

APPLICATION OF BACTERIOCCIN FROM *Lactobacillus acidophilus* FOR SHELF LIFE ENHANCEMENT OF FUJI APPLES

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Abstract: Edible coatings have been used for preserving the quality and safety of fresh fruits and vegetables. The aim of the study was to extend the shelf life of Fuji apples by using bacteriocin of *Lactobacillus acidophilus* NCDC 343 as coating material and comparison of bacteriocin preservative efficacy with paraffin wax. Bacteriocin coated apples showed a significant delay in weight loss, titrable acidity, total soluble solids and decay in comparison to uncoated apples. A substantial reduction in microbial count on apples was observed by bacteriocin (100ug/ml) coating. It reduced the bacterial and fungal count by 2.6 and 3.35 log CFU/gm respectively as compared to uncoated apples after 20 days of storage at 25°C. The count of *Staphylococcus aureus* also decreased by 2.1 log CFU/gm on bacteriocin coated apples as compared with apples only inoculated with *Staphylococcus aureus*. In this study, bacteriocin proved to be an effective preservative and showed a better shelf life enhancing efficacy as compared to paraffin wax. It is concluded that biopreservatives, especially GRAS status bacteriocins, can be a good replacement of chemical preservatives.

Keywords: Bacteriocin; Apple; Biopreservation; *Lactobacillus acidophilus*; Paraffin wax.

1. Introduction

India is one of the key food producing countries in the world only next to China (Anon, 2013). India produced 48 metric tonnes of fruits and 20 metric tonnes of vegetables in 2014-2015 (Anon, 2015). Fruits and vegetables provide optimum conditions for the growth of microbes. This results not only in quantitative loss of food but also affects food quality and safety (Bevilacqua et al., 2011; Lawlor et al., 2009). One of the most extensively consumed fruit in the world is Apple (Koyuncu et al., 2007). Fuji apple is popular variety of apple due to its delicious taste. Due to poor post-harvest practices in India like shortage of cold storage facilities and lack of cold chain transportation, a large amount of fresh produce is wasted. The major microbial contaminants of apples include *Erwinia*, *Bacillus*, *Staphylococcus*, *Penicillium*, *Botrytis* and *Zygosaccharomyces* (Ramos et al., 2012).

Paraffin wax, the commonly used coating material for apples to extend their shelf-life causes whitening or chalking on the surface of fruits giving it an artificial appearance. Moreover, if the wax is uneven on the fruit surface, it may cause surface burns due to high temperature. It also leads to development of off-flavours (Anon,

2004). Due to these disadvantages of wax and consumer awareness regarding use of chemical preservatives, biopreservatives has gained the attention of food industry.

Biopreservation is a powerful and natural tool to extend shelf life and to enhance the safety of foods by applying naturally occurring microorganisms and their antimicrobial compounds. Lactic acid bacteria (LAB) possess a major potential for use in biopreservation because of its GRAS status. The antagonistic and inhibitory properties of LAB are due to production of one or more antimicrobial active metabolites such as organic acids, hydrogen peroxide and antimicrobial peptides i.e. bacteriocins (Ghanbari et al., 2013). Bacteriocins are defined as ribosomal-synthesized peptides, as biologically active proteins or protein complexes with antimicrobial activity against closely related species, and they are produced by different groups of bacteria (Reis et al., 2012).

Various bacteriocins have been used in preservation of fruits by different workers such as Pediocin (Martinez et al., 2000), Enterocin (Molinos et al., 2008; Viedma et al., 2010; Aguayo et al., 2016), Nisin (Takala et al., 2011; Ce et al., 2012; Barbosa et al., 2013; Siroli et al., 2015) and Lacticin (Sung et al., 2013). Bacteriocins are an important tool in hurdle concept of food preservation.

There are limited studies of the application of bacteriocins as coating materials on whole apples for extending their shelf-life. Having in mind the main constraints to the Fuji apple preservation, the aim of the present study was to determine the effectiveness of bacteriocin coatings in increasing the shelf life of Fuji apples.

2. Materials and methods

2.1 Purification of bacteriocin

The microbial cultures used in present study namely, *Lactobacillus acidophilus* NCDC 343 and *Staphylococcus aureus* NCDC 109 were procured from National Collection of Dairy Culture, NDRI, Karnal. *Lactobacillus acidophilus* NCDC 343 (LA 343) was used as bacteriocin producer and cultivated on MRS broth at 37°C. *Staphylococcus aureus* NCDC 109 was employed as indicator organism and cultivated on nutrient agar at 37°C.

Turbidometric method was employed to study the growth kinetics of the bacteriocin producing organism. After studying the growth kinetics, bacteriocin activity was determined after every 3 hours in the culture broth of LA 343. The crude bacteriocin was diluted ten folds and well diffusion assay was performed. The antimicrobial activity is defined as the reciprocal of the highest dilution showing inhibition of the indicator lawn and it is expressed in arbitrary units per ml (AU/ml).

