

Original Research Article

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Effect of Storage Temperature on Exopolysaccharide Yields and Stability in *Bacillus subtilis* (MTCC 121) using Grapes

K. Sureshkumar^{1*}, Nathira Fathima², P.Rajendran¹ and R.Vidyalakshmi¹

¹Indian Institute of Crop Processing Technology, Thanjavur- 613 005, Tamil Nadu, India

²Tamilnadu Agricultural University, Coimbatore – 641003, Tamil Nadu, India

*Corresponding author

ABSTRACT

Keywords

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A non-pathogenic microbes *B.subtilis* which produces exopolysaccharides can be used to prevent the fruits and vegetables from various biotic and abiotic stresses which increase the shelf life of fruits and vegetables. The aim of this study was to examine the influence of storage temperature of, pH, Titratable Acidity, Brix % Weight loss % and Decay percentage (%) for the production of EPS EPS 1% & EPS 5% have antibacterial effect and have been proved to increase the shelf life of grapes with and without the combination of *Aloe vera*. The brix% of the coated grapes at room temperature was higher than at 4°C. *Aloe vera* 20% + EPS 1% treatment has given a satisfactory result by retarding the ripening process compared to other treatments. *Aloe vera* 20% + EPS 1% was found to be best as the decay was too less on comparing with the other treatments in different storage temperatures.

Introduction

Many microorganisms can synthesize exopolysaccharides (EPSs) and excrete them out of cell either as soluble or insoluble polymers. These EPS not only can protect the microorganisms but also can be applied in many biotechnological applications, such as textile, pharmaceutical, cosmetics, food, metal mining, oil recovery and metal recovery. Common microbial EPSs, such as xanthan and dextran, have been used as commercial products for many years. Because of their novel properties, the bioactive microbial polysaccharides β -D-glucans and bacterial cellulose have been

used for immune modulation and tumour stasis and audio membrane. Thus, microbial EPSs have attracted more attentions from scientific and industrial communities. EPS are considered to be the key components that determine the structural and functional integrity of microbial aggregates (cohesion) and for anchoring the biofilms to the substratum (adhesion). A large amount of work has been conducted on characterizing the EPS of various pure and mixed cultures (Llamas, *et al.*, 2012 & Amjres, *et al.*, 2012) activated sludge (Feng, *et al.*, 2012), biogranules (Laspidou *et al.*, 2002, and

biofilm (Liu *et al.*, 2004 & Flemming *et al.*, 1995). The EPS may not be essential for growth and viability in free-living bacterial cultures but protect cells from hostile environments. They are also involved in the degradation of particulate substances, sorption of heavy metals, leaching of minerals from sulphidic ores and biocorrosion, thus aiding in heavy metal bioremediation (Horn, *et al.*, 2006 & Gutnick, *et al.*, 2000)

Materials and Methods

Culturing of *B.subtilis* (MTCC 121)

Bacillus subtilis culture was maintained on nutrient agar plates. It was sub-cultured nutrient agar slants and plates and were incubated at 37°C for 24 hrs. The slants and plates were stored at 4°C.

Production of Exopolysaccharides

The isolated bacteria was inoculated in a standardized medium “EPS basal medium” with composition (for 1000ml): Glucose - 10gm, Yeast extract -3gm, Malt extract - 3gm, Peptone -5gm, MgSO₄.7H₂O -1gm, KH₂PO₄ .0.3gm with final pH 5.2. The 1000ml flasks containing 500ml of the EPS basal medium was inoculated with the bacterium and the flasks were incubated at 37°C for 10 days in rotary shaker at 50 rpm (totally 5.5 liters prepared).

Extraction of EPS

EPS was then precipitated by addition of equal volume of ethanol. The mixture was then agitated during addition of ethanol to prevent local concentration of the precipitate and left overnight at 4°C and centrifuged at 7000 rpm for 20 min to pellet out exopolysaccharides. Then, the precipitate was collected in petriplates and dried at

60°C. EPS was extracted according to the method followed by (Ohno *et al.*, 2000)

Preparation of Aloe Vera gel

The liquid obtained constituted fresh Aloe Vera gel. The gel matrix was pasteurized at 70°C for 45min. The gel was cooled immediately to an ambient temperature to stabilize and ascorbic acid (1.9 - 2.0g l⁻¹) was then added citric acid (4.5 - 4.6g l⁻¹) was added to maintain the pH at 4. It was later be stored in brown Amber bottle to prevent oxidation of the gel (Adetunji *et al.*, 2012).

