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Effect of *Lactobacillus sakei* as Protective Culture on Extended Storage Life of Chicken Breast Fillets kept under Refrigeration Temperature

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A B S T R A C T

Study was conducted to evaluate the effect of *Lactobacillus sakei* as protective culture on extended storage of chicken breast fillets kept under refrigeration temperature. Chicken fillets were allotted in to two different treatment groups namely, T1 (as control) and T2 (with *Lactobacillus sakei*). Total viable count increased significantly in both the samples with the advancement of storage period. *Coli titre* count was found to be nil in all the samples throughout the storage period up to 12th day. No *E. coli* organisms could be isolated on 0, 5th, 7th, 9th and 12th day in both the samples. The inoculation of starter cultures (T2) significantly exerted strong inhibitory effect against *Salmonella* and coagulase positive pathogenic *Staphylococci* compared to T1. Sensory evaluation with respect to taste, flavour and overall acceptability revealed that the T2 enjoyed better panel ratings. From the above study it can be inferred that *Lactobacillus sakei* could be use to increase the storage life of chicken fillets and it was a successful attempt, as superior quality in microbiological and sensory parameters was achieved and also ensured a longer shelf life of the further product.

Introduction

India has largest livestock resources, among which poultry broiler production has been more vibrant than layer production within the poultry sector, with an annual growth rate of 11.44 per cent, production of 3.72 million tonnes and employment for 4.29 million people (Index, 2015). Chicken meat products are more preferred by the peoples worldwide and also demand for the processed chicken meat products is ever increasing due to rapid urbanization, improved standards of living and changing life styles. However, impairment of progress of meat sector is due to inadequate processing technology for effective marketing as meat and meat products are highly perishable materials.

Consumers are nowadays demanding safe food products with minimal processing; the application of bio preservation techniques could be a natural alternative for food preservation (Melero *et al.,* 2013). Thus, lactic acid bacteria (LAB) have major potential for use as a protective culture as they are generally recognized as safe (GRAS) and naturally dominate the microbiota of many foods (Castellano *et al.,* 2008). *Lactobacillus sakei* has been shown to possess in vitro proteolytic and antioxidative abilities which could have an impact on chemical processes such as proteolysis and lipid oxidation and therefore could influence storage life and quality of meat and meat products. This microorganism flourishes at low temperatures and frequently becomes one of the dominant flora during cold storage. The positive influence of *Lactobacillus sakei* on food preservation can be explained mainly by its physiological and genomic adaptations to growth on meat products, which result in outcompeting other disease or spoilage causing microorganisms (Chaillou *et al.,* 2013). The use of bioprotective cultures of LAB and their bacteriocins in the production and preservation of ready-to-eat meat products, is a methodology that has been studied as an alternative to chemical additives for assuring food safety. This biological control also allows to reduce the amount of salt, nitrite and other additives required to effectively preserve food (Galvez *et al.,* 2007). The general objective of the present study was to explore the possibility of extending storage life of chicken breast fillets by using *Lactobacillus sakei* as protective culture and its effect on the physico-chemical qualities and sensory attributes.

Materials and Methods

Raw materials

Broiler chicken of 2.0 to 2.5 kg weight were procured from the poultry farm of College of Veterinary Sciences & Animal Husbandry, CAU, Selesih, Aizawl, Mizoram, and humanely slaughtered and dressed under hygienic conditions at Department of Livestock Products Technology. The deboning was done manually and chicken fillets were cut with the help of cutting knife, packed in polyethylene bags and kept under refrigeration until use. Chicken fillets were divided into two equal parts and were allotted to different treatment groups. T-1 (control) and T-2 (sprayed with *Lactobacillus sakei*). The samples were packed and sealed using vacuum packaging machine in High Density Polyethylene (HDPE, 3Mil) bags and kept under refrigeration temperature (4 ± 1^{0}C) in a domestic refrigerator for 12 days. These were then analyzed for different microbiological and sensory parameters and at a periodic interval upto 12^{th} days.

Microbiological evaluation of the meat samples

Total Viable Count (TPC)

Enumeration of the TPC of the fillet samples was done in standard plate count agar medium, by following the 'spread plate technique' described by Harrigan and McCance (1976), on the $0,5^{th},7^{th}$, 9^{th} and 12^{th} d of storage for all the samples. The plates were incubated at 37 ^{0}C for up to 72 hours.

