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Evaluation of changes in proximate composition of Bacteriocin supplemented Prawn

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ABSTRACT:

Bacteriocins are antibacterial proteins produced by bacteria that kill or inhibit the growth of bacteria. The present study deals with the effect of various form of bacteriocin derived from Lactobacillus brevis as crude culture supernatant, ammonium sulphate precipitate and dialysed product on the changes of proximate composition such as moisture, protein, total ash ,total sugar fat and microbial analysis. These were analysed by seeing total bacterial , fungal, yeast, spore formers, coliform and anaerobes counts in cooked prawn. No effect on proximate composition was observed in all forms of bacteriocin treated food. No microbial growth was recorded but distinct reduction in protein, fat and total sugar, moisture, total ash were observed in non - bacteriocin supplemented food which suggest the possible use of bacteriocin as food preservative for processed food product.

Keywords:
Bacteriocin, *Lactobacillus brevis,* proximate composition.

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INTRODUCTION

One of the concerns in food industry is the contamination by pathogens, which are a frequent cause of food borne diseases. Over the past decade, recurrent outbreaks of diarrhea, combined with the natural resistance of the causative agents, contributed to its status as a hazard. In the recent years the food industry faced the need of increasing the possibilities for better conservation and of the food products (Neysens 2002). Today, the conservation is commonly performed by sterilization or by adding sugar, salt, organic acids or by smoking. However, some of these compounds change the taste quality and the appliance of others is not healthy. For improving the quality of the products the approach by which chemicals are added must be ceased and the sterilization must be avoided as far as possible. A new protection is required, which is healthy and natural. .Biotechnology in the food-processing sector targets the selection, production and improvement of useful microorganisms and their products, as well as their technical application in food quality. (Corr *et al.,* 2007).

The use of non-pathogenic microorganisms and/or their metabolites to improve microbiological safety and extend the shelf life of foods is defined as biopreservation (De Martinis *et al.*, 2001). Antagonistic properties of lactic acid bacteria (LAB) allied to their safe history of use in traditional food fermented products make them very attractive to be used as biopreservatives. (Caplice and Fitzgerald 1999). Antibiotics are at present restricted for use in foods and feeds, and bacteriocins are an interesting group of biomolecules with antimicrobial properties that may represent a good alternative (Jack *et al.,* 1995). The bacteriocins produced by food grade starter bacterial cultures, could be applied as preservatives. Bacteriocins produced by lactic acid bacteria have attracted increasing attention, since they are active in a nanomolar range and have no toxicity. In the present study bacteriocin preparation from Lactobacillus breivis was evaluated against proximate composition of cooked prawn under laboratory condition.

MATERIALS AND METHODS

Isolation of *Lactobacillus brevis*

The *Lactobacillus brevis* was isolated from curd sample by serial dilution technique with modified Lactobacillus agar (Hi - media, Mumbai, India). The bacteriocin culture was identified based on morphological and biochemical characters. The pure culture was maintained on modified Lactobacillus slant.

Bacteriocin production

250 ml of modified Lactobacillus brevis was prepared, sterilized by autoclaving, after sterilization 1 ml of Lactobacillus brevis culture was inoculated and kept under shaking at 37^0 C for 24 hours. After incubation the media was centrifuged at 10,000 rpm for 10 mins. The supernatant was collected in sterile test tubes and used for anti bacterial activity against indicator strain adapting well diffusion assay.

Partial purification:

The collected supernatant was saturated with different concentration of ammonium Sulphate 20, 40 , 60 and 80 % , kept at 4^0 C for 6 to 12 hours. After incubation, the tubes were centrifuged at 10,000 rpm for 10 mins and the collected precipitate was dissolved in Tris buffer and dialysed against 0.1M Tris HCL buffer.

Evaluation of Bacteriocin on cooked Prawn Proximate composition

Sample preparation and treatment:

About 100 g of well cooked prawn was taken in a pre sterilized, air tight, plastic container. 10 ml of filter sterilized supernatant (crude), 10 ml of ammonium sulphate precipitate and 10 ml of dialysed product were added separately. Three replications were maintained for each treatment. One set of treated containers at respective treatment was kept at 30^0 C while the other was maintained at refrigeration without freezing for 15 days.

