

# DEVELOPMENT OF BIOACTIVE PACKAGING: PROBIOTIC COATING FOR IMPROVING FRESH-CUT APPLES QUALITY

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**Abstract-** The objective of this study was to apply an edible coating incorporated with free and encapsulated *Lactobacillus rhamnosus* B-445 cells (T2 & T3, respectively) to minimally processed apple slices (MPAS). The effectiveness of probiotic edible coatings as protective material for the probiotic and their effects on the quality attributes of MPAS were evaluated. Moisture content, total soluble solids, firmness, V.C, pH, total titratable acidity, viability of probiotics, microbial growth and sensory quality were studied during 13 days of storage at  $5\pm 1^\circ\text{C}$  and 70-75% relative humidity. Results showed that all probiotic alginate-based coatings successfully maintained all attributes of coated fresh-cut apples comparison with T1 (without probiotics) and uncoated samples. Any of the assayed coatings exhibited a positive effect on the sensory properties of fresh-cut apples. Furthermore, probiotic edible coatings had a marked effect in reducing mesophilic and yeast and molds counts on fresh-cut apples throughout storage. The probiotics *Lactobacillus rhamnosus* B-445 maintained fresh-cut apples quality during refrigerated storage, demonstrating the feasibility of alginate based edible coatings to carry and support viable probiotics on fresh-cut fruit.

**Keywords:** alginate, edible coatings, fresh-cut apple, probiotics, bioactive packaging

## 1. INTRODUCTION

The demand for healthy diets with fresh pleasant foods, especially minimally processed fruits and vegetables, resulted in a variety of products available in the market. The health benefits of probiotic lactic acid bacteria contribute to increase consumption of such prepared foods. Nowadays there is an increasing demand for non-dairy based probiotic products and for the development of fruits and vegetables with probiotic effect. However, the available information in this respect is limited [1]. In spite of the consumption of food products containing probiotics, has increased worldwide and a lot of attention from the food industries has been received, the edible films and coatings were studied as probiotic carriers with many applications in some few studies [2].

In general, fresh-cut processing of fruits causes quality deterioration and textural damage and changes [3], such as the flesh browning in the apple that develops as a result of the polyphenol oxidase activity [4,5].

Bioactive food packaging systems may provide health benefits to consumers, it is a novel approach in the concept of functional foods. The main difference between the commonly used active packaging technologies and bioactive packaging is that while the former packaging technology deals with maintaining or increasing quality and safety of packaged foods, the latter has a direct impact on the health of the consumer by generating healthier packaged foods. Bioactive packaging materials will ensure the storage and eventually release the bioactive agents into the food product [6]. Probiotic bacteria are important in this respect.

The probiotic microorganisms consist mostly of strains of the genera *Lactobacillus* and *Bifidobacterium* which are types of lactic acid bacteria [7]. *Lactobacillus rhamnosus* GG (L. rham. GG) is one of the extensively studied strains with well documented probiotic properties, it is known to colonize the intestine and to be active against organisms causing traveler's diarrhea and rotavirus infection [8,9]. Some researchers suggested that counts higher than  $10^6$  CFU mL<sup>-1</sup> [10,11] are required to cause the probiotic effect and others suggested a concentration of at least  $10^7$  and  $10^8$  CFU mL<sup>-1</sup> [12, 13]. However, L. rham. GG concentration on apple wedges was maintained at  $10^8$  CFU mL<sup>-1</sup> over a 10 days storage period at 2-4°C with acceptable quality of apple [14]. Probiotics may be carried within edible polymer matrices used by the food packaging industry. These probiotics, as well as many other compounds, can be incorporated into biopolymeric matrices to develop active/bioactive food packaging materials as an alternative method to control pathogenic microorganisms, and to improve food stability and safety [15].

Edible films and coatings from another side are made from naturally occurring polymers such as polysaccharides, and regarded as good oxygen barriers [16]. The major drawbacks of such films/coatings are their relatively low water resistance and poor vapor barrier properties [17]. One of the most commonly used polysaccharides is alginate. Alginates possess good film-forming properties and produce transparent and water-soluble films [18]. On the other hand, encapsulation technique of probiotics is carried to protect these bacteria from the effects of the low pH and bile, during their passage through the gastrointestinal tract [19,20]. Among the most commonly used polymers as encapsulating material sodium alginate. Alginate has been used as the

encapsulating material due to its ability to absorb water, to be easy manipulated and innocuousness, having also other features such as gelling, stabilizing and thickening [21].

