AB}	1.58 ± 0.09^{A}	1.58 ± 0.05^{X}
3rd Day	1.41 ± 0.06^{a}	1.38 ± 0.05^{Aa}	1.78 ± 0.09^{ABb}	1.53 ± 0.06^{X}
5th Day	1.83 ± 0.08^{a}	1.77 ± 0.15^{Ba}	2.30 ± 0.19^{Cb}	1.97 ± 0.10^{Y}
6th Day	1.77 ± 0.16	1.72 ± 0.06^{B}	2.00 ± 0.06^{BC}	1.83 ± 0.06^{Y}
Over all mean	1.65 ± 0.06^{X}	1.61 ± 0.05^{X}	1.92 ± 0.08^{Y}	_

Table.3 Mean ± SE values of ERV (ml) of control and treated chicken carcasses stored at 4±1ºC

Days	Pediocin	Pediocin +EDTA	Control	Overall mean
0th Day	20.67 ± 0.42^{D}	20.67 ± 0.42^{C}	20.67 ± 0.42^{D}	20.67 ± 0.23^{Z}
3rd Day	17.33 ± 0.33^{Cb}	18.00 ± 0.26^{Bb}	15.50 ±0.62^{Ca}	16.94 ± 0.35^{Y}
5th Day	12.50 ±0.43^{Ba}	17.67 ± 0.42^{Bb}	12.00 ± 0.36^{Ba}	14.06 ± 0.66^{X}
6th Day	9.83 ± 0.48^{Aa}	13.00 ± 0.36^{Ab}	9.17 ± 0.48^{Aa}	10.67 ± 0.47^{W}
Over all mean	15.08 ± 0.90^{XY}	17.33 ± 0.60^{Z}	14.33 ± 0.92^{X}	_

Table.4 Mean ± SE values of Thiobarbituric acid number (mg/kg) of control and Treated chicken carcasses stored at 4±1ºC

Days	Pediocin	Pediocin +EDTA	Control	Overall mean
0th Day	0.023 ± 0.001 ^{A}	0.023 ± 0.001^{A}	0.023 ± 0.001^{A}	0.023 ± 0.001^{W}
3rd Day	0.064 ± 0.004^{Bb}	0.047 ± 0.002^{Ba}	0.098 ± 0.003^{Bc}	0.070 ± 0.005^{X}
5th Day	0.094 ± 0.005 ^{Ca}	0.085 ± 0.005^{Ca}	0.120 ± 0.01^{Cb}	0.099 ± 0.005^{Y}
6th Day	0.120 ± 0.005 ^{Da}	0.107 ± 0.005 ^{Da}	0.149 ± 0.01^{Db}	0.125 ± 0.005^{Z}
Over all mean	0.076 ± 0.01^{XY}	0.066 ± 0.01^{X}	0.098 ± 0.01^{Y}	_

Table.5 Mean ± SE Tyrosine values (mg/100gm) of control and Treated chicken carcasses stored at 4±1ºC

Days	Pediocin	Pediocin +EDTA	Control	Overall mean
0th Day	11.78 ± 0.87	11.78 ± 0.87 ^{A}	11.78 ± 0.87 ^{A}	11.78 ± 0.47^{X}
3rd Day	16.16 ± 3.13	16.05 ± 2.65 ^{AB}	18.62 ± 4.34 ^{B}	16.94 ± 1.89^{Y}
5th Day	18.28 ± 3.15	17.94 ± 1.46 ^{B}	19.10 ± 0.72 ^{B}	18.44 ± 1.12^{YZ}
6th Day	21.08 ± 0.13^{b}	19.93 ± 0.26 ^{Ba}	23.57 ± 0.33^{Bc}	21.53 ± 0.39^{Z}
Over all mean	16.82 ± 1.27	16.43 ± 0.97	18.27 ± 1.37	_