Daily observation was recorded for pH changes and off flavor etc. After the 15 days the following parameters such as mositure, protein, total ash, total sugar and microbial analysis were carried out by standard method (APHA 1989).

Microbial Analysis:

The respective bacteriocin treated samples were incubated in 37^0 C and 55^0C for 10 days. After 10 days it was analyzed for Total plate count (TPC), Spore count, Anaerobic growth, and coliform growth.For total plate count , plate count agar was used. For spore count dextrose tryptone agar was used. For determining Anaerobes, dextrose tryptone broth was used and for coliforms Mac konkey broth was used.10g of the sample was weighed accurately, then transferred into the pestle and mortar, macerated wll, and it was transferred into the conical flask, containing 90 ml Ringer's solution which gives 1:10 dilution.From the above dilution 1ml of the sample was inoculated into the

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sterile petriplates aseptically and the respective media were poured , mixed well, allowed for setting and incubated at both 37^0 C and 55^0 C. For spore count, the diluted sample was kept in the boiling water both for 15min (to analyze the heat resistant spore) and then inoculated to the petriplates. Dextrose tryptone broth was inoculated with one ml of the diluted sample and 1ml of the sterile liquid paraffin to maintain anaerobic sample and 1ml of the sterile liquid paraffin to maintain anaerobic conditions. These tubes were also incubated at 37^0 C and 55^0 C.

RESULTS:

Bacteriocin activity against Indicator Organism

The crude bacteriocin prepared from the cultured filterate of Lactobacillus brevis shows distinct anti microbial activity against indicator organism. A clear zone of 30.0mm was observed.

The effect on changes of Proximate composition and Microbial analysis of cooked prawn after the bacteriocin treatment.

Non - Bacteriocin supplemented prawn:

The protein, total ash, total sugar, moisture, fat are found to be decreased in control (non - bacteriocin supplemented prawn) both in refrigerated and non - refrigerated sample. And are presented in **Table 1,2 and 3.**The protein content in control refrigerated and non - refrigerated was found to be 20.11% and 21.8% after 5 days. After 10 days it was reduced to 15% in control non - refrigerated and 21.6% in refrigerated sample. After 15 days, in control non - refrigerated sample 11.2% and in control refrigerated sample it was 21.4%.The Moisture content in control refrigerated and non - refrigerated were found to be 60.5% and 58.9% after 5 days. After 10 days it was reduced to 58.4% in control non - refrigerated and 56.5% in refrigerated sample. After 15 days, in control non - refrigerated sample 55.5% and in control refrigerated sample it was 50.3%

The total ash in control refrigerated and non - refrigerated were found to be 1.6% and 1.8% after 5 days. After 10 days it was reduced to 1.4% in control non - refrigerated and 1.7% in refrigerated sample. After 15 days, in control non - refrigerated sample 1.2% and in control refrigerated sample it was 1.4%..The total sugar content in control refrigerated and non - refrigerated were found to be 4.2% and 5.4% after 5 days. After 10 days it was reduced to 3.5% in control non - refrigerated and 5.2% in refrigerated sample. After 15 days, in control non - refrigerated sample 2.5% and in control refrigerated sample it was 5.0%. The fat content in contol refrigerated and non - refrigerated were found to be 2.5% and 2.4% after 5 days. After 10 days it was reduced to 2.2% in control non - refrigerated and 2.4% in refrigerated sample. After 15 days, in control non - refrigerated sample 1.7% and in control refrigerated sample it was 2.3%.