Concerning fresh-cut vegetables and fruits, they are an expanding sector of the food industry. Consumers generally have high expectation for the quality of these minimally processed foods. Therefore, in the last years, a number of innovative strategies, including technological and microbial approaches, have been suggested to maximize their quality and shelf life [22,23].

In our current study, probiotic *Lactobacillus rhamnosus* B-445 (*Lb. rhamnosus*) free and encapsulated cells were incorporated into edible coatings based on alginate for preserving fresh-cut apple slices. So, the objective of this work was to formulate alginate edible coatings for coating fresh-cut apples. The coating solutions were used to coat apple slices and the feasibility of the alginate based edible coatings as carriers of organism (*Lactobacillus rhamnosus* B-445) was investigated as well as its effect on the chemical, microbial and sensory quality attributes of fresh-cut apple through shelf-life.

## 2. MATERIALS AND METODES

Apples (*Malus domestica*) variety Anna were obtained from the local market. *Lactobacillus rhamnosus* B-445 were provided by Northern Regional Research Laboratory (NRRL), Illinois, USA.

### 2.1 Microbial Strains and Growth Condition

*Lb. rhamnosus* was grown on MRS broth and incubated at 37°C for 24 h. The strain was activated two or three times in order to obtain high biomass in the stationary phase then the cell pellets were harvested by cold centrifugation at 4000 rpm, for 20 min. The pellets were washed using sterile saline solution (0.9% (w/v) NaCl) and recovered under the same centrifugation conditions then stored at  $\approx 5 \pm 1^\circ\text{C}$  till be encapsulated.

#### 2.1.1 Preparation of Encapsulated *Lactobacillus Rhamnosus* Using Spray Drier Method

According to Dolly et al., [24] with some modification, the *Lb. rhamnosus* suspension was mixed with the sodium alginate solution (3%) in the ratio of 1:3 and then subjected to spray drying. The suspension was dried into powder using a co-current Mini Spray Dryer B-290 (BÜCHI, Flawil, Switzerland). The inlet and outlet temperatures were 140°C and 70°C, respectively.

#### 2.1.2 Enumeration of the Microencapsulated Cells

The viability of *Lb. rhamnosus* was assessed as described by Chavarri et al., [25]. One gram of the microcapsules was dissolved in 9 ml of sterile tri-sodium citrate solution (2% w/v) and vortexed till complete dissolution, then the samples were serially diluted to appreciate concentration using saline solution and pour plated in MRS agar. The plates were incubated at 37°C for 48 h. The viable cell number was expressed as colony forming unit per gram (log cfu/g).

### 2.2 Preparation of Apple

Fruits of uniform size and colour maturity were chosen and washed with tap water then rinsed with disinfectant solution (calcium hypochlorite 2%) and air dried. The apples were peeled and the core tissues were completely removed. The flesh was cut into 1.5 cm thick slices lengthwise directly before dipping in coating solution.

### 2.3 Preparation of the Probiotic Edible Coatings

Coating forming solutions were prepared by dissolving alginate (2%) powders in distilled water with heating at 70°C until the solution became clear. Glycerol was added as plasticizer at 50% of alginate used. The heated aliquots were cooled at 40°C and kept isothermally to avoid alginate setting until inoculation with probiotics, then mixed with the prebiotic inulin after adjusting pH to 7.0. The coating solutions were mixed under slow agitation with the *Lactobacillus rhamnosus* B-445 (free, T2 and encapsulated, T3 cells). These solutions are incubated at 37 °C for 24 h to obtain suspensions for the coating of apple slices. The cell viability of the alginate base coating inoculated T2 and T3 was 8.22 and 8.34 log cfu/g, respectively.