Bacteriocin purification was carried out by adsorption desorption method of Yang et al. (1992). One litre of MRS broth was inoculated with overnight grown metabolically active culture of LA 343. The cell culture after 20 hours of incubation was heated in boiling water bath for 20 minutes and was cooled down slowly to room temperature. pH of the culture was adjusted to 6.5 with 4M NaOH and kept for overnight stirring at 4°C and then it was centrifuged at 9000 rpm for 20 minutes. The pellet was taken and washed thrice with 5mM sodium phosphate buffer (pH 6.5). The pellet was resuspended in minimum volume of NaCl (pH 1.5, adjusted with 5% phosphoric acid) and was stirred overnight at 4°C. After stirring, the cell suspension was centrifuged at 9000 rpm for 20 minutes. The pellet was discarded and purified bacteriocin was obtained.

2.2 Characterization of purified bacteriocin

Effect of temperature and pH was studied on antimicrobial activity of bacteriocin by method of Gong et al. (2010). Purified bacteriocin was incubated in water bath at temperatures 4, 25, 30, 37, 45, 60, 80 and 100°C for 30 minutes and in autoclave at 121°C for 15 minutes. pH stability was tested by adjusting the pH value from 2.0 to 9.0 with 0.1N hydrochloric acid (HCl) and 0.1N sodium hydroxide solution (NaOH). The activity of bacteriocin was determined by well diffusion assay. Controls were maintained without any treatment.

Antimicrobial activity of purified bacteriocin was studied against various food spoiling bacteria (*Pseudomonas fluorescens* MTCC 103, *Erwinia sp.* MTCC 2760, *Bacillus subtilis* MTCC 2451, *Escherichia coli* NCDC 135, *Staphylococcus aureus* NCDC 109, *Leuconostoc mesenteroids* NCDC 29, *Listeria monocytogenes* MTCC 657), fungi (*Aspergillus awamori* MTCC 2879, *Fusarium oxysporum* MTCC 284, *Botrytis cinera* MTCC 359) and yeasts (*Candida parapsilosis* NCDC 279, *Saccharomyces cerevisiae* NCDC 42, *Candida albicans* MTCC 183) using the well-diffusion assay.

2.3 Preparation of coatings for apples

Fuji apples obtained from the local market of Patiala (Punjab) were divided into three groups as per different coatings applied to them.

Group I: Apples coated with bacteriocin

Group II: Apples coated with paraffin wax

Group III: Uncoated apples (control)

To coat the apples, bacteriocin of *Lactobacillus acidophilus* NCDC 343 was used at concentration of 100µg/ml. 1gm of paraffin wax (HiMedia Laboratories) was coated per fruit. Uncoated apples were used as control. After treatment, fruits were stored in macro-perforated polypropylene bags at 25°C for 20 days.

2.4 Microbiological analysis

For microbiological analysis, 5gm of apple peel was taken and homogenized in saline water and filtered using a sterile cheese cloth. Serial dilutions of each sample was plated on nutrient agar and Potato dextrose agar for enumeration of total aerobic bacterial and fungal count respectively and the plates were incubated at 37°C for 24h and at 30°C for 72h, respectively. The microbial count was expressed as log CFU/gm.

2.5 Physico-chemical analysis

The physico-chemical tests were conducted on 0, 5th, 10th, 15th and 20th day of storage.

2.5.1 Weight loss

The weight of the apples was measured during storage on each 5th day and was compared with the weight on the first day of storage. Weight loss percentage was calculated as percentage from the initial weight.

2.5.2 Total Soluble Solids

The apple flesh was mashed and then filtered. The total soluble solids (TSS) content was observed by using a portable hand refractometer and expressed as °Brix. Before using, the refractometer was calibrated with distilled water.

2.5.3 Titratable acidity (TA) and pH

TA and pH were calculated by the method given by AOAC (2000). 10gm of the fruit pulp was taken and homogenised with distilled water and then filtered. The pH of the juice was measured by using digital pH meter.

The titratable acidity was determined by the amount of 0.1N NaOH required to titrate the filtrate (5ml) and expressed as the malic acid equivalent (gm malic acid equivalent/100g fresh weight).

2.5.4 Decay Percentage

Decay percentage of apple fruits was calculated as the number of decayed fruit divided by initial number of all fruits i.e. 25 (Anany et al., 2009).

2.6 Inhibition of *Staphylococcus aureus* on fuji apples by bacteriocin of LA 343

Fuji apples were artificially contaminated with sterile saline solution suspension of *Staphylococcus aureus* NCDC 109 (1×10^6 CFU/ml). After inoculation, apples were allowed to dry for 1 hour at room temperature and coated with 100µg/ml of purified bacteriocin (Group IV). In Group V, apples were only inoculated with *Staphylococcus aureus* NCDC 109 (1×10^6 CFU/ml). After treatment, apples were stored at 25°C for 20 days in macro-perforated polypropylene bags. After every 5 days, samples were taken and used to analyse *Staphylococcus aureus* count on Mannitol Salt agar (HiMedia) plates.

2.7 Statistical analysis

All the experiments were performed in triplicates. The experimental data was analysed by one way ANOVA using Daniels's XL Toolbox. All results were expressed as the mean± standard deviation and differences at $p < 0.05$ were considered significant.