Coli titre

The *coli titre* of the fillet samples at time interval similar to TVC was determined by following the 'multiple tube technique' described by Harrigan and McCance (1976). Serial dilution of the meat samples up to 10^{-3} were inoculated into brilliant green lactose bile broth, pH 7.4±0.1 and incubated at 37º C for up to 72 h. The results were expressed as most probable number (MPN) per gm by following the conversion table given by AOAC (1990).

Counts for *E. coli*

Counts for *E. coli* was done at the time interval as in case of TVC were done by inoculating the fillet samples at appropriate decimal dilutions of the samples up to 10^{-3} on Mac Conkeys Agar plate by following the 'spread plate technique' with slight modifications (Boschkova, 1990). The plates were incubated at 37^0C and the plates were counted after 24 h of incubation.

Presence or absence of *Staphylococcus aureus*

Determination of presence or absence of *Staphylococcus aureus* was done by serial dilution of the fillet samples up to 10^{-2} were inoculated into Baird Parker agar plate by following the 'spread plate technique' described by FDA bacteriological analytical manual 2005 18th edn, AOAC, Washington, for all the samples on 0, 5th, 7th, 9th and 12th day of storage.

Presence or absence of *Salmonella*

For determination of the presence or absence of *Salmonella* organisms in the fillet samples, the ISO 6579 method was followed with necessary modifications. All the samples were analyzed on 0, 5th, 7th, 9th and 12th d of storage for all the samples. A 25gm portion of the meat sample were mixed with 225ml of pre-enrichment medium (buffered peptone water) in a conical flask and incubated at 37^0C for 24 h. Following incubation, 10 ml of the cultured were transferred to 100ml of tetrathionate broth and incubated at 43^0C for 72 h.

Presence or absence of *Pseudomonas*

Determination of presence or absence of *Pseudomonas* organisms in the fillet samples was done by inoculating the samples at appropriate decimal dilution on King, Ward and Raney's agar (1954) plates by 'spread plate technique'. Plates were incubated at 37^0C for 24 h and then at 22^0C for 72h.

Organoleptic evaluation of the meat samples

Test for detection of spoilage of meat samples by assessment of odour were assessed by semi-trained panel using 10 point hedonic score card (Pearson 1968) for all the samples on 3rd, 5th, 7th, 9th and 12th day of storage. Sensory attributes of the chicken fillet were assessed organoleptically using 8-point Hedonic scale (Keeton 1983) was followed with slight modification. The samples were heated in microwave oven for two minutes, prior to serve ten semi trained panellists. The average of the individual scores was taken as the score for the particular attribute.

Statistical analysis

The data obtained from the experiment were statistically analyzed as per Snedecor and Cochran (1995) using the SPSS software version 20. One way ANOVA and Duncan test were applied to analyse and test the significant difference between the different treatments.

Results and Discussion

Microbiological analysis of the meat samples

The results on the effect of different treatments and storage periods are presented in Table 1. It showed that TVC of T1 significantly increase from (4.20±.10 to 5.26±.034 $\log_{10}$ cfu/g) from 0th to 12th day of storage. However it shows no significant ($p \geq 0.05$) difference in the storage days except 0dand 5thd where it differs significantly ($p \leq 0.05$) same as in 9th and 12thd of storage. Similar findings have been reported by

Morioka *et al.,* (1999) on soft salami sausage incorporated with starter culture. In T2, the TVC was not detected on 0d, whereas from 5^{th}d (4.10±.100 log_{10} cfu/g), it increases significantly ($p \leq 0.05$) till 12^{th}d of storage (4.96±.040 log_{10} cfu/g). Effect of different treatments and storage periods on the mean *coli* titre counts of the fillet sample expressed as most probable number (MPN) per g was found to be negative. The *coli* titre counts were absent for all the control and treatments on 0, 5^{th}, 7^{th}, 9^{th} and 12^{th}d of storage. No *E.coli* culture was found in all the treatment groups on 0, 5^{th}, 7^{th}, 9^{th} and 12^{th}d of storage. Babji and Murthy (2000) also observed decrease in the counts of *coliform* in goat meat samples treated with *Lc. Lactis* and *L.plantarum than* the control samples.