#### 2.3.1 Coating Procedure for Fruits

Fruits were dipped for 1 min into anti-browning solution composed of a mixture of 1% (w/v) L- ascorbic acid and 1% (w/v) citric acid. The excess liquid was gently removed by draining on stainless steel screen for 3 minutes under fan to ensure dryness, then were dipped into 2% (w/v) calcium chloride solution as solidifying agent. Samples were removed and air dried. The control samples were dipped into distilled water following the same procedure. Then one-third of samples was dipped into probiotic coating solutions (T2) and (T3) and no probiotic coating solution (T1) for 3 min and dried for 5 min at room temperature using air-drier. Samples were removed, air dried, then placed on packaging. Some apple slices were processed in parallel without coating (C). The treated samples and a control were stored in a refrigerator at  $5 \pm 1^\circ\text{C}$  and 70-75 % relative humidity (RH). Samples were withdrawn at 0, 1, 3, 5, 7, 9, 11 and 13 days of storage for analysis.

### 2.4 Physical and Chemical Analyses

- Moisture content, total soluble solid (TSS), pH and total titratable acidity (TTA) were determined following the methods of [26].

- Firmness of the whole fruits was measured using a hand dynamometer model FDP 1000 with a thump (2 mm) in gf (gram- force). The data were transformed into Newton units using standard factor (1 gram- force = 0.00980665 Newton).
- Ascorbic acid (V.C) was determined using 2,6 dichlorophenolindophenol titrimetric method as described in [26]. The results were expressed in milligrams ascorbic acid per 100 ml of fruit juice.

### 2.5 Microbial Counts

The microbiological parameters that were determined included counts of aerobic mesophilic microorganisms, and moulds and yeasts as described by Guerreiro et al., [27].

### 2.6 Sensory Evaluation

It was carried out by seven trained panelists as described by Ramadhan et al., [28].

### 2.7 Statistical Analysis

This was evaluated through analysis of variance (ANOVA) using statistical software SPSS for Windows, version 19.0 (SPSS Inc., Chicago, IL, 245 USA). Duncan's multiple range test ( $P \leq 0.05$ ) was used to detect differences among means [29].

## 3. RESULTS AND DISCUSSION

### 3.1 Moisture content

Table [3.1] shows the changes in moisture contents during storage. The results revealed that the control sample (C) showed a higher level of moisture loss throughout the storage period, and there was significant difference between probiotic coated and coated samples. It can be noticed that treatments T2 and T3 protected the MPAS from moisture loss and subsequently weight loss throughout the storage period. The best significant maintenance of moisture content was found in T2 followed by T3, then T1 coated samples. These results are in agreement with Bourtoom, [30] and Ahmed and Butt [31]. In general moisture contents of apple slices significantly decreased as a function of storage time for both uncoated and coated samples.

**Table- 3.1 Moisture Contents (%) of Minimally Processed Apple Slices Coated with Edible Coatings of Different Treatments and Stored at  $5 \pm 1^\circ\text{C}$  and 70-75% RH for 13 Days\***

Treatment	Storage Periods(day)							
	0	1	3	5	7	9	11	13
C	85.55 <sup>Aa</sup>	82.19 <sup>Bb</sup>	77.85 <sup>Bc</sup>	ND	ND	ND	ND	ND
T1	85.54 <sup>Aa</sup>	86.13 <sup>Aa</sup>	84.95 <sup>Ab</sup>	83.70 <sup>Ac</sup>	82.36 <sup>Bd</sup>	80.01 <sup>Be</sup>	ND	ND
T2	86.42 <sup>Aa</sup>	86.35 <sup>Aa</sup>	85.70 <sup>Ab</sup>	84.60 <sup>Ac</sup>	83.20 <sup>Ad</sup>	82.11 <sup>Ae</sup>	81.21 <sup>Af</sup>	80.37 <sup>g</sup>
T3	86.52 <sup>Aa</sup>	86.50 <sup>Aa</sup>	85.73 <sup>Ab</sup>	84.82 <sup>Ac</sup>	83.56 <sup>Ad</sup>	82.26 <sup>Ae</sup>	82.00 <sup>Af</sup>	ND

C: uncoated apple slices, T1: coated samples without probiotics, T2: coated samples with *Lactobacillus rhamnosus* B-445 fee cell, T3: coated samples with encapsulated *Lactobacillus rhamnosus* B-445.