3. Results and discussion

3.1 Production of bacteriocin

Growth kinetics of LA 343 was studied to identify various growth phases. No significant lag phase was observed. The log phase started after 3 hour of incubation and extends upto 24 hour of incubation. After the log phase, the organism exhibited stationary growth phase. Bacteriocin samples obtained from different growth phases of *Lactobacillus acidophilus* were subjected to well diffusion assay using indicator strain *Staphylococcus aureus*. Bacteriocin production started in log phase. The inhibitory activity was maximal (6400 AU/ml) after 20 hours of incubation i.e. late log phase. There is substantial decrease in bacteriocin activity upon further incubation.

3.2 Purification of bacteriocin

The purification of bacteriocin was carried out by pH dependent Adsorption- Desorption method of Yang et al. (1992).

Bacteriocin producing organism i.e. *Lactobacillus acidophilus* NCDC 343 was incubated in MRS broth for 20 hours. After adsorption of bacteriocin on producer cells, no inhibitory activity was reported in supernatant. It proves that all bacteriocin was adsorbed on cells. The results of purification are summarized in Table 1. After final step, the bacteriocin was purified 13.5-folds. The purified bacteriocin preparation contained 6400 AU/ml.

Table 1: Bacteriocin purification profile from *Lactobacillus acidophilus* NCDC 343

	Volume (ml)	Total Activity (AU/ml)	Total protein (mg)	Specific activity (AU/mg)	Purification folds
Cell free supernatant (CFS)	50	12800	13.5	948.2	1
Purified bacteriocin	10	6400	0.5	12800	13.5

3.4 Characterization of purified bacteriocin

To determine the stability of the bacteriocin, the effect of temperature and pH was studied. Antimicrobial spectrum of bacteriocin was studied against various food spoilage organisms.

3.4.1 Effect of temperature

The antimicrobial activity was not affected significantly upon heating the bacteriocin upto a temperature of 100°C. After heating at 121°C, there was a decrease in the antimicrobial activity by 12.5% (Table 2). This indicates that bacteriocin is thermostable upto 100°C and can be effectively used as a preservative in food items.

Table 2: Effect of temperature on bacteriocin activity

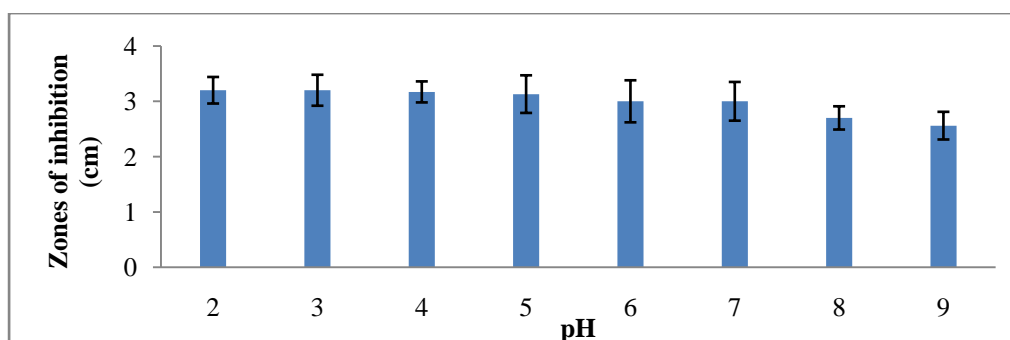
S.No.	Temperature	Zone of inhibition (cm)	Residual Activity (%)
1.	Control (untreated)	3.2±0.23	100
2.	4°C	3.2±0.3	100
3.	25°C	3.2±0.36	100
4.	30°C	3.2±0.24	100
5.	37°C	3.2±0.42	100
6.	45°C	3.2±0.15	100
7.	60°C	3.2±0.36	100
8.	80°C	3.2±0.33	100
9.	100°C	3.2±0.4	100
10.	121°C	2.8±0.39	87.5

Data is represented as Mean± standard deviation. Differences are found to be significant (p<0.05).

3.4.2 Effect of pH

There is a clear significant effect of pH on the antimicrobial activity of bacteriocin. The zone of inhibition decreases with the increase in pH. The bacteriocin has stable antimicrobial activity in a range of pH from 2.0 to 5.0 but above this pH the activity considerably decreased and the bacteriocin becomes less effective. At pH 8.0 and 9.0, bacteriocin activity decreased by 15.63% and 20% respectively (Fig 1).

Bacteriocins of *Lactobacillus acidophilus* NCDC 343 showed an advantage for its application as food preservative in acidic foods, as it can remain stable at acidic pH. Apple, cherry, citrus, peach, plum, yoghurt and vinegar are highly acidic foods (pH 1.0-3.0). Banana, melon, papaya, pineapple and tomato are medium acidic foods (pH 4.6). It can lower the use of chemical preservatives in foods, which may cause food allergies in the consumers.



