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Exopolysaccharide (Eps) Based Edible Films to Enhance Shelf-Life of Fruits and Vegetables

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ABSTRACT

As the world population increases, supply and quality of fresh foods becomes a challenge. The development of food packaging films and coatings is one of the modern strategies for preserving food products and ensuring their freshness and quality during storage. Exopolysaccharides (EPSs), usually derived from Lactic Acid Bacteria (LAB), are molecules that are released by most bacteria which act as a protection for the bacteria. In this investigation, a dissolved EPS, combined with a plasticizer, was employed in the formulation of an edible film. The findings revealed that fruits coated with lyophilized EPS exhibited superior resilience against environmental factors that contribute to spoilage. The utilization of microbial exopolysaccharides emerges as a promising and sustainable alternative to contemporary food packaging techniques. This study underscores the potential of this approach to enhance the shelf life and quality of food products, thereby addressing the pressing challenges associated with the growing global population.

Keywords: LAB; Exopolysaccharides (EPS); Edible film; Food packaging

1. Introductions

Lactobacillus or Lactic Acid Bacteria (LAB) is a commonly found organism in several dairy products and other fermented food products. They are Gram-positive, anaerobic, or microaerophilic, rod-shaped, non-sporing bacteria. They are usually recognized as safe for consumption and consist of health-promoting potential for food applications, including probiotic characteristics [1]. Most bacteria produce or secrete biological polymers as a metabolic by-product which primarily functions as an adhesive in most cases [2] but also acts as a protective barrier for the microbe and shields it from many harmful effects. These polymers are called Exopolysaccharides (EPS). In recent years, there has been a growing amount of interest in understanding EPS and its applications and functions. The unique structural features have made bacterial EPSs of particular interest in the fields of chemistry, medicine, and food industry [3]. Because of their ability to increase water holding capacity, EPSs are widely used as viscous, stabilizing, and emulsifying agents in the food industry and to further improve the rheological property and texture of bread and fermented milk products such as yogurt and cheese. Apart from these they also have potential health benefits as antioxidant, anticancer, anti-inflammatory, and antiviral activities [6].

It has been found that a wide range of lactic acid bacteria (LAB) can produce intra or extracellular polysaccharides, with various chemical compositions and properties [7]. These EPSs can either be

heteropolymers or homopolymers. Dextrans are a very commonly secreted Exopolysaccharide by Lactic acid bacteria. It is an exopolysaccharide produced by different microbial strains, mostly of the genera *Leuconostoc, Lactobacillus, and Streptococcus*, when grown on the sucrose-rich media. Dextrans consists of consecutive α- (1 → 6) linked glucose units which makes it a homopolymer. Dextran is extremely water soluble and shows no post drug delivery cellular toxicity. Additionally, it can be fully metabolized by the body which can minimize the chance of renal failure [8] and is hence accepted by the body. Dextrans also help in prevention of crystallization of sugar, improves moisture retention, and maintains the flavour of food products and hence they are used in the production of jams [11]. Due to the increase in the world's population, supplying fresh food to everyone must be a priority. Maintaining the quality of the fresh supply obtained, represents a major challenge for the food industry. Hence, there have been many endeavours and attempts in finding many strategies to increase the shelf life of many food products. Many storage techniques have been developed in order to extend the shelf lives of food products, such as refrigeration at high humidity, modified atmosphere packaging, UV irradiation or Vacuum sealing. But considering the time and processes involved in the production of the final product, there is a need for methods which could further help prolong the shelf life of these food products.

To aid with this issue, the use of edible films/coatings is another concept for preservation that has been receiving considerable attention [9]. Due to these reasons, the field of biopolymers and their applications has been gaining a lot of attention recently and has been extensively studied. Generally, an edible coating is nothing but a very thin layer of the desired material that is formed on the surface of the food which can be ingested and remains non-toxic to the human body. By being ingestible and from renewable sources, edible coatings represent a distinct category of packaging materials that puts itself apart from other conventional packaging materials [11]. Most of these Biopolymers or polysaccharides have a unique characteristic of forming films and gel-forming capacity. Since they have a natural tendency to act as an adhesive, they can easily form a film with the help of electrostatic, hydrophobic, or ionic bonds or other intermolecular forces such as covalent bonds (e.g., disulfide bonds and crosslinking) [10]. Hence these Polysaccharides