Table.6 Mean ± SE values of Hunters color lab – Lightness (L*) of control and Treated chicken carcasses stored at 4±1ºC

Days	Pediocin	Pediocin +EDTA	Control	Overall mean
0th Day	52.89 ± 1.01	52.89 ± 1.01	52.89 ± 1.01	52.89 ± 0.55
3rd Day	52.30 ± 1.22	51.76 ± 1.26	52.30 ± 1.22	52.12 ± 0.67
5th Day	52.76 ± 1.25	53.70 ± 0.67	52.59 ± 0.57	53.02 ± 0.49
6th Day	52.74 ± 0.69	53.66 ± 1.32	52.45 ± 0.87	52.95 ± 0.55
Over all mean	52.67 ± 0.50	53.00 ± 0.54	52.56 ± 0.44	_

Table.7 Mean ± SE values of Hunters color lab – Redness (a*) of control and Treated chicken carcasses stored at 4±1ºC

Days	Pediocin	Pediocin +EDTA	Control	Overall mean
0th Day	4.09 ± 0.24	4.09 ± 0.24	4.09 ± 0.24^{AB}	4.09 ± 0.13^{Y}
3rd Day	3.70 ± 0.18	3.90 ± 0.15	3.69 ±0.16^{A}	3.76 ± 0.09^{X}
5th Day	3.80 ± 0.19	4.00 ± 0.23	4.35 ± 0.07^{B}	4.04 ± 0.11^{XY}
6th Day	4.19 ± 0.16	4.19 ± 0.17	4.33 ± 0.19^{B}	4.24 ± 0.10^{Y}
Over all mean	3.94 ± 0.10	4.04 ± 0.09	4.11 ± 0.10	_

Table.8 Mean ± SE values of Hunters color lab – Yellowness (b*) of control and Treated chicken carcasses stored at 4±1ºC

Days	Pediocin	Pediocin +EDTA	Control	Overall mean
0th Day	12.09 ± 0.24	12.09 ± 0.24^{A}	12.09 ± 0.24^{A}	12.09 ± 0.13
3rd Day	12.16 ± 0.18	12.40 ± 0.25^{AB}	12.17 ± 0.22^{A}	12.24 ± 0.12
5th Day	12.02 ± 0.17	12.06 ± 0.24^{A}	12.56 ± 0.39^{AB}	12.21 ± 0.16
6th Day	12.60 ± 0.40	13.06 ± 0.19^{B}	13.10 ± 0.24^{B}	12.92 ± 0.17
Over all mean	12.21 ± 0.13	12.40 ± 0.14	12.48 ± 0.16	_

Fig.1 Spraying of chicken carcass with cell free supernatant (Pediocin NCDC252)

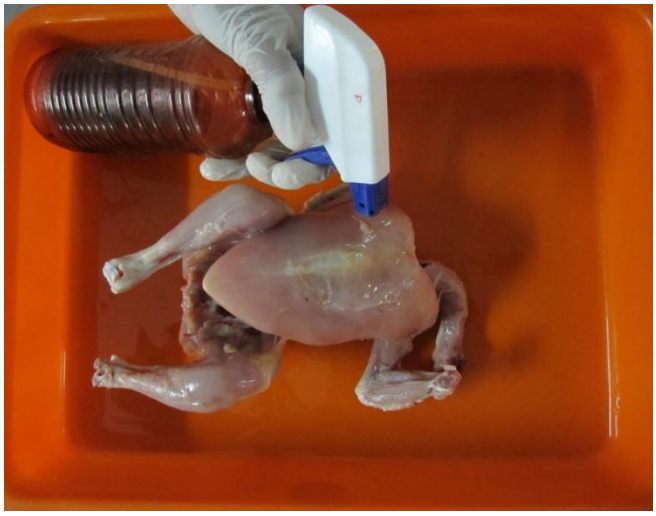


Fig.2 Spraying of chicken carcass with cell free supernatant (Pediocin NCDC252) and disodium EDTA