Optimization of Different Treatment

The experiments conducted in storage temperature of, pH, Titratable Acidity, Brix % Weight loss % and decay percentage (%) for the production of EPS using Aloe Vera gel and exopolysaccharides from *Bacillus subtilis*

Results and Discussion

Effect of Storage Temperatures on pH

Figure 1 reveals that the pH of the grapes had marginal decrease on storage. There was no remarkable difference in pH between the coated and uncoated grapes. This might be due to the semi permeability created by coatings on the surface of the fruit, which might have modified the internal atmosphere i.e. endogenous O₂ and CO₂ concentrations in the fruit, thus retarding ripening (Lowings *et al.*, 1982; Bai *et al.*, 1988).

Effect of Storage Temperatures on Brix %

From the observations recorded in the changes in brix percentage (%) on storage of coated grapes in Figure 2, it is found that

brix% of the coated grapes at room temperature was higher than at 4° C as the higher temperatures retard the ripening process. The brix% of EPS 1 % was higher when compared to the uncoated grapes and thus this might have led to the decay of grapes on the 15th day. ALOE 20% + EPS 1% treatment has given a satisfactory result.

Effect of Storage Temperatures on Titratable Acidity

Figure 3 shows that there was no significant difference between the different treatments but there was difference noted between coated and uncoated grapes. The coating has led to the Titratable Acidity retention, which indicated that control fruits presented a more pronounced maturation development than coated berries, similarly to that found in starch-coated strawberry (Mali *et al.* 2003), and could be related to the higher respiration rate found in uncoated fruits. Some hike in titratable acidity values might also be due to

the grapes taken for the analysis.

Effect of Storage Temperatures on Weight Loss %

From the Figure 4, it was observed that coating of Aloe Vera gel and exopolysaccharides had decreased the weight loss on comparing with the control. The weight loss at room temperature was more than those at 4°C. EPS 5% was found to be the best while Aloe Vera coated grapes were found to be unsatisfactory on comparing with the other treatments. This positive effect of edible coatings was based on their hygroscopic properties, which enables formation of a water barrier between the fruit and the environment, and thus avoiding its external transference (Morillon *et al.*, 2002). As other edible coatings, AV gel prevented moisture loss and controlled respiratory gases exchange (Valverde *et al.*, 2005).