can further be altered structurally to fit the needs. They also are colourless and have an oil-free appearance. The film thus formed can be used to coat various fruits and vegetables. Along with other preliminary food processing techniques, application of this edible coating or film can help in increasing the shelf life of various fruits and vegetables. It offers protection from environmental factors such as heat, light, presence or absence of moisture, oxygen, dust particles, gas emission, etc [10]. They can significantly reduce Oxidative effects on food such as rancidity, darkening of surfaces and reducing dehydration.

Apart from helping in increasing shelf-life of food products, these EPS based edible film can also act as a prebiotic which in turn stimulate the growth of healthy bacteria in the gut. Combining the produced EPS with a plasticizer such as glycerol, allows us to make thin films of edible coating. This can easily be used to package food products. Besides this, the latest innovations in food packaging techniques include the development of intelligent packaging films to be able to the quality and safety of food products during storage and transportation, which is where most of the fruits and vegetables get spoilt, and to provide the extension of shelf life [10].

The purpose of this study is to understand the production, characteristics and properties of Exopolysaccharides and apply the knowledge in the field of Food industry. This study allows us to understand real life problems and to come up with innovative solutions for the same.

Since the food industry involves many inevitable processes like accumulation of raw material, food processing and it is packaging it is of utmost priority that the highest level of quality is maintained during all these steps. The finished food product must have an increased shelf life as compared to the raw products. This poses a requirement for advanced and modern food packaging techniques. To address this need, this project aims at applying the knowledge and characteristics of Exopolysaccharides onto the food packaging industry and coming up with an innovative packaging idea.

2. Materials and Methodology

2.1 Materials

Unpastuerized milk was used as the source for Lactic Acid Bacteria (LAB), which was obtained from a local cattle farmer in Bangalore, Karnataka, India. Other materials required included MRS Media (TM Media), Agar powder (HIMEDIA), Glycerol (NICE Chemicals Pvt. Ltd.) (MW 92.10), Polyethylene glycol (PEG) (Sigma Aldrich), Tricholoracetic acid (TCA) (HIMEDIA), L-Ascorbic acid (ACS Chemicals) (MW 176.12).

2.2 Methodology

The production and extraction of useful Exopolysaccharides involves many steps which include selecting a source that is rich in the desired bacteria. This is followed by increasing the production by sub-culturing it in selective media and finally, extracting it. The methodology used was the standard procedure given in [12].

2.2.1 Culturing LAB using unpasteurized milk

Unpasteurized milk was used as the source as it contains Lactic Acid Bacteria (LAB) in abundance that produce Exopolysaccharides. MRS media is a selective media that only allows the growth of LAB. Modified MRS Agar was maintained at pH 5, making it highly specific and promoting the growth of LAB. Streak plate method was used to aseptically swab by using the unpasteurized milk as the source, onto the MRS agar, which was then incubated for 48 hours. Once there were visible colonies, confirmatory and biochemical tests like Gram staining and catalase tests were performed. The cultures were then transferred to modified MRS broth which was done to make the extraction of EPS easier. The inoculated broths were then kept in the orbital shaker at and incubated at 37˚C for 48 hours.

2.2.2 Extraction and isolation of EPS

Once there was sufficient growth of LAB in the MRS broth, extraction of EPS was performed. Cooling centrifugation was performed and the supernatant was collected and further treated with TCA (trichloroacetic acid) to denature all the proteins. After treating it with TCA, it was left in the orbital shaker to homogenise the solution.

The precipitated proteins were again removed by centrifugation. It was followed by ethanol precipitation. This was again subjected to centrifugation. The pellets that settled resulted in the crude precipitate of EPS. DI water was added to this in equal volumes and dissolved.

2.2.3 Identification and analysis of EPS molecules

The composition and purity of the extracted EPS molecules was observed using LCMS (Liquid Chromatography – Mass Spectrometry) and UV – Visible Spectroscopy.