Table 1.Changes in Proximate composition of Crude bacteriocin supplemented prawn at different time ntervals (%)

S. No	Treatment	Parameters	Changes in Proximate composition (%) at different time periods (days)		
1.	Control (Non - refrigerated)		**5**	**10**	**15**
			60.5	58.4	55.5
		Moisture	20.11	15	11.2
		Protein	1.6	1.4	1.2
		Total Ash	4.2	3.5	2.5
		Total Sugar	2.5	2.2	1.9
		Fat			
2.	Control Refrigerated	Moisture	58.9	56.5	50.3
		Protein	21.8	21.6	21.4
		Total Ash	1.8	1.7	1.4
		Total Sugar	5.4	5.2	5.0
		Fat	2.4	2.4	2.3
3.	Test Non - refrigerated	Moisture	71	70.5	69.8
		Protein	22.0	21.7	21.5
		Total Ash	1.7	1.5	1.2
		Total Sugar	5.4	5.4	5.1
		Fat	2.3	2.1	1.8
4.	Test refrigerated	Moisture	70.4	69	67
		Protein	22.0	21.8	21.7
		Total Ash	1.7	1.5	1.3
		Total Sugar	5.4	5.4	5.3
		Fat	2.4	2.1	1.9

Evaluation of Ammonium sulphate precipitate on proximate composition on prawn at different time intervals:

The significant difference could be observed on all the test parameters in bacteriocin treated food under refrigerated and non- refrigerated, than on non - bacteriocin supplemented food.

Protein

The protein content in Bacteriocin treated sample under refrigeration was found to be 22.0% and without refrigeration was also found to be the same after 5 days. After 10 days there was slight degradation in protein content which was found to be 21.7% but in refrigerated bacteriocin treated sample there found to have less change compared to the non - refrigerated bacteriocin treated sample and the values were recorded as 21.8%. After 15 days, there was not much degradation in the protein content in refrigerated as well as non - refrigerated sample, the values were recorded as 21.5% and 21.7%

Total Ash:

The Total Ash content in Bacteriocin treated sample under refrigeration was found to be 1.7% and without refrigeration was also found to be the same after 5 days. Even after 10 days, there were no change in the values, both in bacteriocin treated refrigerated and non - refrigerated sample, values were noted as 1.5%. After 15 days, There were no negotiable change in the total ash of bacteriocin treated non - refrigerated sample when compared to the referigerated sample. The values were recorded as 1.2% and 1.3%

Total Sugar

The Total sugar in bacteriocin treated under refrigerated and non - refrigerated sample were found to be same after 5 days and the value was recorded as 5.4%. There were no considerable change in non - refrigerated bacteriocin treated sample, the value was recorded as 5.4%. The Total sugar content in refrigerated bacteriocin treated sample was well maintained, the values were recorded as 5.4% after 10 days. There was a further change found in non - refrigerated bacteriocin treated sample and the value was recorded as 5.1% whereas in bacteriocin treated refrigerated sample, it was recorded as 5.3% after 15 days.

Moisture

After 5 days,in bacteriocin refrigerated sample there was retention in the moisture content and the value was 70.4 % and in bacteriocin non – refrigerated sample there was not much retention and the value was found to be 71%.After 10 days there was more retention found in bacteriocin treated refrigerated sample and the value was and in bacteriocin treated non – refrigerated sample the value was maintained as 70.5% and in bacteriocin treated refrigerated, the value was recorded as 69%.After 15 days, the bacteriocin treated non – refrigerated sample was still

Table 2..Changes in Proximate composition of Ammonium sulphate precipitated bacteriocin supplemented prawn at different time intervals (%)

S.No	Treatment	Parameters	Changes in Proximate composition (%) at different time periods (days)		
			5	10	15
1.	Control (Non - refrigerated)	Moisture	60.5	58.4	55.5
		Protein	20.11	15	11.2
		Total Ash	1.6	1.4	1.2
		Total Sugar	4.2	3.5	2.5
		Fat	25	2.2	1.9
2.	Control Refrigerated	Moisture	58.9	56.5	50.3
		Protein	21.8	21.6	21.4
		Total Ash	1.8	1.7	1.4
		Total Sugar	5.4	5.2	5.0
		Fat	2.4	2.4	2.3
3.	Test Non - refrigerated	Moisture	71	70.8	70.5
		Protein	22.2	22	21.8
		Total Ash	1.6	1.4	1.3
		Total Sugar	5.4	5.3	5.2
		Fat	2.3	2.1	2
4.	Test refrigerated	Moisture	71.1	70.8	70.5
		Protein	22.3	22	21.9
		Total Ash	1.6	1.5	1.3
		Total Sugar	5.4	5.4	5.3
		Fat	2.4	2.3	2.2

maintained as 69.8% and in bactericoin treated refrigerated sample there was still more retention and the value was found to be 67%.