-Averages with different capital letters (due to treatments) and averages with different small letters (due to storage) differed significantly ( $P \leq 0.05$ ). ND= not determined for spoilage.

### 3.2 Total Soluble Solid (TSS)

Total soluble solid (TSS) significantly increased in coated samples when compared with uncoated samples [Table 3.2]. This was true at any storage time. This finding might be due to less moisture loss during storage of coated MPAS. It was quite important to note that, no significant differences were recorded between probiotic coated samples. The lowest increment of TSS in general was found in T2 samples (13.5%) followed by T3 and T1 (13.0%) at day11 of storage, then T1 samples (13.5%) at 9<sup>th</sup> day of storage compared with uncoated samples (13.5%) at 3<sup>rd</sup> days of storage. These results are similar to those obtained by Roßle et al., [14].

**Table- 3.2 Total Soluble Solids (TSS) of Apple Slices Coated with Edible Coatings of Different Treatments and Stored at  $5 \pm 1^\circ\text{C}$  and 70-75% RH for 13 Days\***

Treatment	Storage Periods(day)							
	0	1	3	5	7	9	11	13
C	11.5 <sup>Ac</sup>	12.0 <sup>Ab</sup>	13.5 <sup>Aa</sup>	ND	ND	ND	ND	ND
T1	11.5 <sup>Ae</sup>	11.5 <sup>Be</sup>	12.0 <sup>Bd</sup>	12.5 <sup>Bc</sup>	13.0 <sup>Ab</sup>	13.5 <sup>Ba</sup>	ND	ND
T2	11.5 <sup>Ae</sup>	11.5 <sup>Be</sup>	11.5 <sup>Be</sup>	12.0 <sup>Be</sup>	12.0 <sup>Ae</sup>	12.5 <sup>Ac</sup>	13.0 <sup>Ab</sup>	13.5 <sup>a</sup>
T3	11.5 <sup>Ad</sup>	11.5 <sup>Bd</sup>	11.5 <sup>Bd</sup>	12.0 <sup>Bc</sup>	12.5 <sup>Ab</sup>	12.5 <sup>Ab</sup>	13.0 <sup>Aa</sup>	ND

\*See footnotes of Table [3.1] for details.

### 3.3 Fruit Firmness

Table [3.3] indicates that significant differences were found in firmness between untreated (C) and treated samples with *Lactobacillus rhamnosus* B-445. (T2 and T3) also, significant differences were recorded during storage period of all samples. While in T2 and T3 significant differences were observed for *Lb. rhamnosus*

treatments and samples treated with coating only (T1). These results are in agreement with Rico et al., [32] and Alegre et al., [33].

**Table- 3.3 Firmness (N) of Apple Slices Coated with Edible Coatings of Different Treatments and Stored at 5±1°C and 70-75% RH for 13 Days\***

Treatment	Storage Periods(day)							
	0	1	3	5	7	9	11	13
C	28.5 <sup>Aa</sup>	27.0 <sup>Bb</sup>	24.0 <sup>Bc</sup>	ND	ND	ND	ND	ND
T1	28.3 <sup>Ba</sup>	28.0 <sup>Ab</sup>	26.7 <sup>Ac</sup>	25.8 <sup>Bd</sup>	25.0 <sup>Bc</sup>	24.1 <sup>Bf</sup>	ND	ND
T2	28.3 <sup>Ba</sup>	28.0 <sup>Ab</sup>	27.0 <sup>Ac</sup>	27.0 <sup>Ac</sup>	25.7 <sup>Ad</sup>	24.8 <sup>Ae</sup>	23.8 <sup>Af</sup>	23.0 <sup>g</sup>
T3	28.2 <sup>Ba</sup>	28.0 <sup>Ab</sup>	27.3 <sup>Ac</sup>	27.0 <sup>Ad</sup>	25.8 <sup>Ae</sup>	25.0 <sup>Af</sup>	24.0 <sup>Ag</sup>	ND

\*See footnotes of Table [3.1] for details.

### 3.4 pH

The data presented in Table [3.4] showed that no significant effects of probiotics treatment on pH samples were observed until 5 day of storage. Significant differences were observed between control and treated samples and almost between probiotics coated samples and coated samples without probiotics. The pH values of stored coated samples were significantly lower compared to the control of the same age. The results showed a significant increase in pH for all samples all during storage.