2.2.4 Preparation of film using the dissolved EPS

The EPS that had been dissolved had now been split into two portions, one of which was used to make the film by combining it with a plasticizer. Glycerol and PEG (polyethylene glycol) were employed. PEG was

used at 3 different concentrations of 5%, 10%, and 15% whereas glycerol was used at 10%, 20%, and 30% concentrations (w/v). The prepared mixture was used to coat the fruit samples by dipping them in it.

2.2.5 Lyophilization of EPS and preparation of film

The Lyophilization procedure was carried out to produce the dry EPS powder using the other half of the dissolved EPS. It ensures that all the moisture is removed to give only EPS powder. The film was then made by combining the lyophilized EPS with 30% glycerol (since it showed the best result in the first shelf-life testing). After that, the produced mixture was applied onto fruits by dipping them.

2.2.6 Testing for shelf life and interpreting the results

The shelf life of the coated fruits and vegetables were tested by checking their weights, firmness, colour and shrinkage. The fruits and vegetables used for this were banana, avocado, strawberry and broccoli. Day to day observtions were made.

2.3 Tests performed

2.3.1 UV-Visible Spectroscopy

UV Visible Spectroscopy was used to find the absorption of electrical transitions of EPS. This analysis also resulted in the purity of the extracted EPS samples. The absorbance was measured from 150nm – 850nm and the graph was analysed.

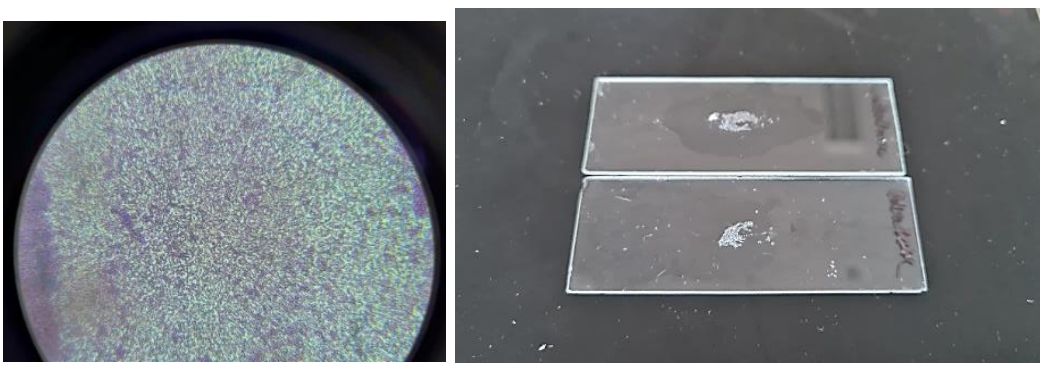
2.3.2 LC-MS (Liquid Chromatography – Mass Spectroscopy)

LC-MS is a hyphenated technique used for separation, identification, and quantification of both unknown and known compounds as well as to describe the structure and chemical properties of different molecules .The technique has a very high sensitivity which makes it appropriate for the detection of small or trace molecules in a mixture.

3. Results and Discussion

3.1 Gram Staining and Catalase test

Gram staining is a test done for the identification ofbacterias. The gram staining test showed a purple colour which indicates that the species was Gram Positive, as seen in Fig 1(a). Catalase test is one of the biochemical tests in IMViC the catalase test which came out to be negative, as seen in Fig 1(b). Since it is known that the LAB species are gram positive and catalase negative [13], it further confirms the presence of LAB colonies.



(a) (b)

Fig 1: (a) Gram staining was performed and a purple staining was seen when observed under a microscope. (b) Catalase test was performed by adding Hydrogen peroxide and was observed for air bubbles. None were seen.

3.2 UV-Visible Spectroscopy Analysis

UV Visible Spectroscopy was used to find the absorption of electrical transitions of EPS. This analysis also resulted in the purity of the extracted EPS samples. As the results showed in Fig 2(a), a strong peak occurred at around 200 nm, in the UV range. The peaks in 190-230 nm wavelength are mostly due to π–π* transitions, which are found in many functional groups like alcohols and mostly organic structures [14]. Since there was no peak observed at the 260-280nm region it can be said the EPS did not contain any proteins or nucleic acids. This would further mean that the EPS obtained from the LAB source was free of any DNA/RNA/Protein contamination.