The results on the presence or absence test for *Salmonella* in the fillet samples were presented in Table 2. The samples under T1 and T2 were found to be positive for *Salmonella* on 0^{th}d and 5^{th} d and from 7^{th}d of storage onwards found to be negative. Holfazapfel *et al.,* (1995) also suggested the use of protective cultures of LAB for control of *Salmonellae* in fresh meat and poultry.

The results on the presence or absence test for *Pseudomonas* in the meat samples were presented in Table 3. The samples under T1 and T2 were found to be positive for *Pseudomonas* on 0d and 5^{th}d and from 7^{th}d of storage onwards found to be negative. Hechelmann *et al.,* (1977) also reported that the *Pseudomonads* were usually sensitive to all salt and nitrite of the cured meat products. The results on the presence or absence test for coagulase positive pathogenic *staphylococci* in the meat samples were presented in (Table 4). The samples under Treatment I and Treatment II were found to be positive for coagulase positive pathogenic *staphylococci* on 0d and 5^{th} d and from 7^{th} d of storage onwards found to be negative. Cintas *et al.,* (1992) studied the antibacterial activity of LAB isolated from Spanish dry fermented sausages and reported that supernatants from *L. sake* 148 was inhibitory towards *Staph. aurues*.

Sensory evaluation of the meat samples

Results on the panel evaluation of odour score of the fillet samples as affected by different treatments and storage periods are presented in figure 1. The mean odour score of the meat samples of T1 significantly decreased ($p \leq 0.05$) from 10.00±.000 (on the 3^{rd}d) to 5.0 ±.000 by the end of 12^{th}d of storage. However T2 could maintain the level of acceptability in terms of odour score as assessed by the panellists up to 12^{th}d of storage with a mean panel rating of 6.33±.333. There is no significant ($p \geq 0.05$) difference between the storage days of 3^{rd} and 5^{th}d and there after it decreases significantly ($p \leq 0.05$) from 7^{th} to 12^{th} day. T1 and T2 are significantly different ($p \leq 0.05$) on 7^{th} and 12^{th} day of storage. Nathappan *et al.,* (1985) also reported that the odour score of mutton samples decrease with the advancement of the storage period and the odour score of the mutton samples stored at 5 ± 1^0C was found to be just within the limit of acceptability up to 72 hours of storage. Result for appearance (Fig. 2) and flavour (Fig. 3) followed a decreasing trend with the advancement of storage period. By the end of 12^{th}d of storage, the mean appearance of the T1 was found to be 7.00±.091 and T2 7.14±.09 respectively. There is a no significant ($p \geq 0.05$) difference between T1 and T2 in all storage days. The T2 samples were highly acceptable whereas the T1 shows least acceptable. By the end of 12^{th} d of storage, the mean appearance of the T1 sample was found to be 6.80±.21. The value decreases significantly ($p \leq 0.05$) on 3^{nd} and 5^{th} d of storage and shows no significant difference ($p \geq 0.05$) from 5^{th} to 11^{th} d and again it decreases significantly ($p \leq 0.05$) on

12th d of storage. The samples of Treatment 2 could also maintain the level of acceptability in terms of flavour as assessed by the panellists up to 12th d of storage with a mean panel rating of 6.94±.10. There is no significant ($p \geq 0.05$) difference between the storage days. There is a no significant ($p \geq 0.05$) difference between T1 and T2 in all storage days except on 12th d where it differ significantly ($p \leq 0.05$). The result for texture (Fig. 4) too followed a decreasing trend as the storage period advanced. The mean texture of T1 (6.60±.09) whereas for T2 (7.02±.11) respectively, where T2 could maintain the level of acceptability as assessed by panellists by the end of 12th d of storage. T1 and T2 did not differ significantly ($p \geq 0.05$) in all storage days except on 5th d where it differ significantly ($p \leq 0.05$).

