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Impact of probiotics in preserving the microbiological property and nutritional quality in carrots

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Abstract
A study was carried out to determine the beneficial effects of probiotic culture in preserving the nutritional properties and extending the shelf life of carrots. The microbial and physiochemical attributes of carrots was tested by washing in water treated with probiotic culture *L. plantarum* 020 *and Lactobacillus acidophilus* 016. The probiotic culture- *L. plantarum* 020 and *Lactobacillus acidophilus* 016 were found to be viable during storage under room temperature. The total bacterial count after probiotic treatment reduced to 2 log cycles on carrots. A reduced fungal growth was noticed on carrots during storage after probiotic treatments. The carotene content and weight loss in carrots were maintained after treatments. The carrots treated with *L. plantarum* 020 were able to maintain the quality up to 8 days of storage whereas carrots treated with *L. acidophilus* 016 maintained its quality up to 6th day with initiation of spoilage. Thereafter untreated carrots started to spoil by the 4th day when stored under room temperature.

Keywords: Probiotics, carrot, postharvest quality

1. Introduction
Carrot is one of the major root vegetable cultivated in Nilgiris. Majority of the carrots harvested in Nilgiris are processed in carrot washing machinery units. Generally, harvested carrots are washed in washing machinery plants with water and then packaged in gunny bags. Washed carrots are sent to the market for auction and then sent to supply chain to reach the consumers. The major constraint in this process is postharvest losses caused due to microbial spoilage. Processed carrots in these units have less than 48 h of shelf life. Poor quality water used along with the mechanical injuries like bruising and cracking in carrots caused during processing in washing plants render them more prone to attack by organisms and significantly increases the rate of water loss and gaseous exchange. Mechanical stress during harvesting and processing has a negative effect on sensory quality of carrots, as it enhances microbial growth leading to spoilage, resulting in 34% reduction in sweet taste, 45% increase in ethanol flavour and 27% increase in sickeningly sweet taste (Seljåsen *et al.*, 2001) [19].
Carrots undergo deterioration, mainly due to changes in colour, texture, odour, biochemical parameters and improper storage condition. Various treatments are provided to reduce the postharvest losses in carrots. Edible films and edible coating emulsions have been reported to be effective in reducing the white blush defect in carrots (De Jesús Avena-Bustillos *et al.*, 1994) [6]. Several other studies have reported the effect of irradiation, X-ray, UV-C light and cold plasma for extending the shelf-life on fresh-cut vegetables (Palekar *et al.* 2015) [16], Mahmoud (2012) [12], Manzocco *et al.* (2013) [13], Tappi *et al.* (2016) [22] respectively. However, high costs with intensive labour requirement and complexities in the use of these methods limits the commercial applications for treating fresh or fresh-cut fruits and vegetables. Probiotics are the beneficial microorganisms which are beneficial to human health when consumed (Nichols, 2007) [15]. These microorganisms are naturally observed in gastrointestinal tract of human beings and also can be provided orally. As the probiotics are beneficial to the human health their presence in food will not have any harmful affect on the human health. The selection of new probiotic strains and its application in food industries has gained much importance as probiotics incorporated food have increased nutritional quality and storage stability. However, the potential health benefit will depend on the characteristic profile of the probiotics. *Lactobacillus* and *Bifidobacterium* are most commonly used probiotics in food and agricultural produce. Probiotics could produce antimicrobial substances like bacteriocins, which inhibits the growth of other microbes by competing for nutrition in the host. Some probiotic strains can reduce intestinal transit time, improve the quality of migrating motor complexes and temporarily increase the rate of mitosis in enterocytes (Husebye *et al.* 2001) [10]

Banasaz *et al.* (2002) [1]. As the probiotics are known to control pathogenic infections in human and are safe microbial formulations, their ability as a postharvest disease management system was studied.

Hence, the aim of the present study was to compare the viability of probiotic cultures *Lactobacillus plantarum* 016 and *Lactobacillus acidophilus* 020 in maintaining the postharvest qualities of carrots when stored at ambient temperature.

2. Materials and Methods

Samples of Carrots (New Korda variety) were collected from a carrot machinery plant in Ooty, Nilgiris, Tamilnadu, India. Samples were collected before and after processing in carrot wash unit during November 2018 and stored under refrigeration 4ºC until analysis.