3.3 LC-MS Analysis

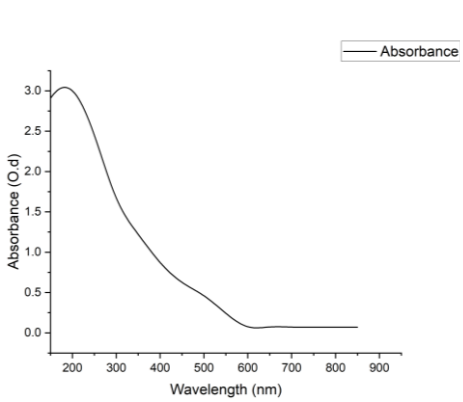
LC-MS was done in order to understand the composition and the structure of the EPS obtained. Fig 2(b) shows the graphs obtained from LC-MS analysis where the X axis represents the mass to charge ratio (or the molecular mass when the charge is 1) whereas the Y axis represents the percentage of concentration. As it can be observed from the graphs that there were very significant peaks observed. This indicates that certain molecules with the observed molecular weight was present at very high concentrations.

The identification of the molecules was done by using the NIST Molecular weight search – web book. The molecular weights obtained from the graph was searched for on the website, and molecules of the similar weights were noted to create a table of the possible molecules that can be present the EPS. This is represented in Table 1.

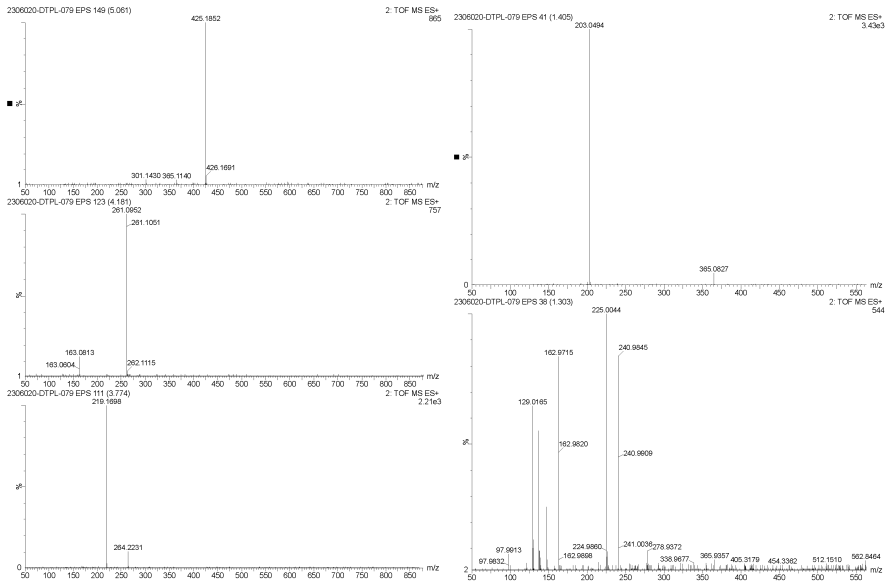
LC-MS MW	Possible molecules	MW of carb
203.04	α-D-Glucose hydrate	198.07
	D-sorbitol monohydrate	200.18
365.08	ribitol, acetylated	362.12
	Glucose, 2-methyl, acetylated	362.12
129	Malic acid	134.08
162.97	Rhamnose	164.16
225	α-D-Glucofuranose, 1,2-O- (1-methylethylidene)-	220.05
241	1,4-Pyrone-2,6-dicarboxylic acid, diethyl ester	240.06
	α-D-Mannopyranoside, permethylated	250.05
	1,5-Anhydro-4-O-acetyl- 2,3,6-tri-O-methyl-D-mannitol	248
	2,3-Di-O-acetyl-1,5- Anhydro-4-O-methyl-L-rhamnitol	246.11
425.18	Didrovaltrate	424.1

219	α-D-Glucofuranose, 1,2-O- (1-methylethylidene)-	220
	Trimethyl (1z)-1-propene- 1,2,3-tricarboxylate	216

Table 1: Shows the molecular weights of compounds obtained from LC-MS and the corresponding possible molecules which are around the same molecular weight (obtained from NIST Web Book)



(a)



(b)

Fig 2: (a) Graph obtained from UV-Visible spectroscopy, shows the absorbance of the EPS solution from 150 to 850nm (b) Graph obtained from LC-MS, the peaks indicate that the molecule of that weight is present in higher concentration.