**Table- 3.4 pH Values of Apple Slices Coated with Edible Coatings of Different Treatments and Stored at 5±1°C and 70-75% RH for 13 Days\***

Treatment	Storage Periods(day)							
	0	1	3	5	7	9	11	13
C	4.12 <sup>Ac</sup>	4.26 <sup>Ab</sup>	4.53 <sup>Aa</sup>	ND	ND	ND	ND	ND
T1	4.13 <sup>Ad</sup>	4.13 <sup>Bd</sup>	4.15 <sup>Bc</sup>	4.18 <sup>Ab</sup>	4.19 <sup>Bb</sup>	4.26 <sup>Aa</sup>	ND	ND
T2	4.12 <sup>Af</sup>	4.12 <sup>Bef</sup>	4.12 <sup>Ce</sup>	4.17 <sup>Ad</sup>	4.19 <sup>Bc</sup>	4.27 <sup>Ab</sup>	4.28 <sup>Ab</sup>	4.30 <sup>a</sup>
T3	4.14 <sup>Ae</sup>	4.13 <sup>Be</sup>	4.13 <sup>Ce</sup>	4.18 <sup>Ad</sup>	4.21 <sup>Ac</sup>	4.23 <sup>Bb</sup>	4.26 <sup>Ba</sup>	ND

\*See footnotes of Table [3.1] for details.

### 3.5 Total Titratable Acidity (TTA)

Table [3.5] shows the total titratable acidity (TTA, expressed as % of malic acid) of both uncoated and coated fruit. Significant differences in TTA were noticed between control and all coated samples. TTA of all samples decreased as a function of storage time. TTA of coated samples slightly decreased during storage compared to control. These results are in agreement with results obtained by Roñle et al., [14].

**Table- 3.5 Titratable Acidity (TTA) of Apple Slices Coated with Edible Coatings of Different Treatments and Stored at 5±1°C and 70-75% RH for 13 Days (Expressed as % of Malic Acid) \***

Treatment	Storage Periods(day)							
	0	1	3	5	7	9	11	13
C	0.62 <sup>Aa</sup>	0.53 <sup>Bb</sup>	0.46 <sup>Bc</sup>	ND	ND	ND	ND	ND
T1	0.65 <sup>Aa</sup>	0.63 <sup>Ab</sup>	0.61 <sup>Ac</sup>	0.57 <sup>Ad</sup>	0.55 <sup>Ae</sup>	0.50 <sup>Bf</sup>	ND	ND
T2	0.64 <sup>Aa</sup>	0.62 <sup>Ab</sup>	0.60 <sup>Ac</sup>	0.58 <sup>Abd</sup>	0.56 <sup>Ae</sup>	0.54 <sup>Af</sup>	0.54 <sup>Af</sup>	0.52 <sup>g</sup>
T3	0.65 <sup>Aa</sup>	0.62 <sup>Ab</sup>	0.60 <sup>Ac</sup>	0.59 <sup>Ac</sup>	0.55 <sup>Ad</sup>	0.53 <sup>Ae</sup>	0.52 <sup>Af</sup>	ND

\*See footnotes of Table [3.1] for details.

### 3.6 Ascorbic Acid (vitamin C) Content

Table [3.6] shows that ascorbic acid of fresh MPAS was slightly higher than 9.0 mg/100g with insignificant differences between the control and coated samples. At any storage time the control had significant less content when compared with the coated samples. It could be noticed from Table [3.6] that ascorbic acid contents in both coated and uncoated apple slices significantly decreased during cold storage with less rate in coated samples.

In fact, ascorbic acid content is used to estimate the mean life of several products and as an indicator for preservation of vitamin content and other components responsible for the organoleptic characteristics of fruit and vegetable preserves.