Table.1 TVC (log10 cfu/g) chicken fillets treated with *Lactobacillus sakei* kept under refrigeration storage at ± 4ºC

Treatment groups	Period of analysis					
	0d	5th d	7th d	9th d	12th d	Overall
T1	$4.20\pm.10^{Ba}$	$4.59\pm.06^{Bb}$	$4.69\pm.05^{Bb}$	$5.03\pm.04^{cB}$	$5.26\pm.03^{dB}$	$4.75\pm.10^{*}$
T2	ND	$4.10\pm.10^{Ab}$	$4.35\pm.05^{Ac}$	$4.65\pm.09^{dA}$	$4.96\pm.04^{eA}$	3.61±.48*

Means ± SE with different uppercase superscripts in the same row and lowercase superscripts in the same columns are significantly different (P<0.05). T1-Control, T2 (with *Lactobacillus sakei*)

Table.2 Presence and absence test of *Salmonella* of chicken fillets treated with *Lactobacillus sakei* kept under refrigeration storage at ± 4ºC.

Treatment groups	Period of analysis				
	0d	5th d	7th d	9th d	12th d
T1	+	+	-	-	-
T2	+	+	-	-	-

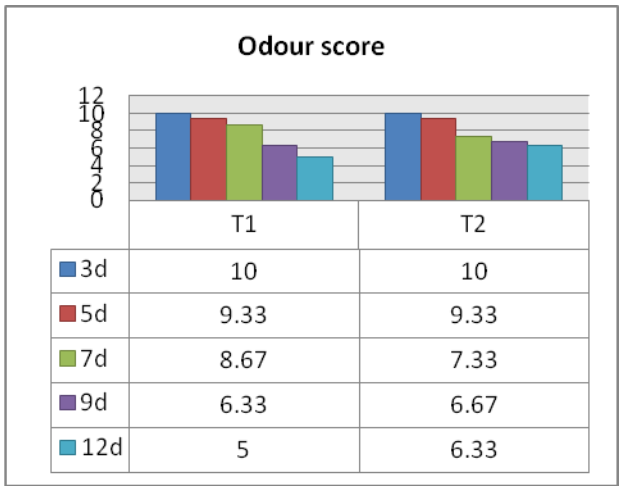
Table.3 Presence and absence test of *Pseudomonas* of chicken fillets treated with *Lactobacillus sakei* kept under refrigeration storage at ± 4ºC

Treatment groups	Period of analysis				
	0d	5th d	7th d	9th d	12th d
T1	+	+	-	-	-
T2	+	+	-	-	-

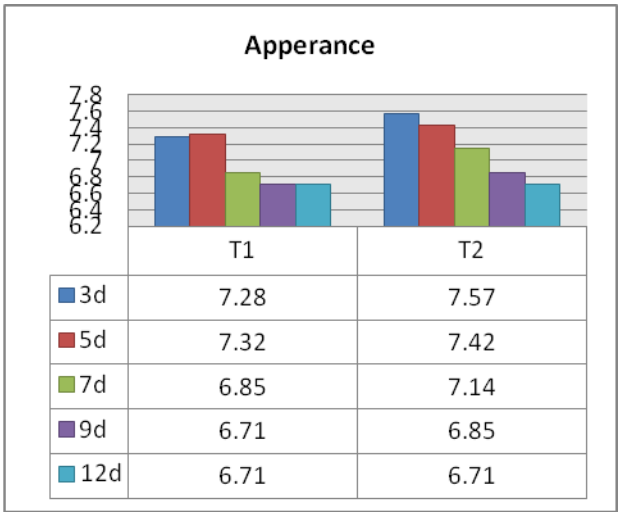
Table.4 Presence and absence test of Staphylococcus aureus of chicken fillets treated with *Lactobacillus sakei* kept under refrigeration storage at ± 4ºC

Treatment groups	Period of analysis				
	0d	5th d	7th d	9th d	12th d
T1	+	+	-	-	-
T2	+	+	-	-	-

Fig.1&2 Odour scores of chicken fillets treated with *Lactobacillus sakei* kept under refrigeration storage at ± 4ºC (Mean ± SE) & Appearance scores chicken fillets treated with *Lactobacillus sakei* kept under refrigeration storage at ± 4ºC (Mean ± SE)