Fat

The Fat content was found to be slightly degraded with a very minute difference in non - refrigerated bacteriocin treated samples as 2.3% and for refrigerated bacteriocin treated sample was 2.4% after 5 days. The fat content started to degrade more after 10 days in both refrigerated and non - refrigerated bacteriocin treated sample. Both the values were recorded as 2.1%. After 15 days, both the bacteriocin treated refrigerated and non - refrigerated samples were found to be still more degraded. The values were recorded as 1.8% and 1.9% for non - refrigerated and refrigerated bacteriocin treated samples.

Evaluation of Ammonium sulphate precipitate on proximate composition on prawn at different time intervals:

There were no distinct difference on proximate composition observed in ammonium sulphate precipate (Table 2).

Protein

The Protein content in Bacteriocin treated sample under refrigeration was found to be 22.3% and without refrigeration was found to be 22.1%. After 10 days, the protein content was found with significant difference both in Bacteriocin treated refrigerated and non - refrigerated sample and the values were recorded with the same value as 22%. After 15 days, the protein has found to be slightly degraded in both the bacteriocin treated refrigerated and non - refrigerated sample and the values were found to be 21.9% and 21.8%.

Total Ash

In Bacteriocin treated sample under refrigeration, it was found to be 1.6% and without refrigeration was also found to be the same after 5 days. After 10 days there was no significant difference in Bacteriocin referigerated and non - refrigerated sample.The values were recorded as 1.5% and 1.4%.After 15 days, there seen a slight degradation in Bacteriocin treated sample, both refrigerated and non - refrigerated. The values were recorded as 1.3% and 1.3%

Total Sugar

After 5 days, the Total sugar in bacteriocin treated sample under refrigeration and non - refrigeration were found to be same and is noted as 5.4%. After 10 days, there was a significant change in bacteriocin treated non - refrigerated sample whereas in bacteriocin treated refrigerated samples, it was maintained, the values are recorded as 5.3% and 5.4%. After 15 days, the Total sugar content was found to be the same in both bacteriocin treated samples. The values were recorded as 5.2% in bacteriocin treated refrigerated sample ad 5.3% in bacteriocin tread non - refrigerated sample.

Table 3. Changes in Proximate composition of Dialysed product bacteriocin supplemented prawn at different time intervals (%)

S. No	Treatment	Parameters	Changes in Proximate composition (%) at different time periods (days)		
			5	10	15
1.	Control (Non - refrigerated)	Moisture	60.5	58.4	55.5
		Protein	20.11	15	11.2
		Total Ash	1.6	1.4	1.2
		Total Sugar	4.2	3.5	2.5
		Fat	2.5	2.2	1.9
2.	Control Refrigerated	Moisture	58.9	56.5	50.3
		Protein	21.8	21.6	21.4
		Total Ash	1.8	1.7	1.4
		Total Sugar	5.4	5.2	5.0
		Fat	2.4	2.4	2.3
3.	Test Non - refrigerated	Moisture	71.1	70.9	70.6
		Protein	22.2	22	21.8
		Total Ash	1.7	1.7	1.5
		Total Sugar	5.4	5.4	5.3
		Fat	2.4	2.2	2.1
4.	Test refrigerated	Moisture	70.8	70.7	70.6
		Protein	22.3	22.1	22
		Total Ash	1.8	1.8	1.7
		Total Sugar	5.4	5.4	5.4
		Fat	2.4	2.3	2.3

Moisture

After 5 days, the bacteriocin treated refrigerated and non – refrigerated samples was found to be same with small difference in the values as 71.1% and 71%. After 10 days, both bacteriocin treated refrigerated and non – refrigerated samples were found to be the same and the value was 70.8%. After 15 days, the bacteriocin treated refrigerated and non – refrigerated samples were found with small difference and their values were recorded as 70.6% and 70.5%.