(Error bar represent standard deviation of mean)

Fig 1: Effect of pH on activity of bacteriocin

3.4.3 Antimicrobial spectrum

To use a compound as a preservative in food materials, it is important to elucidate its antimicrobial spectrum. The antimicrobial spectrum of purified bacteriocin was studied and results are listed in Table 3.

Bacteriocin from *Lactobacillus acidophilus* NCDC 343 showed inhibitory activity against all the tested bacteria. *Staphylococcus aureus* was the most sensitive towards the bacteriocin with an inhibition zone of 3.2cm and *E.coli* was found to be most resistant to bacteriocin with inhibition zone of 1.55cm. *Leuconostoc mesenteroides* was the second most sensitive with zone of inhibition of 2.9cm. Bacteriocin of *Lactobacillus plantarum* also showed antibacterial activity against *Listeria monocytogenes* which is among the most commonly found pathogens on raw fruits. Bacteriocin also inhibited the growth of *Aspergillus awamori*, *Fusarium oxysporum* and *Botrytis cinera*, which are the major food spoiling fungi. All the yeasts tested for their sensitivity towards the bacteriocin, were found to be sensitive with maximum inhibition zone if 2.5cm in case of *Saccharomyces cerevisiae*, 2.2cm for *Candida parapsilosis* and 2.0cm for *Candida albicans*.

Table 3: Antimicrobial spectrum of purified bacteriocin from *Lactobacillus acidophilus* NCDC 343 against food spoiling microorganisms

Food spoiling organisms	Accession no.	Zone of inhibition (cm)
<i>Pseudomonas fluorescens</i>	MTCC 103	2.3 ± 0.4
<i>Erwinia sp.</i>	MTCC 2760	2.5±0.3
<i>Bacillus subtilis</i>	MTCC 2451	2.1±0.2
<i>Escherichia coli</i>	NCDC 135	1.55±0.14
<i>Staphylococcus aureus</i>	NCDC 109	3.2±0.3
<i>Leuconostoc mesenteroids</i>	NCDC 29	2.9±0.35

<i>Listeria monocytogenes</i>	MTCC 657	2.3 ± 0.4
<i>Aspergillus awamori</i>	MTCC 2879	2.3± 0.4
<i>Fusarium oxysporum</i>	MTCC 284	2.1± 0.2
<i>Botrytis cinera</i>	MTCC 359	1.9± 0.26
<i>Saccharomyces cerevisiae</i>	NCDC 42	2.5±0.3
<i>Candida parapsilosis</i>	NCDC 279	2.25±0.2
<i>Candida albicans</i>	MTCC 183	2.0±0.2

(Data is represented as Mean± standard deviation)

3.5 Shelf-life enhancement of Fuji apples by bacteriocin of *Lactobacillus acidophilus* NCDC 343

Fuji apples from local market were purchased and coated with different coating materials. Microbiological and physico-chemical analysis was done to determine the quality of fruits.

3.5.1 Microbiological analysis

Peels taken from apples of different groups were subjected to microbiological analysis and the changes in the populations of total aerobic bacteria, yeasts and molds were determined at different storage periods.

3.5.1.1 Total Aerobic Bacteria count

During storage at 25°C, bacteriocin coating significantly reduced the bacterial count compared with uncoated control samples. After storage of 20 days, bacteriocin and paraffin wax coating reduced the bacterial population by 2.6 log CFU/gm and 2.0 log CFU/gm respectively, compared to uncoated apples (Table 4; Fig 2).

As reported by Leverentz et al. (2003), apple slices treated with nisin reduced bacterial count by approximately 0.9 to 2.0 log units and after 7 days of storage.