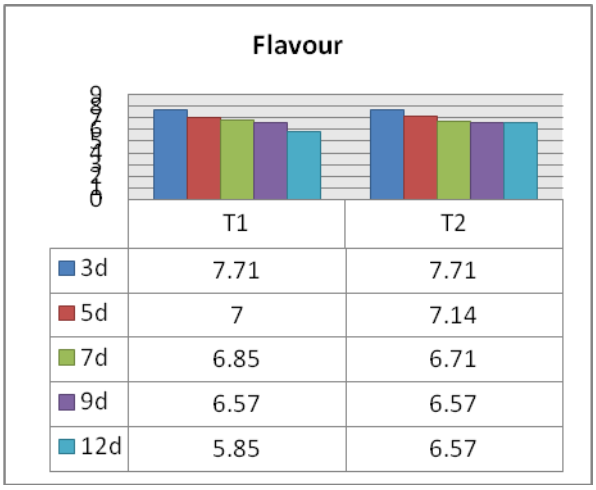


	T1	T2
3d	10	10
5d	9.33	9.33
7d	8.67	7.33
9d	6.33	6.67
12d	5	6.33

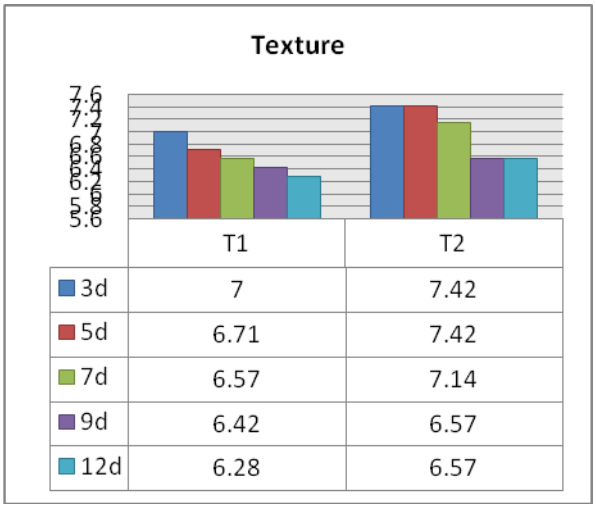


	T1	T2
3d	7.28	7.57
5d	7.32	7.42
7d	6.85	7.14
9d	6.71	6.85
12d	6.71	6.71

Fig.3&4 Flavour scores chicken fillets treated with *Lactobacillus sakei* kept under refrigeration storage at ± 4ºC (Mean ± SE) and Texture scores chicken fillets treated with *Lactobacillus sakei* kept under refrigeration storage at ± 4ºC (Mean ± SE)