Fat

After 5 days, the fat content was found to be slightly degraded in bacteriocin treated non refrigerated sample and in refrigerated bacteriocin sample the fat content was maintained. The values were recorded as 71% and 71.1% . After 10 days, there was still more changes in bacteriocin treated non - refrigerated sample and in refrigerated bacteriocin treated sample, there was no change. The values were same and recorded as 70%. After 15 days, there was further degradation in bacteriocin treated non - refrigerated sample and in refrigerated bacteriocin treated sample was found with negotiable changes. The values were recorded as 2% and 2.2%.

Evaluation of Dialysed form of bacteriocin on proximate composition on prawn at different time intervals:

There was not much difference on proximate composition observed in Partially purified product.

Protein

After 5 days, there was not much change in protein content in both bacteriocin treated non - refrigerated and refrigerated samples. The values were recorded as 22.2 and 22.3%. After 10 days, the protein content was maintained without little degradation both in bacteriocins treated non - refrigerated and refrigerated sample. The values were recorded as 22.0% and 22.1%.After 15 days, the protein content was maintained in both bacteriocin treated non - refrigerated and refrigerated samples and the values were recorded as 21.8% and 22%.

Total Sugar

After 5 days, the total sugar content was found to be maintained without degradation in bacteriocin treated non - refrigerated and refrigerated sample. The value is recorded as 5.4% for both the samples. After 10 days, there were no degradation found in total sugar content in both bacteriocin treated non - refrigerated and refrigerated samples. The value was recorded as 5.4% for both the samples. After 15 days, there was still no much change in the values, for bacteriocin treated refrigerated sample it was 5.4% and 5.3% for bacteriocin treated non - refrigerated sample.

Total Ash

After 5 days, the total ash content was found to be nearly similar in bacteriocin treated refrigerated and non - refrigerated samples. The values were recorded as 1.7% and 1.8%.After 10 days, the total ash content was found to be maintained in both the bacteriocin treated refrigerated and non - refrigerated samples. The values were recorded as 1.7% and 1.8%. After 15 days, the total ash content was found to be with slight difference in bacteriocin treated non - refrigerated samples but in bacteriocin treated refrigerated sample there was no change inferred. The values were recorded as 1.7% and 1.8%

Moisture

After 5 days, the Moisture content in bacteriocin treated non – refrigerated and refrigerated sample has no significant difference . The values were found to be 70.8% for bacteriocin treated non – refrigerated and 71.1% for bacteriocin treated refrigerated sample. After 10 days, the moisture content in the bacteriocin treated non – refrigerated sample has very slight difference when compared to refrigerated bacteriocin supplemented sample. The values were recorded as 70.9% for non – bacteriocin treated sample and 70.7% refrigerated bacteriocin treated sample. After 15 days, the moisture content in bacteriocin treated non – refrigerated sample has considerable change when compared to bacteriocin treated refrigerated sample. The values were recorded as 70.6% and 70.6%

Fat

After 5 days, in Bacteriocin treated refrigerated and non – refrigerated samples there were no change and the values were found to be the same and recorded as 2.4%. After 10 days, there was a significant change observed both in refrigerated and non – refrigerated bacteriocin treated sample and the values were recorded as 2.3% for refrigerated bacteriocin treated sample and 2.2% for non – refrigerated sample. After 15 days the values were maintained in both the samples and the value was revealed as 2.3% for bacteriocin treated refrigerated sample and for bacteriocin treated non – refrigerated sample was 2.1%

Microbial Analysis:

Significant difference was observed in bacteriocin supplemented food than the control. The microbial growth was recorded in all the tested time

Table 4. Microbial Analysis in Crude Bacteriocin supplemented prawn at different time intervals

Treatment	Microbial Count (cfu/G.)														
	Bacteria			Mould and Yeast			Spore formers			Anaerobes			Coliform		
	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15
Control (Non - Refrigerate)	10.1 x10^2	15.1x10^4	25.1x10^6	7x10^3	14.1x10^4	17.2x10^2	2.5X 10	3X 10	46X1O^2	-	-	-	+	+	+
Control (Refrigerated)	-	1.4x10^1	11.3x10^1	-	-	3.1x10^1	-	-	-	-	-	-	+	+	+
Test (Non - Refrigerated)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Test (Refrigerated)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 5. Microbial Analysis in Ammonium Sulphate precipitate supplemented prawn at different time intervals