3.4 Conducting Shelf-life test

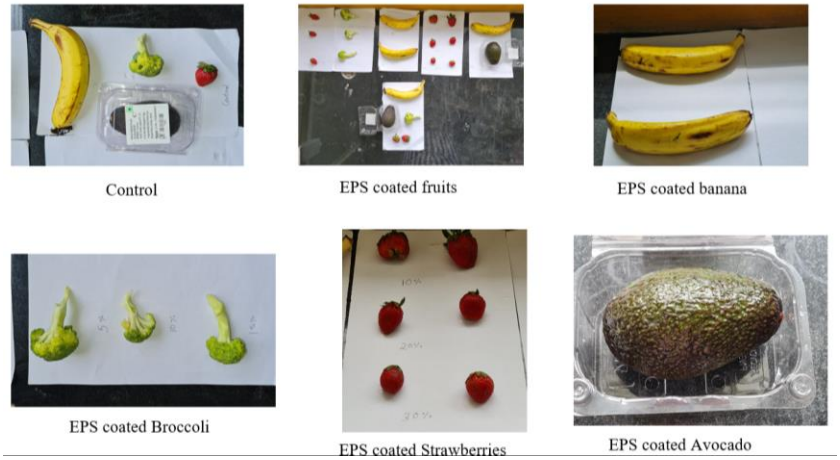
This was conducted in two parts. The first part where the dissolved EPS was mixed with the plasticizer and the second part where the lyophilized EPS was mixed with the plasticizer. To conduct the shelf-life test, banana, strawberries, avocado and broccoli were used as samples and as control. The coatings were done by dipping the samples in the prepared solution.

The dissolved EPS was mixed with PEG (Polyethylene Glycol) and Glycerol, and coated to the selected fruits. Banana, strawberries, and avocado were coated with dissolved EPS by varying concentration of glycerol. As for broccoli it was coated with plasticizer containing PEG. The observations were made over a span of 8 days. On day 1, fruits and vegetables coated with EPS dissolved in DI water and kept outside in room temperature (Fig 3(a)). No major changes were observed on day 2 (Fig 3(b)). On day 5, the strawberries showed shrinkage. Samples coated with 10% Glycerol showed the most shrinkage.

The bananas did not show any significant difference visually, but the banana kept for the control started to lose its firmness and became mushy. The banana coated with 30% Glycerol showed the best results. The

dissolved EPS with 15% PEG plasticizer showed the best results for broccoli as of day 5 as it did not show any brown colour on the surface and was still firm (Fig 3(c)).

By day 8 the other coated samples started showing signs of spoilage whereas the control rotted (Fig. 3 (d)). The EPS dissolved with 30% glycerol banana on the other hand showed promising results. As there was no significant improvement in the shelf life of other samples, use of lyophilised EPS was considered. Learning that dissolved EPS with 30% glycerol showed better results, lyophilised EPS with 30% glycerol was used and coated onto the grapes, as shown in Fig 4.



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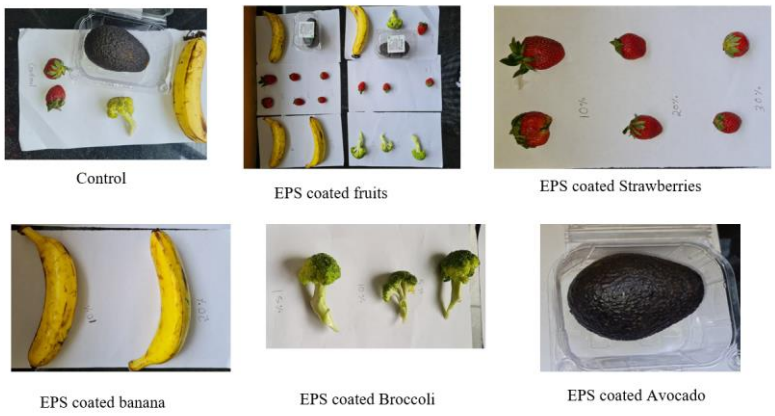