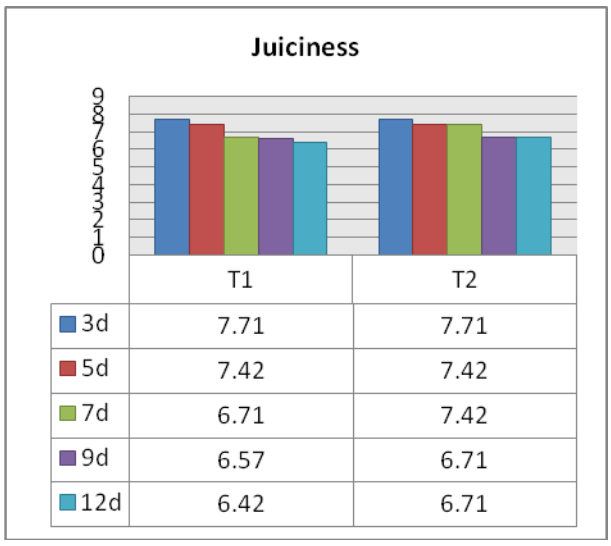


	T1	T2
3d	7.71	7.71
5d	7	7.14
7d	6.85	6.71
9d	6.57	6.57
12d	5.85	6.57

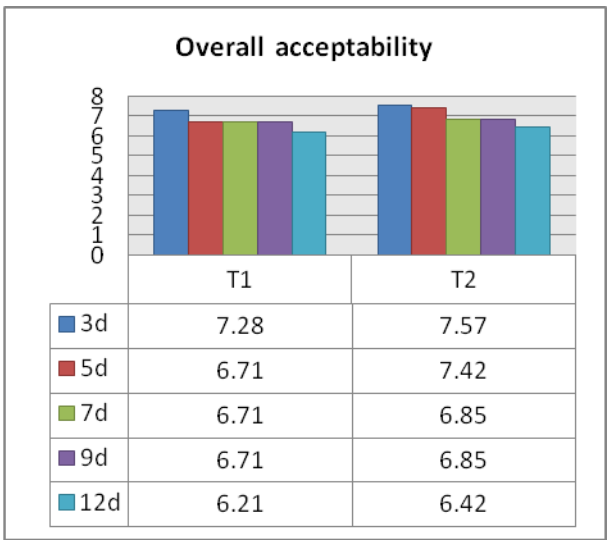


	T1	T2
3d	7	7.42
5d	6.71	7.42
7d	6.57	7.14
9d	6.42	6.57
12d	6.28	6.57

Fig.5 & 6 Juiciness scores chicken fillets treated with *Lactobacillus sakei* kept under refrigeration storage at ± 4ºC (Mean ± SE) & Overall acceptability chicken fillets treated with *Lactobacillus sakei* kept under refrigeration storage at ± 4ºC (Mean ± SE)



	T1	T2
3d	7.71	7.71
5d	7.42	7.42
7d	6.71	7.42
9d	6.57	6.71
12d	6.42	6.71



	T1	T2
3d	7.28	7.57
5d	6.71	7.42
7d	6.71	6.85
9d	6.71	6.85
12d	6.21	6.42

Juiciness (Fig. 5) of the meat products followed a decreasing trend as the storage period advanced. By the end of 12th d of storage, the mean juiciness of the meat samples of T1 was found to be 6.97±.11. The value decreases non significantly (p≥0.05) on 3nd and 5th d whereas a significant (p≤0.05) difference was observed between 5th and 7th day of storage and non significant (p≥0.05) decrease in value from 7th to 12th d of storage days. However T2 could maintained the level of acceptability up to 12th d of storage with a mean panel rating of 7.20±.10. There is a no significant (p≥0.05) difference between the T1 and T2 in all storage days except on 7thd where it differ significantly (p≤0.05). The result of taste panel evaluation for overall acceptability of T1 and T2 are presented in Figure 6. It showed a decreasing trend with advancement of storage days. By the end of 12th d of storage, the mean overall acceptability at samples of T1 (6.52±.08) and T2 (7.02±.10) respectively. Which shows T2 could maintain the level of overall acceptability up to 15th d of storage. The value differs significantly (p≤0.05) in 5th d and again it shows a no significant (p≥0.05) difference between 5th, 7th and 9th day of storage and again significantly (p≤0.05) decrease from 9th to 12th d of storage. There is no significant (p≥0.05) difference on 3rd and 5th d and it differs significantly (p≤0.05) on 7th d and again from 7th to 9th d it decreases non significantly (p≥0.05). There is a no significant (p≥0.05) difference between the T1 and T2 in all storage days except on 5th and 12th d where it differ significantly (p≤0.05). Everson *et al.*, *(*1970) reported that use of starter culture *P. cerevisiae*, a much greater degree of uniformity of flavour, appearance and texture could be obtained in the product. Anandh and Lakshmanan (2010) mentioned that decrease in overall acceptability scores with increase in storage period might be due to decrease in appearance and colour, flavour, juiciness and texture scores. Decrease in appearance scores in refrigerated storage with advancement of storage scores in different meat products at the end of storage might be due to release period might be mostly due to non-enzymatic browning of the product as the sequel of pigment and lipid oxidation (Madelwar *et al.*, 2016; Suradkar, 2008).

In conclusion, the present study revealed that the use of *Lactobacillus sakei* for the study of the shelf life under refrigeration temperature can be regarded as an effective tool for increasing safety of fresh meat. Addition of starter cultures alone has been found to extend the shelf-life of chicken fillets under refrigeration temperature. Employed bacterial culture showed strong inhibitory effect against meat borne pathogens and spoilage organisms like *Salmonella, staphylococci, E. coli,* coliform organisms. Inhibitory effect against *pseudomonads* was found to be more pronounced. On the basis of the study of the microbiological and sensory evaluation of the meat samples under different treatment groups, the samples treated with *Lactobacillus sakei* was found better than non treated group.

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