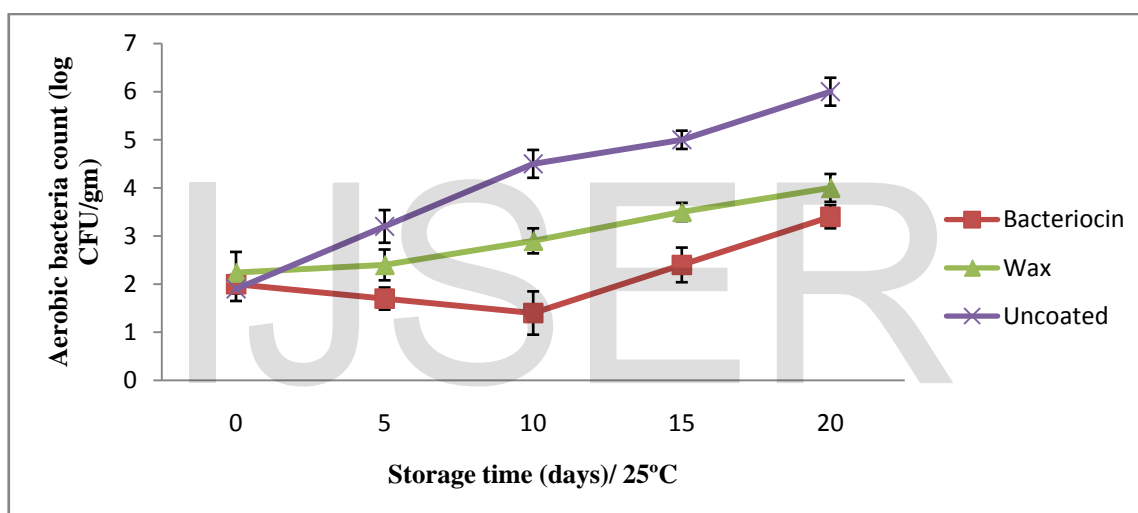
3.5.1.2 Yeast and mold count

The yeast and mold count decreased by 3.1 log CFU/gm and 1.9 log CFU/gm on apples coated with bacteriocin and paraffin wax respectively in comparison to untreated apples after 10 days of storage. Whereas, after 20 days of storage, fungal count decreased by 3.4 log CFU/gm and 2.8 log CFU/gm on apples coated with bacteriocin and paraffin wax respectively as compared to control (Table 4, Fig 3).

Bacteriocin produced by LA 343 is capable of inhibiting microbial spoilage of apples more efficiently as compared to paraffin wax during storage at 25°C. Bacteriocin of LA 343 has shown both antibacterial as well as antifungal properties *in vitro* and also in food model systems. Since fungus is the major food spoilage agent and is major concern of safety among consumers, bacteriocin of LA 343 can play a major role in providing microbial safety of food products.

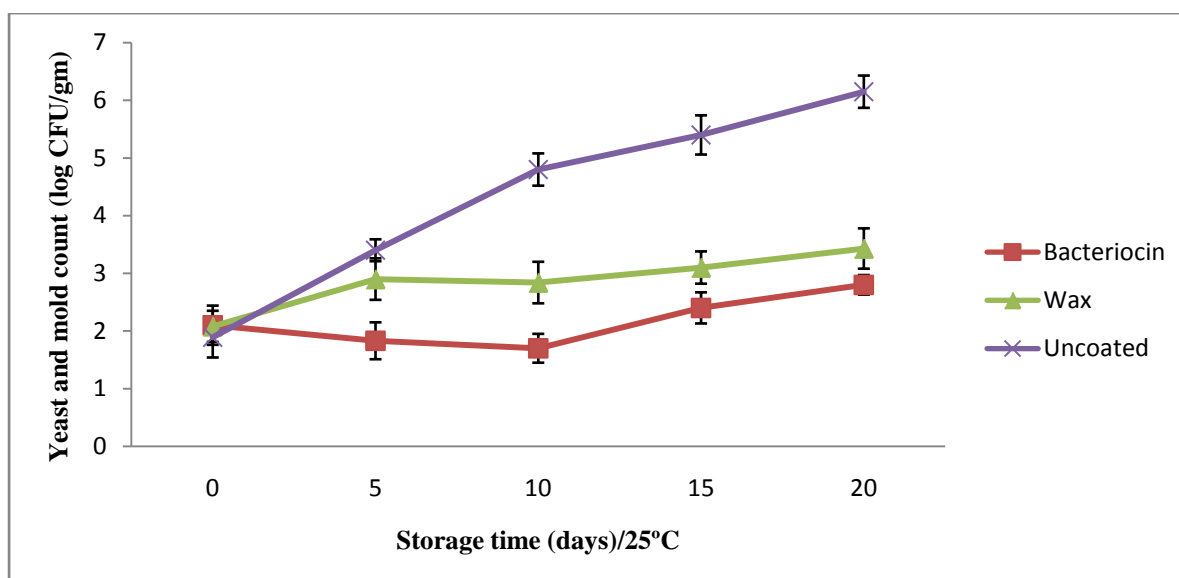
Table 4: Change in population of microbes on Fuji apples during storage

Microorganisms	Coating material	(log CFU/gm)				
		Storage time(days)/25°C				
		0	5	10	15	20
Total aerobic bacteria count	Bacteriocin	2.0±0.19	1.7±0.23	1.4±0.45	2.4±0.36	3.4±0.24
	Paraffin wax	2.2±0.43	2.4±0.32	2.9±0.26	3.5±0.9	4.0±0.29
	Control	1.9±0.25	3.2±0.34	4.5±0.29	5.0±0.9	6.0±0.29
Yeast and molds count	Bacteriocin	2.1±0.34	1.83±0.3	1.7±0.25	2.4±0.27	2.8±0.17
	Paraffin wax	2.1±0.27	2.9±0.36	2.9±0.36	3.1±0.28	3.4±0.35
	Control	1.9±0.35	3.4±0.19	4.8±0.28	5.4±0.34	6.2±0.28



(Error bar represent standard deviation of the mean)

Fig 2: Effect of different coatings materials on total aerobic bacterial count of apple



(Error bar represent standard deviation of the mean)

Fig 3: Effect of different coating materials on yeast and mold count of apples

3.5.2 Physico-chemical analysis

Various physical and chemical parameters of apples like weight loss percentage, total soluble solids, titratable acidity, pH and decay percentage were studied to determine their quality during storage at 25°C.