**Table- 3.6 Ascorbic Acid Content (mg/100g sample) of Apple Slices Coated with Edible Coatings of Different Treatments and Stored at 5±1°C and 70-75% RH for 13 Days\***

Treatment	Storage Periods(day)							
	0	1	3	5	7	9	11	13
C	9.12 <sup>Aa</sup>	5.23 <sup>Bb</sup>	4.12 <sup>Cc</sup>	ND	ND	ND	ND	ND
T1	9.13 <sup>Aa</sup>	7.21 <sup>Ab</sup>	7.12 <sup>Bc</sup>	6.96 <sup>Bd</sup>	6.00 <sup>Bc</sup>	5.16 <sup>Cf</sup>	ND	ND
T2	9.12 <sup>Aa</sup>	7.23 <sup>Ab</sup>	7.20 <sup>Ac</sup>	7.20 <sup>Ac</sup>	6.88 <sup>Ad</sup>	6.16 <sup>Ae</sup>	5.90 <sup>Af</sup>	5.13 <sup>g</sup>
T3	9.11 <sup>Aa</sup>	7.22 <sup>Ab</sup>	7.18 <sup>Ac</sup>	7.17 <sup>Ad</sup>	6.85 <sup>Ae</sup>	6.10 <sup>Bf</sup>	5.56 <sup>Bg</sup>	ND

\*See footnotes of Table [3.1] for details.



### 3.7 Microbiological Analysis

Table [3.7] shows the results of the microbiological quality of the product during storage. The maximum number of aerobic mesophylls (TC) was  $5.4 \log \text{cfu g}^{-1}$ , and the maximum yeast and mould count (Y&M) was  $3.9 \log \text{cfu g}^{-1}$ , suggesting that the 3 coating treatments of apples slices analyzed were not at critical levels that could lead to sensory changes of the stored product.

It could be observed from Table [3.7] that, all coatings reduced yeast and mould growth compared to the control. Significant differences were found between samples and the probiotic treatments that had the best effect in maintaining minimum microbial count (TC), yeast and moulds (Y&M) and extending fruits storage life. This suggests that *Lb. rhamnosus* species are involved in the production of anti-microbial substances that act against other bacteria, viruses, protozoa and fungi [34,35] In this respect, it was reported that lactic acid bacteria (LAB) not only improve health when consumed, but they can also play a protective role against pathogens in the product [36]. The presence of such cultures could improve the shelf life of food while reducing the need to use increasing levels of chemical additives [37].

**Table- 3.7 Effect of Different Coating Treatments during Cold Storage of Minimally Processed Apple Slices (MPAS) on the Microbial Growth ( $\log \text{cfu/ g}$ )\***

Treatment	Storage Periods(day)													
	0		3		5		7		9		11		13	
	TC	Y&M	TC	Y&M	TC	Y&M	TC	Y&M	TC	Y&M	TC	Y&M	TC	Y&M
C	2.4 <sup>Ac</sup>	2.2 <sup>Ac</sup>	5.4 <sup>Aa</sup>	3.2 <sup>Aa</sup>	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
T1	2.4 <sup>Ad</sup>	2.2 <sup>Ad</sup>	2.6 <sup>Bd</sup>	2.9 <sup>Bc</sup>	3.3 <sup>Ac</sup>	3.2 <sup>Ab</sup>	3.9 <sup>Ab</sup>	3.5 <sup>Aa</sup>	4.9 <sup>Aa</sup>	3.6 <sup>Aa</sup>	ND	ND	ND	ND
T2	2.5 <sup>Ae</sup>	2.2 <sup>Af</sup>	2.7 <sup>Bf</sup>	2.6 <sup>Cc</sup>	2.9 <sup>Be</sup>	2.9 <sup>Bd</sup>	3.1 <sup>Cd</sup>	3.0 <sup>Cc</sup>	4.2 <sup>Cc</sup>	3.2 <sup>Bb</sup>	5.0 <sup>Bb</sup>	3.6 <sup>Ba</sup>	5.3 <sup>a</sup>	3.7 <sup>a</sup>
T3	2.4 <sup>Ae</sup>	2.2 <sup>Af</sup>	2.7 <sup>Bd</sup>	2.6 <sup>Cc</sup>	2.8 <sup>Bd</sup>	3.0 <sup>Bd</sup>	3.3 <sup>Bc</sup>	3.2 <sup>Bc</sup>	4.5 <sup>Bb</sup>	3.7 <sup>Ab</sup>	5.2 <sup>Aa</sup>	3.9 <sup>Aa</sup>	ND	ND