2.1 Probiotic strains and culture

Probiotic culture like *Lactobacillus plantarum* 020 and *Lactobacillus acidophilus* 016 were procured from NDRI, Karnal, India. Cultures were multiplied separately in the De Man, Rogosa and Sharpe (MRS) broth for 24 h at 37ºC. Ten ml of broth from each of *Lactobacillus* species grown in MRS broth were mixed in equal ratio and centrifuged at 4000 rpm for 10 min to harvest the cells (Mousavi *et al.*, 2011) [14]. Harvested cells were mixed in sterile distilled water to get 10^9 CFU per ml and used for different carrot treatments.

2.2 Treating carrots with probiotic cultures

The prepared starter cultures each (10 ml) were introduced in to pure water (1000 ml) to obtain 1% inoculation. It was allowed to stay at room temperature for 1 h. Known quantity of carrot samples were washed with *L. plantarum* 020 treated water (T_1), *L. acidophilus* 016 treated water (T_2) and untreated samples were kept as a control (T_0). The microbiological and chemical properties like total carotene content and percent weight loss were assessed on the 0, 2, 4, and 6th day of ambient storage.

2.3 Microbial analysis of probiotic treated carrots

The viable count of treated culture was determined by the standard plate count method using Man-Rogosa-Sharpe agar (MRS agar) on the 0, 2, 4, and 6th day and the results were expressed as CFU per ml. The total bacterial, fungal count was determined using Nutrient agar and Rose Bengal agar respectively. Plates were incubated at 37ºC for 48-72 hours (Bell *et al.*, 2005) [2]. To study the surface microbial count, the whole carrot sample was immersed in peptone water and a dilution of 10^{-1} was made and serial dilutions were made for further analysis.

2.4 Chemical analysis of probiotic treated carrots during storage

2.4.1 Total carotene content

The total carotene content was measured on days 0, 2, 4 and 6. A known quantity of carrot sample was taken and homogenized in a pestle and mortar using acetone. The pulp was extracted repeatedly using acetone until the residue is colourless. This mixture is then added to a separating funnel containing petroleum ether. Five percent sodium sulfate solution was then added to the solution. The petroleum ether extract was removed from the funnel and added to anhydrous sodium sulfate. Carotene content was determined using spectrophotometry method, in which the yellowish colour formed is measured against spectrophotometer at the wavelength of 453 nm.

Total quantity of carotene content in 100 g of carrot sample is calculated using the equation (1)

$$\text{Total carotene} = \frac{\text{Absorbance of sample}}{0.2592} \times \frac{\text{total volume} \times 100}{\text{weight of sample(g)} \times 1000} \ldots (1)$$

2.4.2 Weight loss

Weight loss of carrots during storage was found using an analytical balance with a precision of 1×10^{-4} g. The weight loss was calculated using the equation (2)

$$WL_t(\%) = \frac{W_0 - W_t}{W_0} \times 100 \quad \ldots (2)$$

Where;

WL_t = weight loss percentage at time "t".

W_0 = initial weight of the sample.

W_t = weight of the sample at time "t".

2.5 Statistical Analysis

The statistical analysis was carried out using AGRES software. Differences were considered to be significant at $p < 0.05$. Analysis of variance (ANOVA) was performed to study the effect of probiotics on shelf life of carrots.

3. Results and Discussion

3.1 Effect of storage on viability of probiotic cultures

The changes in the survival of probiotic cultures *Lactobacillus plantarum* 020 and *Lactobacillus acidophilus* 016 over the surface of carrots during the storage are given in Fig. 1. The cultures were inoculated in water and the carrot samples were washed in the treated water. It was observed that the probiotic cultures were capable of surviving on the outer surfaces of carrots at room condition although a decline in the counts ($p<0.05$) was observed due to poor adaptation onto the new substrate. On the 0th day of inoculation, the cultures were able to survive at a concentration of 7.015 and 7.045 logs which gradually decreased to 3.3 and 3.5 logs in case of *L. plantarum* 020 and *L. acidophilus* 016 respectively on the 6th day. The decreased viability of cultures may be due to high metabolic activity at room temperature, which gradually decreased their growth.