3.5.2.1 Weight loss

Weight losses of the apples stored at 25°C increased with storage period, and was significantly higher for the uncoated apples (1.04%) than for the apples coated with bacteriocin (0.78%) and paraffin wax (0.91%) after 20 days of storage (Table 5). Coating of bacteriocin and paraffin wax played an important role in prevention of water loss from apples but least water loss was in case of apples coated with bacteriocin.

Coating material reduced the permeability of fruits and does not allow water to escape. As a result, less weight loss occurs in apples treated with coating materials. In this study, bacteriocin of LA 343 has proven helpful in preserving the firmness and natural sheen of the apples during storage whereas uncoated apples showed browning and other effects and therefore are unacceptable by the consumers. Study conducted by Bai et al. (2003) also showed that the weight loss in case of Gala apples coated with zein was less in comparison to uncoated apples.

Table 5: Weight loss percentage in apples during storage at 25°C

S.No.	Coating material	0 day	5 th day	10 th day	15 th day	20 th day
1.	Bacteriocin	0±0.24	0.36±0.19	0.53±0.18	0.75±0.24	0.78±0.15
2.	Paraffin wax	0±0.25	0.46±0.21	0.62±0.15	0.86±0.09	0.91±0.19
3.	Uncoated (control)	0±0.21	1.45±0.18	1.31±0.13	1.79±0.16	1.04±0.19

Data is represented as Mean± standard deviation. Differences are found to be significant (p<0.05).

3.5.2.2 Total Soluble Solids (TSS)

There was a significant increase in the TSS values in coated as well as uncoated apples during storage at 25°C. But this increase was higher in uncoated apples because there was no barrier to the rate of respiration (Table 6). TSS of bacteriocin and paraffin wax coated apples increased by 1° and 1.2° respectively as compared to an increase of 3° in case of uncoated apples after 20 days of storage at 25°C.

According to Synowiec et al. (2014), the coated apples provide a barrier for the respiration and the TSS content only increased a little as compared to uncoated apples. More ripening occurred in apples which were uncoated as compared to coated apples. Moreover low oxygen permeability of coating delayed the deteriorative oxidation reaction of malic acid content. Kittur et al. (2001) also reported similar results that the TSS value of polysaccharide coated banana and mango were lower as compared to uncoated one.

Table 6: Effect of coating material on TSS of apples during storage at 25°C

S.No.	Coating material	0 day	5 th day	10 th day	15 th day	20 th day
1.	Bacteriocin	14±0.9	14.23±0.07	14.47±0.18	14.76±0.67	15±0.49
2.	Paraffin wax	14±0.2	14.7±0.3	14.9±0.26	15.2±0.78	15.2±0.54
3.	Uncoated (control)	14±0.3	14.9±0.47	15.7±0.56	16.3±0.57	17±0.6

Data is represented as Mean± standard deviation. Differences are found to be significant (p<0.05).

3.5.2.3 Titratable acidity (TA) and pH

Titrateable Acidity: A decline in acidity values of coated as well as uncoated apples was observed in this study and this decline was highly pronounced in uncoated apples. Apples having bacteriocin coating showed least decline in acidity as compared to paraffin wax coated and uncoated apples. Titratable acidity in bacteriocin coated apples reduced by 0.8 g/L whereas it reduced by 0.12 g/L and 0.22 g/L in case of paraffin wax and uncoated apples respectively, after 20 days of storage.

Acids are an important substrate for respiratory metabolism. Greater is the respiration, greater will be the decline in acidity. During ripening the acid content of the food decreases (Synowiec *et al.* 2014).

pH: In terms of pH, uncoated apples had highest pH as compared to coated apples. pH of bacteriocin coated apples was upto 3.7 as compared to 4.5 in case on untreated apples after 20 days of storage, both having an initial pH value of 3.0 (Table 7).

Titrateable acidity and pH are inversely proportional to each other in case of highly metabolizing fruits. Since acids are used as substrates for metabolism, there is reduction in acidity and hence an increase in pH (Anany *et al.*, 2009). According to Yaman and Bayoindirli (2002), coatings on fruits reduce the utilization of acids and therefore reduce the respiration rate. The results of our study are in accordance with Pre-Aymard *et al.* (2005) which showed that the Anna apples coated with 1-methylcyclopropene had highest acidity during storage at 20°C whereas the acidity of untreated apples decreased proportionally during storage period.

Table 7: Effect of coating material on pH and Titrateable acidity (TA, g/L) on apples during storage

S.No.	Coating material	TA (g/L)					pH				
		Storage time (days)					Storage time (days)				
		0	5	10	15	20	0	5	10	15	20
1.	Bacteriocin	0.25	0.21	0.19	0.18	0.17	3.0	3.2	3.3	3.5	3.7
2.	Paraffin wax	0.25	0.20	0.18	0.16	0.13	3.0	3.4	3.7	4.0	4.3
3.	Uncoated (control)	0.25	0.20	0.15	0.05	0.03	3.0	3.6	3.9	4.3	4.5

3.5.2.4 Decay percentage

With storage period, the fruits undergo decay and show the signs of senescence. In this study, bacteriocin and paraffin wax coating significantly decreased the decay of apples as compared to uncoated apples. Table 8 shows decay percentage of apples during storage at 25°C. Decay percentage of uncoated apples (control) was approximately 5 times higher than bacteriocin coated apples after 20 days of storage.