\*See footnotes of Table [3.1] for details, cfu/g: Colony forming unit/gram

### 3.8 Viable Probiotic Bacterial Count

Upon culture, *Lb. rhamnosus* was added to the coating solutions, it yielded viable populations of *Lb. rhamnosus* free cell and spray dried in counts of 8.22 and 8.34  $\log \text{cfu/g}$ , respectively in alginate coatings after inoculation and incubation. Table [3.8] shows that immediately after coating (day 0), viable counts of *Lb. rhamnosus* on the coated apple slices decreased to 6.99 and 7.54  $\log \text{cfu/g}$  for T2 and T3, respectively. This decrease was caused by dilution effects. Table [3.8] also shows that the lactobacilli population remained viable and constant during the cold storage. The survival and maintenance of *Lb. rhamnosus* may be regarded as satisfactory, as their values remained between 6 and 7  $\log \text{cfu/g}$ . These results agree with those of Alegre et al., [33] and RoBle et al., [14]. Although the minimum recommended level of viable probiotics which should be present in foods for any health benefits to be achieved can vary, in general the food industry has adopted the recommended level of  $10^6 \text{cfu g}^{-1}$  at the time of consumption [10,38]. Thus, probiotics have a promising potential for exploitation as functional supplements in fruit products due to their impressive tolerance to acidic environments [39].

In general, it may be of benefit to mention that probiotics are defined by Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) in 2002, as "live microorganisms which, when administered in adequate amounts, confer health benefit on the host" [40]. Members of the genera *Bifidobacterium* and *Lactobacillus* are the most frequently used probiotics [41,9].

**Table- 3.8 Viable Cells ( $\log \text{CFU/g}$ ) of *Lactobacillus rhamnosus* B-445 of Minimally Processed Apples during Cold Storage at  $5 \pm 1^\circ \text{C}$  for 13 Days\***

Treatment	Storage Periods (day)						
	0	3	5	7	9	11	13
C	abs	Abs	abs	Abs	abs	abs	abs
T1	abs	Abs	abs	Abs	abs	abs	abs
T2	6.99 <sup>Ba</sup>	6.72 <sup>Bb</sup>	6.67 <sup>Bc</sup>	6.64 <sup>Bd</sup>	6.57 <sup>Be</sup>	6.30 <sup>Bf</sup>	5.98 <sup>Bg</sup>
T3	7.54 <sup>Aa</sup>	7.24 <sup>Af</sup>	7.50 <sup>Ab</sup>	7.44 <sup>Ac</sup>	7.44 <sup>Ac</sup>	7.42 <sup>Ad</sup>	7.41 <sup>Ad</sup>

\*See footnotes of Table [3.1] for details. abs: absent

### 3.9 Sensory Evaluation

Data presented in Table [3.9] showed the mean scores of sensory evaluation of apple slices. At zero time of storage no significant differences were found among uncoated and coated samples. Apple slices coated with different treatments had higher score than those of uncoated samples. No significant differences in scores of organoleptic qualities were found between all coated samples for all attributes until 7 day of storage, after that T2 coated samples were the best treatments since the samples remained good without any spoilage.

The fruits treated with T2 received maximum score followed by T3, and T1 [Table 3.9]. Colour, odour, taste and texture of these fruits were relatively maintained to 13 days of storage period due to protective, antifungal and barrier effects of probiotics edible coating while, non-coated samples received less scores that due to high shrinkage, less colour, low quality and fungal deterioration after the 3<sup>rd</sup> day of storage. These results are similar to those of [14,33].

**Table- 3.9 Mean Sensory Scores of Apple Slices as Affected by Coating Treatments and Storage at 5±1°C and 70-75%RH\*.**