Thakur and Sharma (2017) [23] studied the viability of *Lactobacillus bulgaricus* and *Lactobacillus. plantarum* in pomegranate juice under room temperature and reported similar results as the present study. The functionality of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* was studied as edible coatings in fish to preserve the quality and a reduction was observed on the viability of cells during storage (De Lacey *et al.*, 2012) [7]. Loss in viability of probiotics in room temperature is mainly due to an increased cell metabolism and death when temperature is increased as compared to refrigerated storage (Ferdousi *et al.*, 2013) [9]. Viability of probiotic bacteria during storage is inversely related to storage temperature (Gardiner *et al.*, 2000).

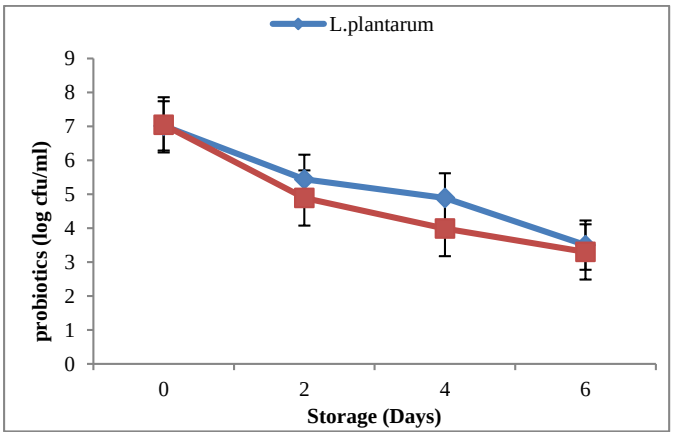

Fig 1: Viability of *L. plantarum* 020 and *L. acidophilus* 016 during storage

In the present study, the survival of *L. plantarum* 020 and *L. acidophillus* 016 was similar on the first day of treatment. As the storage continued, there was a sharp decrease in the viability of both cultures reducing from 1.035×10^7 and 1.109×10^7 to 0.00037×10^7 and 0.00031×10^7 CFU/ml for *Lactobacillus plantarum* 020 and *Lactobacillus acidophillus* 016 respectively, proving the influence of temperature during storage.

3.2 Effect of storage on microbiological properties of probiotic treated carrots

3.2.1 Bacterial and fungal growth after treatments

The total bacterial and fungal growth was assessed in the samples treated with probiotic culture when stored at room temperature. As the storage period increases, there was a sharp reduction in the survival of probiotic culture. The untreated carrots showed a reduced population of 1.28×10^3 on the 0^{th} day when compared with the probiotics treated samples having a population of 3.63×10^7 and 9.77×10^7 CFU/ml of *L. plantarum* 020 and *L. acidophillus* 016 respectively. The growth of bacteria in untreated samples multiplied gradually from 1.28×10^3 on 0^{th} day to 8.22×10^7 CFU/ml by the 6^{th} day. Whereas a gradual reduction in bacterial population was observed in probiotics treated samples. The bacterial growth reduced from 3.63×10^7 CFU/ml and 9.77×10^7 on 0^{th} day to 4.36×10^5 and 9.77×10^5 CFU/ml by the 6^{th} day when treated with Lp 016 and La 020 respectively. The decline in the bacterial count ($p< 0.05$) was observed due to the instability of probiotics culture during storage. However, the culture maintained the quality of the samples throughout storage.

The appearance of carrots under storage is shown in the Fig. 2. Most of the untreated carrots spoiled by the 6^{th} day of storage, whereas the carrots treated with *L.plantarum* 020 remained fresh and free of spoilage. There was an initiation of microbial growth in *L.acidophillus* 016 treated carrots.

(a) (b) (c)

Fig 2: (a) Carrots without treatment (b) *L.plantarum* 020 (c) *L. acidophillus* 016

On the other hand, there was a reduced fungal population during storage in probiotic treated samples. The fungal count observed were 1.62×10^2, 0.07×10^2 and 0.09×10^2 on the 0^{th} day and increased gradually to 9.77×10^3, 0.45×10^2 and 1.02×10^2 CFU/ml on untreated carrots and treated with *Lactobacillus plantarum* 020 and *L. acidophillus* 016 respectively by the 6^{th} day of storage. The result proved the dominance of bacteria during storage suppressing the fungal growth on treated carrots. The inactivation mechanism of probiotic against pathogens is by microbial antagonisms and also by preparing the plant to defend itself from external attack, termed as "induced systemic resistance" (Conrath *et al.*, 2002) [5].