Treatment	Microbial Count (CFU/g.)														
	Bacteria			Mould and Yeast			Spore formers			Anaerobes			Coliform		
	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15
Control (Non - Refrigerate)	10.1 x10^2	15.1x10^4	25.1x10^6	7x10^3	14.1x10^4	17.2x10^2	2.5X 10	3X 10	46X1O^2	-	-	-	+	+	+
Control (Refrigerated)	-	1.4x10^1	11.3x10^1	-	-	3.1x10^1	-	-	-	-	-	-	+	+	+
Test (Non - Refrigerated)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Test (Refrigerated)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

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Table 6. Microbial Analysis in Dialysed product Bacteriocin supplemented prawn at different time intervals

Treatment	Microbial Count (CFU/g.)															
	Bacteria			Mould and Yeast			Spore formers			Anaerobes			Coliform			
	5	10	15	5	10	15	5	10	15	5	10	15	5	10	15	
Control (Non - Refrigerate)	10.1×10^2	15.1×10^4	25.1×10^6	7×10^3	14.1×10^4	17.2×10^2	2.5X 10	3X 10	$46X1O^2$	-	-	-	+	+	+	
Control (Refrigerated)	-	1.4×10^1	11.3×10^1	-	-	3.1×10^1	-	-	-	-	-	-	+	+	+	
Test (Non - Refrigerated)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	
Test (Refrigerated)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	

period in non - bacteriocin supplemented food (**Table 4, 5 and 6**).

In control non - refrigerated:

The bacterial count at 5, 10 and 15 days were found to be 10.1×10^2, 15.1×10^2, 25.1×10^6 Cfu/gThe fungal count at 5, 10, and 15 days were found to be 7.0×10^4, 14.1×10^4, 17.2×10^5 Cfu/ gThe spore formers count for 5, 10, 15 days were found to be 2.5X 10, 3×10^1, 4.6×10^2 Cfu/g.Nil count was recorded in anaerobes for 5, 10 , 15 days respectively.The coliform was found to be positive on 5, 10, 15 days respectively.

Control refrigerated

The bacterial count at 5, 10 and 15 days were recorded as 0, 1.4×10^1 and11.3×10^1 Cfu/g. The fungal count at 5, 10 and 15 days were found to be 0,0 and 3.1×10^1 Cfu/gNil count was recorded in spore formers count for 5, 10 and 15 days respectivelyNil count was recorded in anaerobes for 5, 10 and 15 days respectively. The coliform was found to be positive on 5, 10, 15 days respectively. In bacteriocin supplemented food products (both in refrigerated and non - refrigerated) no growth were recorded in all the tested time period at bacteriocin (crude, ammonium sulphate precipitate, partially dialysed product) supplemented food products.

DISCUSSION

Biopreservation systems such as bacteriocinogenic LAB cultures and/or their bacteriocins have received increasing attention, and new approaches to control pathogenic and spoilage microorganisms have been developed. Some lactic acid bacteria (LAB) demonstrated antagonism towards pathogenic and spoilage organisms.Although bacteriocins are produced by many Gram-positive and Gram-negative species, those produced by LAB are of particular interest to the food industry, since these bacteria have generally been regarded as safe (Joshi *et al*., 2005).

Bacterial fermentation of perishable raw materials has been used for centuries to preserve the nutritive value of food and beverages over an extended shelf life.The production of bacteriocins by LAB is advantageous for survival of the producing bacteria in a competitive ecological niche; therefore, they could be exploited by the food industry as a tool to control undesirable bacteria in a food-grade and natural manner, which is likely to be more acceptable to consumers.

From this study it is clear that. the bacteriocin supplemented prawn, retained its original proximate and nutrient composition at

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tested time interval under refrigerated and non - refrigerated conditions. This study will be helpful to use exploit bacteriocin as a food preservative to prevent the microbial spoilage without other conventional preservation methods.

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