Table 8: Effect of coating material on decay percentage (%) during storage at 25°C

S.No.	Coating material	0 day	5 th day	10 th day	15 th day	20 th day
1.	Bacteriocin	0	0	0	0	4±0.23
2.	Wax	0	0	2±0.17	7±0.41	11±0.49
3.	Uncoated (control)	0	0	6±0.39	11±0.51	19±0.58

Data is represented as Mean± standard deviation. Differences are found to be significant (p<0.05).

3.6 Inhibition of *Staphylococcus aureus* on fuji apples by bacteriocin of LA 343

Fuji apples inoculated with *Staphylococcus aureus* (10⁶ CFU/ml) were coated with bacteriocin of LA 343 to determine the coating effectiveness against *Staphylococcus aureus*. Bacteriocin was effective in controlling the growth of *Staphylococcus aureus* to a large extent. After 5 days of storage period, the bacteriocin reduced the *S. aureus* count by 1.1 log CFU/gm as compared to untreated apples (only inoculated with *Staphylococcus aureus*). The count decreased by 2.1 log CFU/gm after 20 days of storage as compared to untreated apples (Table 9). This describes the effect of bacteriocin in hindering the growth of *Staphylococcus aureus*.

Kumar et al. (2012) demonstrated that addition of crude bacteriocin HKT-9 (100µg/ml) to artificially contaminated vegetables inhibited the growth of *Staphylococcus aureus* by 6 log CFU/ml as compared to control within 24 hours of incubation. Similar results were also reported by Barbosa et al. (2013) in which the antimicrobial films of nisin inhibited the growth of *Staphylococcus aureus* inoculated in mango slices and reduction of 6 log CFU/gm was observed after 6 days of storage.

Among predominant bacteria involved in diseases, *Staphylococcus aureus* is leading cause of gastroenteritis due to consumption of contaminated food. This poisoning in food is due to absorption of staphylococcal enterotoxins present in the food (Loir et al., 2003). Bacteriocin produced by LA 343 has shown to inhibit growth of *Staphylococcus aureus* on apples, hence it can be used as coating material to prevent food contamination by *Staphylococcus aureus*.

Table 9: Evaluation of coating effectiveness against *Staphylococcus aureus* NCDC 109

Microorganisms	Coating material	(log CFU/gm)				
		Storage time(days)/25°C				
		0	5	10	15	20
<i>Staphylococcus aureus</i> count	<i>Staphylococcus aureus</i> + Bacteriocin coating	2.6±0.45	2.8±0.29	3.5±0.34	3.9±0.31	4.3±0.39
	<i>Staphylococcus aureus</i> (Control)	2.6±0.29	3.9±0.38	4.8±0.26	5.7±0.43	6.4±0.27

Data is represented as Mean± standard deviation. Differences are found to be significant (p<0.05).

3.7 Overall Appearance of apples during storage at 25°C

Overall appearance of apples was checked during storage. After storage for 10 days at 25°C, apples coated with bacteriocin of LA 343 (Group I) showed no signs of deterioration. Apples did not suffer from any mechanical damage and wrinkling whereas uncoated apples (Group III) showed signs of decay (Fig 4 i).

After storage for 20 days at 25°C, apples coated with bacteriocin (Group I) maintained their quality in terms of overall appearance, firmness, texture and aroma. However apples coated with paraffin wax (Group II) gave a clear wrinkling surface and browning. Uncoated apples (Group III) lost its market value and consumer acceptance because of very high respiration rate and metabolism (Fig 4 ii). Coating of bacteriocin of LA 343 has also proven effective to inhibit the growth of *Staphylococcus aureus* on apples. Hence, apples inoculated with *Staphylococcus aureus* and having coating of bacteriocin (Group IV) were in good condition as compared to apples only inoculated with *Staphylococcus aureus* (Group V).