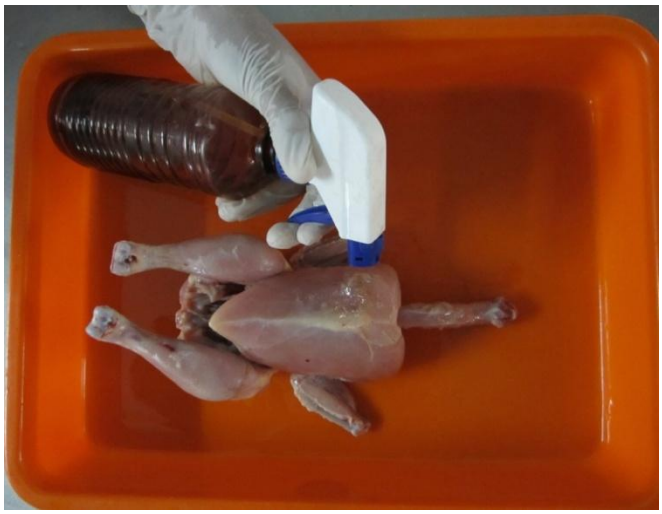


Fig.3 *Pediococcus acidilactici* NCDC252 on MRS agar

Fig.4 Gram staining *of Pediococcus acidilactici* NCDC252

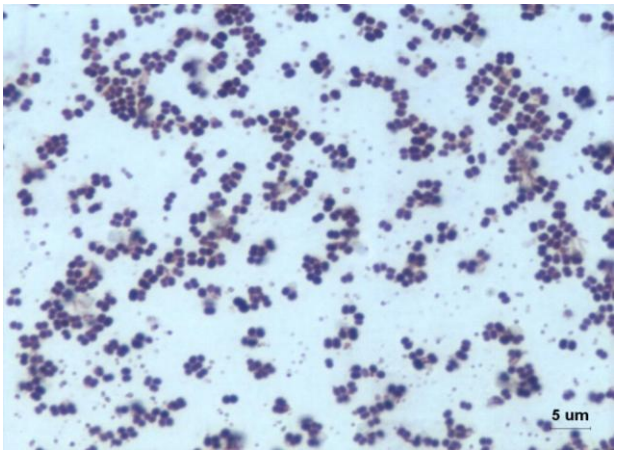


Fig.5 Antibacterial activity of Pediocin NCDC252 against *S. aureus*

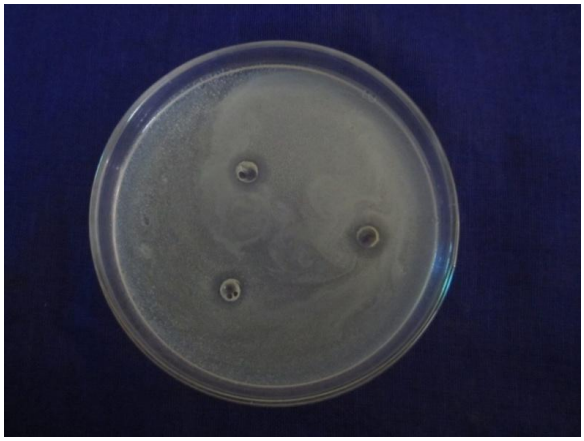
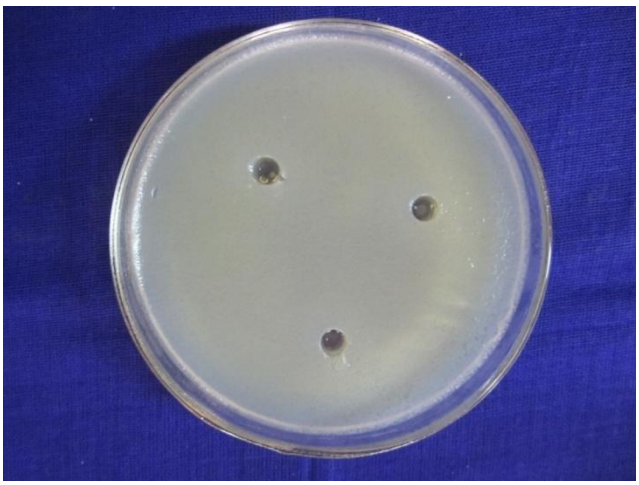