Storage(days)	Attributes	C	T1	T2	T3
0	Colour	9.70 <sup>A</sup>	9.74 <sup>A</sup>	9.73 <sup>A</sup>	9.72 <sup>A</sup>
	Odour	9.82 <sup>A</sup>	9.89 <sup>A</sup>	9.87 <sup>A</sup>	9.86 <sup>A</sup>
	Taste	9.85 <sup>A</sup>	9.92 <sup>A</sup>	9.80 <sup>A</sup>	9.82 <sup>A</sup>
	Texture	9.90 <sup>A</sup>	9.94 <sup>A</sup>	9.91 <sup>A</sup>	9.92 <sup>A</sup>
	Overall accessibility	9.80 <sup>A</sup>	9.95 <sup>A</sup>	9.88 <sup>A</sup>	9.86 <sup>A</sup>
3	Colour	6.10 <sup>B</sup>	9.11 <sup>A</sup>	9.10 <sup>A</sup>	9.20 <sup>A</sup>
	Odour	7.11 <sup>B</sup>	8.76 <sup>A</sup>	8.69 <sup>A</sup>	8.64 <sup>A</sup>
	Taste	7.12 <sup>B</sup>	8.69 <sup>A</sup>	8.85 <sup>A</sup>	8.69 <sup>A</sup>
	Texture	7.00 <sup>B</sup>	8.30 <sup>A</sup>	8.33 <sup>A</sup>	8.40 <sup>A</sup>
	Overall accessibility	5.99 <sup>B</sup>	8.52 <sup>A</sup>	9.11 <sup>A</sup>	9.35 <sup>A</sup>
5	Colour	ND	8.59 <sup>A</sup>	8.55 <sup>A</sup>	8.75 <sup>A</sup>
	Odour	ND	8.00 <sup>B</sup>	8.11 <sup>A</sup>	8.22 <sup>A</sup>
	Taste	ND	8.08 <sup>B</sup>	8.75 <sup>A</sup>	8.64 <sup>A</sup>
	Texture	ND	7.70 <sup>B</sup>	8.45 <sup>A</sup>	8.73 <sup>A</sup>
	Overall accessibility	ND	7.85 <sup>B</sup>	8.39 <sup>A</sup>	9.40 <sup>A</sup>
7	Colour	ND	7.10 <sup>B</sup>	8.50 <sup>A</sup>	8.38 <sup>A</sup>
	Odour	ND	7.14 <sup>B</sup>	8.19 <sup>A</sup>	8.14 <sup>A</sup>
	Taste	ND	7.03 <sup>B</sup>	8.30 <sup>A</sup>	8.26 <sup>A</sup>
	Texture	ND	6.89 <sup>B</sup>	8.10 <sup>A</sup>	8.14 <sup>A</sup>
	Overall accessibility	ND	6.60 <sup>B</sup>	8.20 <sup>A</sup>	8.12 <sup>A</sup>
9	Colour	ND	6.99 <sup>C</sup>	8.50 <sup>A</sup>	8.00 <sup>B</sup>
	Odour	ND	7.21 <sup>B</sup>	8.10 <sup>A</sup>	8.10 <sup>A</sup>
	Taste	ND	7.11 <sup>B</sup>	8.00 <sup>A</sup>	8.00 <sup>A</sup>
	Texture	ND	6.48 <sup>B</sup>	8.00 <sup>A</sup>	8.10 <sup>A</sup>
	Overall accessibility	ND	6.13 <sup>C</sup>	8.51 <sup>A</sup>	8.00 <sup>B</sup>
11	Colour	ND	ND	8.11 <sup>A</sup>	7.16 <sup>B</sup>
	Odour	ND	ND	7.78 <sup>A</sup>	7.00 <sup>B</sup>
	Taste	ND	ND	7.99 <sup>A</sup>	7.10 <sup>B</sup>
	Texture	ND	ND	7.69 <sup>A</sup>	7.32 <sup>B</sup>
	Overall accessibility	ND	ND	7.99 <sup>A</sup>	7.12 <sup>B</sup>
13	Colour	ND	ND	7.89	ND
	Odour	ND	ND	7.43	ND
	Taste	ND	ND	7.33	ND
	Texture	ND	ND	7.12	ND
	Overall accessibility	ND	ND	7.51	ND

\*Averages with unlike letters differed significantly ( $p \leq 0.05$ )

## CONCLUSION

Alginate-based coatings can be formulated satisfactorily with the incorporation of the free cells and encapsulated *Lb. rhamnosus* cells. The alginate based edible coatings seem to be efficient in supporting *Lb. rhamnosus* with reasonable count on fresh-cut apple throughout 13 days of storage at 5±1°C without any quality rejection.

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