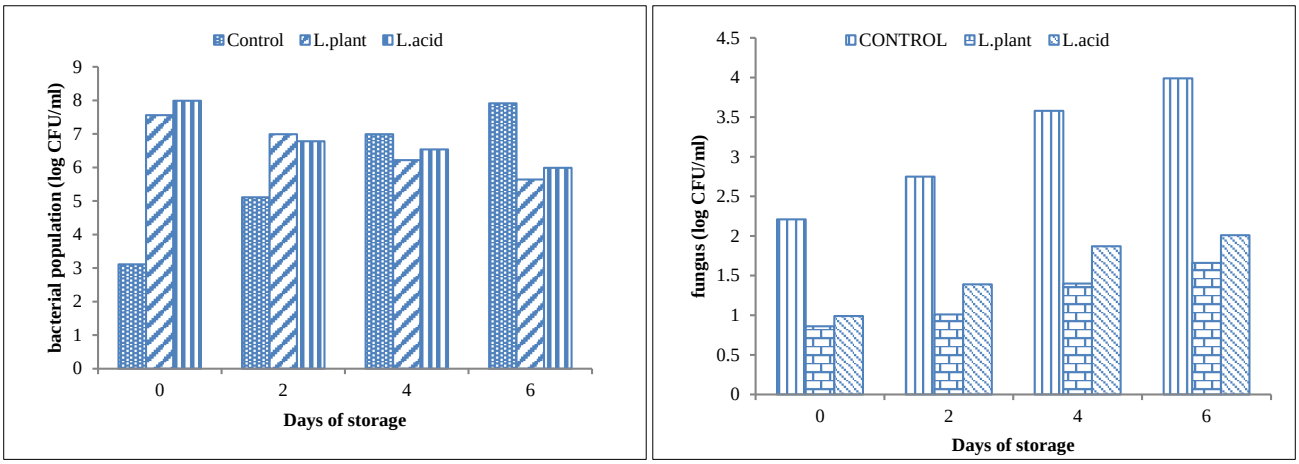

Fig 3: Effect of *L. plantarum* 020 and *L. acidophilus* 0216 on bacterial and fungal growth on carrots during storage

The Fig. 3 shows the bacterial and fungal growth during 0, 2, 4 and 6th day of storage. Probiotic culture has the potential to inhibit the growth of saprophytic and pathogenic microorganism (Denkova *et al.*, 2013) [8]. Microbial antagonism includes the inhibition of microbial growth, competition for colonization sites and nutrients, competition for minerals, and degradation of pathogenicity factors ((Berg, 2009) (Compant *et al.*, 2010) [3, 4]).

3.4 Effect of storage on chemical properties of probiotic treated carrots

3.4.1 Carotene content in carrots

There was a gradual degradation of carotene content in carrots during storage. The effect of probiotics in maintaining the carotene content of carrots during storage is given in Fig. 3. Carotene content and moisture loss were significantly affected by storage time and treatment ($p<0.05$). On the 0th day, the carotene content recorded were 9.77, 9.65, 9.88 mg/100 g of sample in untreated, and treated with Lp 020 and La 016 samples respectively. The initial carotene content in samples was similar in value. During storage days, there was a sharp decline in the total carotene content with untreated sample, whereas the carotene content was maintained in treated samples. The carotene content reduced from 9.77, 9.65, 9.88 mg/100 g on 0th day to 5.344, 7.99 and 7.24 mg/ 100 g in untreated and treated with Lp 016 and La 020 respectively by the 6th day. The study showed the ability of probiotic cultures in maintaining the carotene content during storage under room temperature.

The degradation in carotene content of untreated sample during storage is noted mainly due to spoilage. On the 6th day of storage, there was a maximum spoilage in untreated samples degrading the total carotene content whereas the carotene was maintained in treated samples. The use of *Bifidobacterium* strains like *B.lactis* Bb-12, *B.bifidum* B7.1 and B3.2 could preserve the biochemical changes in fermented carrot juice with minimal degradation of carotenoids and the nutritional value of the product without any nutrient supplementations (Kun *et al.*, 2008) [11]. It has been reported that the probiotic strains of *L. casei*, *L. acidophilus*, *L. plantarum*, and *L. delbrueckiiare* being resistant to low pH did not influence the lycopene content and its chemical properties (Koh *et al.*, 2010).