Fig.6 Antibacterial activity of Pediocin NCDC252 against *E. coli*



pH

The mean pH value of chicken carcasses on 0 day was 6.21 ± 0.04, which is in agreement with Petrou *et al.,* (2012). A highly significant difference was found in pH values of control and treated carcasses on 5th day. Control showed high pH than other two treatments on 5th day. Kandeepan and Biswas (2007) stated that the increase in pH is due to the autolysis and increase in microbial load in chiller stored meat.

Water holding capacity

Water holding capacity of muscle affects the quality of meat during almost all processing operations after slaughter. The mean value of water holding capacity for fresh chicken carcasses on 0 day was 1.58 ± 0.09 cm^2. This is in agreement with Jayesh (1999) who reported the WHC of fresh mutton was 1.65±0.07 cm^2. The overall mean value of water holding capacity showed a highly significant difference in between treatments and in between storage periods.

On 3rd and 5th day pediocin NCDC252 alone and pediocin NCDC252 + EDTA group showed significantly higher water holding capacity. On 6th day there was no significant difference found in water holding capacity values in between three groups. The results revealed that water holding capacity of

all groups decreased on 5th and 6th day of storage. Kandeepan and Biswas (2007) stated that the loss of WHC was partly due to increased denaturation of proteins and partly due to enhanced movement of water into extracellular spaces.

ERV (Extract Release Volume)

It was observed that in all groups ERV value decreased as storage period increased due to spoilage of meat. It is in agreement with Sinhamahapatra *et al.,* (2004) and Jay *et al.,* (2003). It was found that mean ERV value showed highly significant difference on 3rd, 5th and 6th day in between three treatments. On 3rd day Pediocin NCDC252 alone and pediocin NCDC252 with disodium EDTA treated carcasses showed highly significant ($P<0.01$) difference in ERV values. Whereas, on 5th and 6th day Pediocin NCDC252 with disodium EDTA shown significantly ($P<0.01$) high ERV values compared to other two groups. It indicates lower microbial spoilage in pediocin NCDC252 with disodium EDTA treated groups compared to other two groups on respective days.

TBA

The results revealed a significant increase in TBA value in all treatments as storage period increases. This is in agreement with Botsoglou *et al.,* (2002) and Rindhe *et al.,* (2012). Kandeepan and Biswas (2007) reported increase in TBA value which was mainly attributed to the oxygen permeability of the packaged meat leading to lipid oxidation. The overall mean values of TBA showed significant difference in between treatments and highly significant difference in between storage period. The pediocin NCDC252 alone and pediocin NCDC252 with disodium EDTA treated carcasses showed highly significant difference with control group. Both groups showed lower TBA value than control. It indicates high lipid oxidation and rancidity in control group compared to other two groups.

Tyrosine Value

Strange *et al.,* (1977) stated that the tyrosine value was an effective method to monitor meat quality and it was an indicator of proteolysis as it measured the amino acids tyrosine and tryptophan present in non-protein extract of meat. The results revealed that both control and pediocin NCDC252 with disodium EDTA treated carcasses showed significant increase in TV value as storage period increases. This is in agreement with Pearson (1968), Kandeepan and Biswas (2007) and Chueachuaychoo *et al.,* (2011).