Figure.1 Effect of Storage Temperatures on Ph

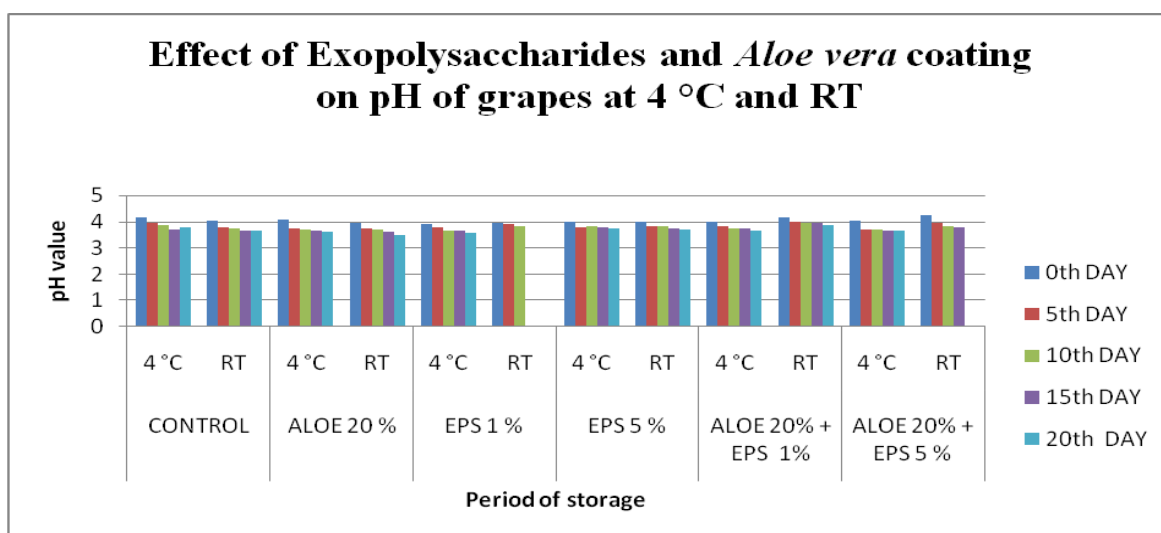


Figure.2 Effect of Storage Temperatures on Brix %

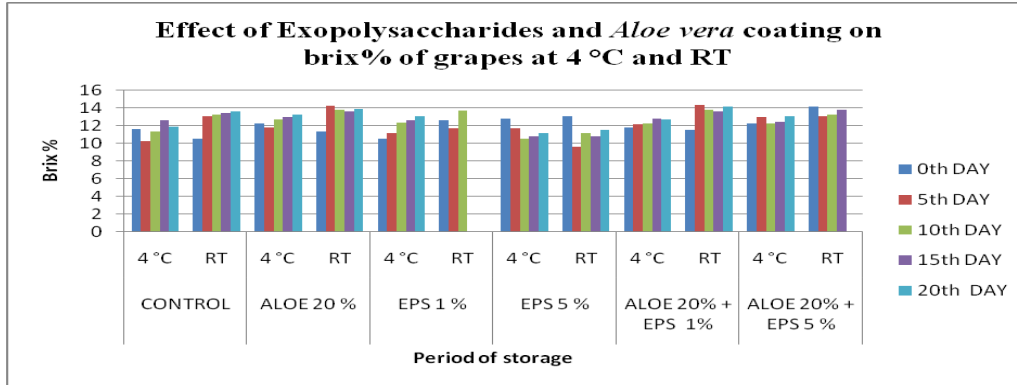


Figure.3 Effect of Storage Temperatures on Titratable Acidity

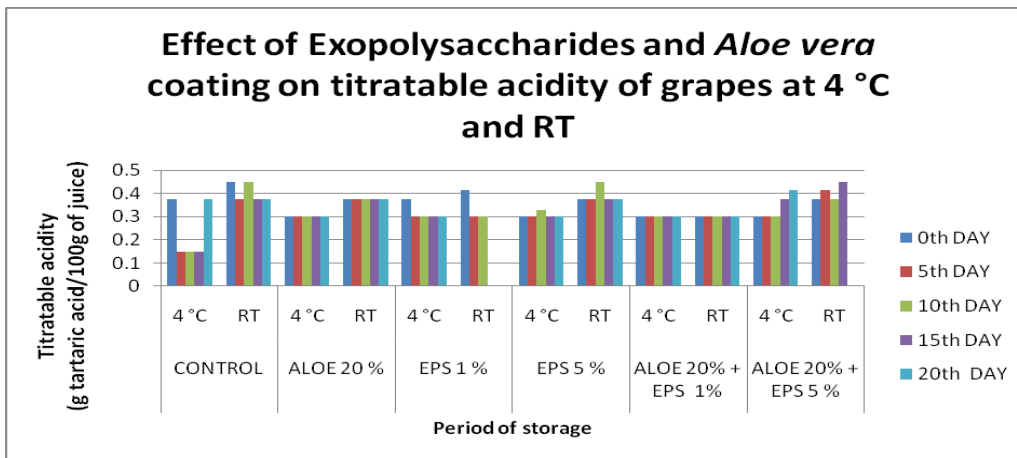


Figure.4 Effect of Storage Temperatures on Weight Loss %

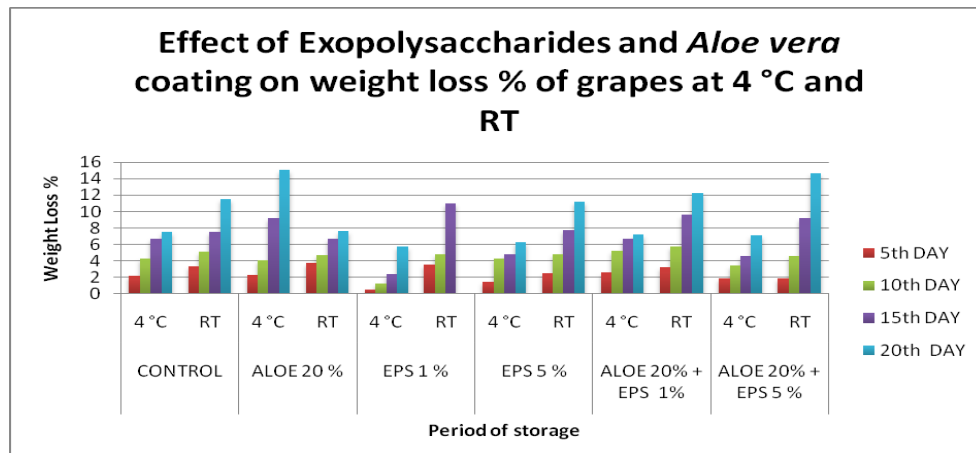
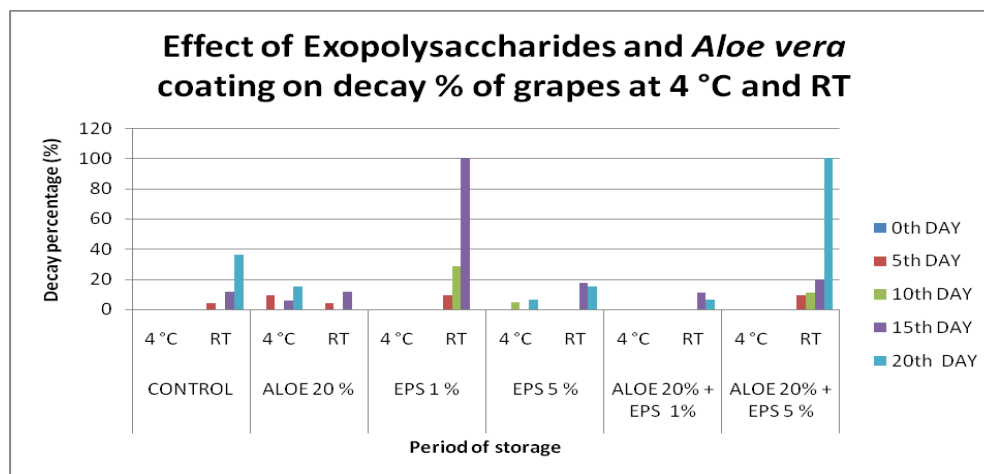


Figure.5 Effect of Storage Temperatures on Decay Percentage (%)



Effect of Storage Temperatures on Decay Percentage (%)

Decay is one of the most important postharvest factors in reducing quality of horticultural crops. From the CHART 5, it was observed that the least effective treatment was the grapes coated with EPS 1%. In ALOE 20% + EPS 5% as 100% decay was observed in room temperature on 15th and 20th day respectively. ALOE 20% + EPS 1% was found to be best as the decay was too less on comparing with the other treatments. The factors that contributed to the decay were fungal contamination and other small fissures in grapes.

In conclusion, EPS 1% & EPS 5% have antibacterial effect against *Staphylococcus aureus* and have been proved to increase the shelf life of grapes with and without the combination of *Aloe vera*. The brix% of the coated grapes at room temperature was higher than at 4°C. *Aloe vera* 20% + EPS 1% treatment has given a satisfactory result by retarding the ripening process compared to other treatments. *Aloe vera* 20% + EPS 1% was found to be best as the decay was too less on comparing with the other treatments in different storage temperatures.

From the research findings it is evident that coating grapes with *Aloe vera* and Exopolysaccharides or in combination of both had a positive impact on increasing the shelf life of grapes. Exopolysaccharides production should be increased in large scale to bring out much more hidden secrets in this compound. In future, these treatments can also be applied in other fruits and vegetables to prevent post harvest losses.

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