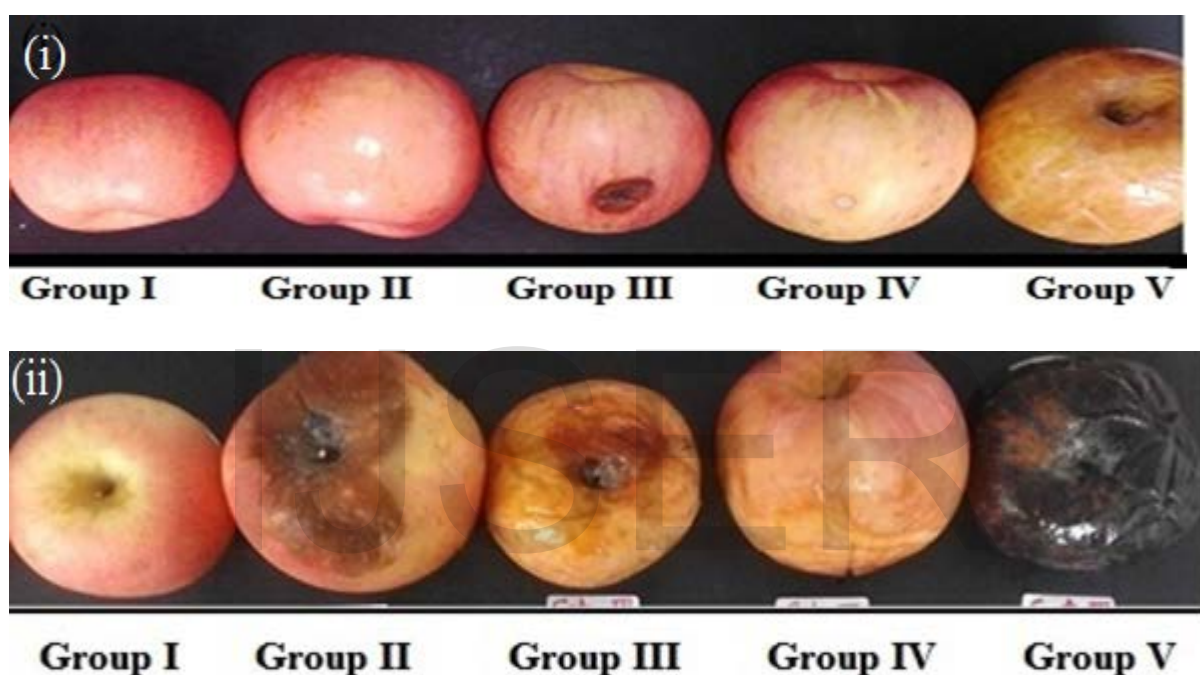


Fig 4: Overall appearance of apples during storage at 25°C (i) After 10 days (ii) After 20 days
Group I: Bacteriocin Coating, Group II: Paraffin wax coating,
Group III: Uncoated (Control), Group IV: Inoculation of *Staphylococcus aureus* + bacteriocin coating,
Group V: Inoculation of *Staphylococcus aureus*

There are few reports on the use of bacteriocins in preservation of apples and results of present study are in accordance with reported studies. Leverentz et al. (2003) proved that, nisin coating inhibited the growth of *Listeria monocytogenes* on apples. Similarly, biopreservation of apples using nisin was done by Siroli et al. (2015) and showed that nisin lead to inhibition of *Escherichia coli*, *Salmonella sp.*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* on apples. In another study, coatings on apple cubes containing enterocin AS-48 inactivated *Listeria monocytogenes* to lower levels as compared to uncoated apple cubes during storage at refrigeration (Aguayo et al., 2016).

Biopreservation of fruits is done by applying coatings of polysaccharides (Rhim, 2004), proteins (Gennadios, 2002) and lipids. Mainly lipids (paraffin wax) are used as a coating material for preservation of fruits. In this study, biopreservation of Fuji apples was done by the bacteriocin produced by *Lactobacillus acidophilus* NCDC 343 and its efficacy was tested in comparison to paraffin wax. Paraffin wax has shown less preservative efficacy as compared to bacteriocin. Results of study has shown that the physical and chemical properties of Fuji apples were maintained during storage in terms of weight loss, total soluble solids, titratable acidity, pH and external appearance. Hence, use of bacteriocin produced by LA 343 as biopreservatives seems to be advantageous in extending shelf-life of Fuji apples.

Post harvest practices damage the integrity of fruits and vegetables and make them vulnerable to microbial spoilage. Out of total world food production, 5-10% is spoiled by fungi (Giviazdowska et al., 2008). Mycotoxins produced by them are toxic to humans. They persist in food chains from harvest to transport and storage stage. Bacteriocin can be an alternative tool to prevent microbial spoilage of food items. In this study, coating of bacteriocin on apples proved to be helpful in preventing microbial spoilage during storage. Even after 20 days of storage at 25°C, the microbial count was much less as compared to uncoated apples. Coating of bacteriocin of *Lactobacillus acidophilus* NCDC 343 on apples proved to have both antibacterial and antifungal effect. Results obtained in the study suggest that bacteriocins can be used as biopreservatives in fruits as they do not interfere in any of their physical and chemical properties. They can be a component of the wash water solution used at farm level, thereby enhancing the shelf life of food products and aiding the farmer in phased marketing of his produce.

Conclusions

In this study, bacteriocin of *Lactobacillus acidophilus* NCDC 343 has shown broad antimicrobial spectrum against food spoilage organism *in vitro*. Coating of bacteriocin (100ug/ml) on Fuji apples prolonged their shelf life by maintaining physiochemical properties and inhibiting microbial spoilage during storage at 25°C. Coating act as barrier and prevent the exchange of gases leading to reduction in respiration rate and weight loss. It contributed to maintain the firmness, acidity and TSS of apples during storage. Bacteriocin coating also inhibited the growth of *Staphylococcus aureus* on apples significantly. It is recommended to use bacteriocin coatings as preservatives to extend shelf life of fresh produce.

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