Hunter color lab analysis

Lightness (L^*)

The mean L^* value of fresh chicken breast on 0 day was 52.89 ± 0.55 which is in agreement with Liu *et al.,* (2011), Chouliara *et al.,* (2007) and Patsias *et al.,* (2008). The results revealed that there was no significant difference in between the treatments and in between the storage period indicates that pediocin NCDC252 alone or with disodium EDTA does not affects the lightness of chicken breast meat stored at 4±1˚C.

Redness (a*)

The mean values of redness (a*) on 0 day was 4.09 ± 0.13 which is in agreement with Patsias *et al.,* (2008). The overall mean values of redness (a*) did not show significant difference between treatments but there was significant difference in between storage periods. Werner *et al.,* (2009) stated that during cold storage a* value of broiler breast muscle decreases. In the present study also all the groups showed a non-significant decrease in a* value upto 3rd day. But later a slight increase was observed in a* value in control group.

Yellowness (b*)

The mean value of yellowness (b*) on 0 day was 12.09 ± 0.24 which was in agreement with Chouliara *et al.,* (2007) and Patsias *et al.,* (2008). Highest b* value was noticed in control group on 6th day of storage. The results revealed a significant increase in b* value in pediocin NCDC252 with disodium EDTA treated and control group, similar to that reported by Werner *et al.,* (2009). The overall mean shows that there was no significant difference in b* values in between treatments and in between storage period. It indicates that pediocin NCDC252 alone or with disodium EDTA did not affect the yellowness of chicken meat stored at 4±1˚C.

It can be concluded that Antibacterial activity of cell free supernatant was assessed against indicator organisms. After this cell free supernatant was used for spraying of chicken carcasses. Along with pediocin, disodium

EDTA was added for spraying to extend its antibacterial activity against gram positive organisms. Carcasses were stored at refrigeration temperature and their by meat quality parameters like pH, water holding capacity, extract release volume, thiobarbituric acid value, tyrosine value, Hunter color analysis were assessed. The results of the study indicated that the chicken carcasses treated with pediocin NCDC252 with disodium EDTA had better physico-chemical quality characteristics than the other two groups upto 5th day of storage life.

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References

Barefoot, S.F. and T. R. Klaenhammer, 1984. Purification and characterization of the *Lactobacillus acidophilus* bacteriocin lactacin B. *Antimicrob. Agents Chemother,* 26(3): 328-334.

Botsoglou, N.A., E. Christaki, D.J. Fletouris, P. Florou-Paneri and A.B. Spais, 2002. The effect of dietary oregano essential oil on lipid oxidation in raw and cooked chicken during refrigerated storage. *Meat Sci.,* 62: 259–265.

Chouliara, E., A. Karatapanis, I.N. Savvaidis and M.G. Kontominas, 2007. Combined effect of oregano essential oil and modified atmosphere packaging on shelf-life extension of fresh chicken breast meat, stored at 4˚C. *Food Microbiol*, 24: 607–617.

Chueachuaychoo, A., S. Wattanachant, and S. Benjakul, 2011. Quality characteristics of raw and cooked spent hen *Pectoralis major* muscles during chilled storage: Effect of salt and phosphate. *International Food Research Journal,* 18: 601-613.

Economou, T., N. Pournis, A. Ntzimani and I.N. Savvaidis, 2009. Nisin–EDTA treatments and modified atmosphere packaging to increase fresh chicken meat shelf-life. *Food Chem.,* 114: 1470–1476.

Grau, R. and R. Hamm, 1957. Biochemistry of meat hydration. *Advances in Food Research*, 10: 356.

Jay, J. M., J. P. Vilai and M. E. Hughes, 2003. Profile and activity of the bacterial biota of ground beef held from freshness to spoilage at 5–7 ^{0}C. *International Journal. Food. Micro,* 81: 105-111.

Jayesh, V., 1999. Effect of chilling and freezing on quality characteristics of mutton. M.V.Sc. thesis submitted to Tamil Nadu veterinary and Animal Sciences University.