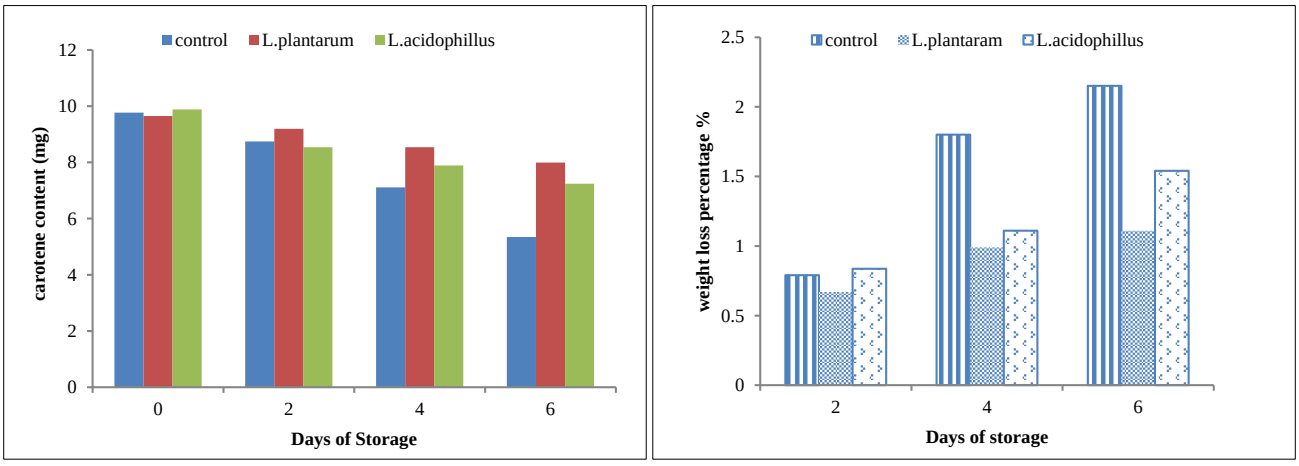

Fig 4: Effect of *L. plantarum* 020 and *L. acidophilus* 016 on total carotene content and weight loss percentage in carrots under ambient storage

3.4.2 Total percent weight loss

The total percent weight loss was calculated for 0, 2, 4 and 6th day of storage. There was a slight increase in the percent weight loss in all samples during the storage period. The highest weight loss was observed in untreated samples showing 2.151% loss from total weight. There was a minimum of 1.11 and 1.54% loss from total weight when treated with Lp 020 and La 016 by the 6th day of storage respectively. At the end of storage, probiotics treated samples retained the weight in samples, as probiotics cells in the surface reduced the microbial load causing spoilage. Spoilage microbes soften the tissues and cells of carrots, ultimately reducing the weight and poor moisture retention in roots. The treated samples had a reduced spoilage compared to untreated samples maintaining the weight during storage at room temperature.

Lactobacillus acidophilus when incorporated in Beetroot juice was better in terms of pigments, vitamins and minerals. The microorganism could also maintained a good cell vitality during storage preserving its moisture (Rakin *et al.*, 2004) [18]. Probiotic used as an edible coating to improve the quality factor of minimally processed carrots showed a reduced effect in color and showed a greater retention in moisture content of carrots (Shigematsu *et al.*, 2018) [20]. The use of probiotics as an edible coating demonstrated a slowdown in moisture loss from food products (Soukoulis *et al.*, 2014) [21]. Parvez *et al.* (2006) [17] found a similar result in strawberries when probiotic treated edible coating was given.

5. Conclusion

The finding tends to support the concept of using probiotics as postharvest disease management system in carrots. Probiotics treated water used for washing carrots maintained the quality and appearance of carrots during storage for a period of 6 days under ambient condition. The viability of probiotic cultures *L. plantarum* 020 and *L. acidophillus* 016 is reduced during storage due to its poor adaptation to new substrate and also the increased temperature leads to cell mortality under room temperature. However, they had a positive effect in reducing the total bacterial and fungal contamination and in maintaining the physicochemical properties of carrots. The total carotene content was maintained with reduced weight loss in treated carrots. The study showed the potential ability of probiotics in preserving the nutritional quality by reducing the postharvest losses in carrots.

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