Jeevaratnam, K., M. Jamuna and A.S. Bawa, 2005. Biological preservation of food - Bacteriocins of lactic acid bacteria. Indian Journal of Biotechnology, 4: 446-454.

Kandeepan G. and S. Biswas, 2007. Effect of domestic refrigeration on keeping quality of buffalo meat. *J Food Tech*., 5(1): 29-35.

Klare, I., C. Konstabel, S. Muller-Bertling, R. Reissbrodt, G. Huys, M. Vancanneyt, Jean Swings, Herman Goossens and Wolfgang Witte, 2005. Evaluation of new broth media for microdilution antibiotic susceptibility testing of *Lactobacili, Pediococci, Lactococci* and *Bifidobacteria*. *Appl. Environ. Microbiol,* 71(12): 8982-8986.

Liu, S., Y. Han, Z. Zhou, 2011. Fusion expression of *pedA* gene to obtain biologically active pediocin PA-1 in *Escherichia coli*. *JZUS-B.*, 12(1): 65-71.

Osmanauaoulu, O., Y. Beyatli and U., Gunduz, 2001. Isolation and Characterization of Pediocin

Producing *Pediococcus pentosaceus* Pep1 from Vacuum-Packed Sausages. *Tr. J. of Biology*, 25: 133-143.

Patsias, A., A. V. Badeka, I. N. Savvaidis and M. G. Kontominas, 2008. Combined effect of freeze chilling and MAP on quality parameters of raw chicken fillets. *Food Microbiol*, 25: 575-581.

Pearson D., 1968. Assessment of meat freshness in quality control employing chemical techniques: A review. *J Sci Food Agric.*, 19(7): 357-363.

Pearson, D., 1967. Assessing beef acceptability. Food Manufacture, November, 1967: 42-43.

Petrou, S., M. Tsiraki, V. Giatrakou and I.N. Savvaidis, 2012. Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. *Int J Food Microbiol*, 156: 264–271.

Piard, J. C., P. M. Muriana, M. J. Desmazeaud and T. R. Klaenhammer, 1992. Purification and partial characterization of lacticin 481, a lanthionine-containing bacteriocin produced by *Lactococcus lactis* subsp. *lactis* CNRZ 481 *Appl. Environ. Microbiol*, 58(1): 279-284.

Rindhe, S., S. U. Suryavanshi, K. K. Khose, R. N. Waghmare, M. J. Saxena, K.Ravikanth and S. Maini, 2012. Role of polyherbal liquid antistressor product in improving meat quality attributes in broilers. *International Research Journal of Pharmacy and Pharmacology*, 2(9): 209-214.

Schillinger, U. and F. Lucke, 1989. Antibacterial activity of lactobacillus sake isolated from meat. *Appl. Environ. Microbiol*, 55 (8): 1901-1906.

Sinhamahapatra, M., S. Biswas, A. K. Das and D. Bhattacharyya, 2004. Comparative study of different surface decontaminants on chicken quality. *Brit. Poult. Sci*., 45(5): 624-630.

Snedecor, G. W. and W.G. Cochran, 1994. Statistical methods. Eighth edition. Oxford and IBH Publishing Co., Calcutta.

Strange, E. D., R.C. Benidict, J.C. Smith and C.E. Swift, 1977. Evaluation of rapid test monitoring alteration in meat quality during storage. Intact meat. *J. Food Prot.*, 40: 843-847.

Tufail, M., S. Hussain, F. Malik, T. Mirza, G. Parveen, S. Shafaat, A.Wajid, R. Mahmood, R. A. Channa and A. Sadiq, 2011. Isolation and evaluation of antibacterial activity of bacteriocin produced by *Lactobacillus bulgaricus* from yogurt. *Afr. J. Microbiol. Res.*, 5(22): 3842-3847.

Werner, C. S. Janisch, U. Kuembet and M. Wicke, 2009. Comparative study of the quality of broiler and turkey meat, *J. Brit. Poult.Sci*, 50(3): 318-324.

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