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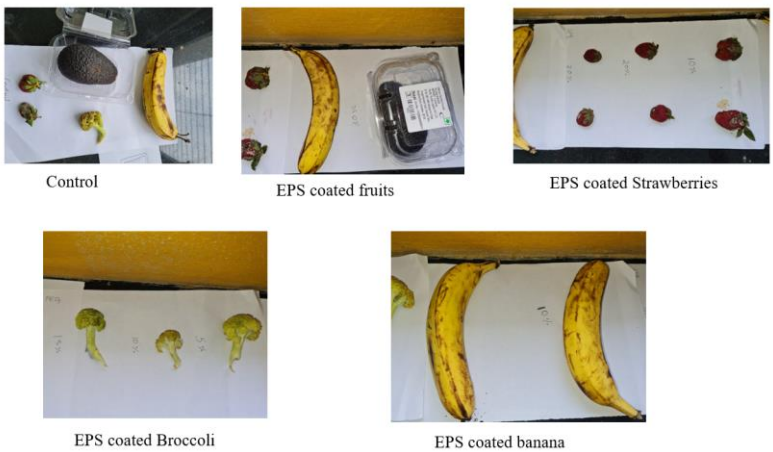


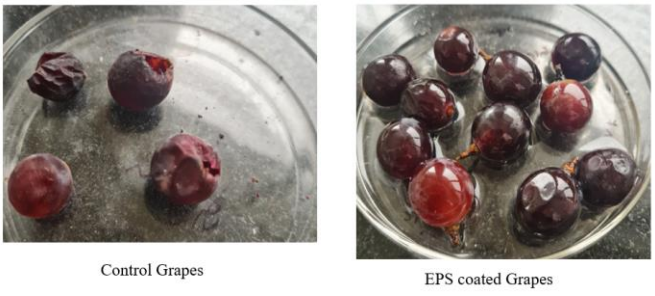
(a)

(b)

Fig 3: (a) The samples and control on day 1 (b) Observations on day 2 (c) Observations on day 5 (d) Observations on day 8

(a)

(b)



(c)

Fig 4: (a) Coated and control grapes on day 1. (b) Control grapes starting to rot and show shrinkage on day 4 and EPS coated grapes still appearing to be normal. (c) Control grapes show complete shrinkage and appear to be dry on day 8 whereas EPS coated grapes appear to be intact.

3.4.1 Changes in weigh recorded during shelf-life testing

The weight loss of fruit is an important factor for predicting the quality of fruit. It usually happens due to the migration of water from fruit to the environment (10). This poses to be an important factor to be accounted for as EPS coated fruits showed significant differences in shrinkage and moisture retention as compared to the control fruits in the previously observed results.

The weights of the grapes were recorded every 3 days (Table 2) and the percentage of weight loss was calculated (Fig 5) which was found to be 88.5% for the control grapes and 25.11% for the EPS coated grapes.

	Control	EPS coated
Grape 1	4.213g	4.010g
Grape 2	4.34g	4.227g
Grape 3	4.156g	4.354g
Grape 4	4.232g	4.402g

(a)

	Control	EPS coated
Grape 1	3.571g	3.968g
Grape 2	3.611g	4.198g
Grape 3	3.589g	4.301g
Grape 4	3.412g	4.379g

(b)

	Control	EPS coated
Grape 1	2.508g	3.845g
Grape 2	2.700g	4.001g
Grape 3	2.499g	4.023g
Grape 4	2.546g	4.112g

(c)

	Control	EPS coated
Grape 1	1.921g	3.327g
Grape 2	1.945g	3.599g
Grape 3	1.912g	3.798g
Grape 4	1.930g	3.854g

(d)

	Control	EPS coated

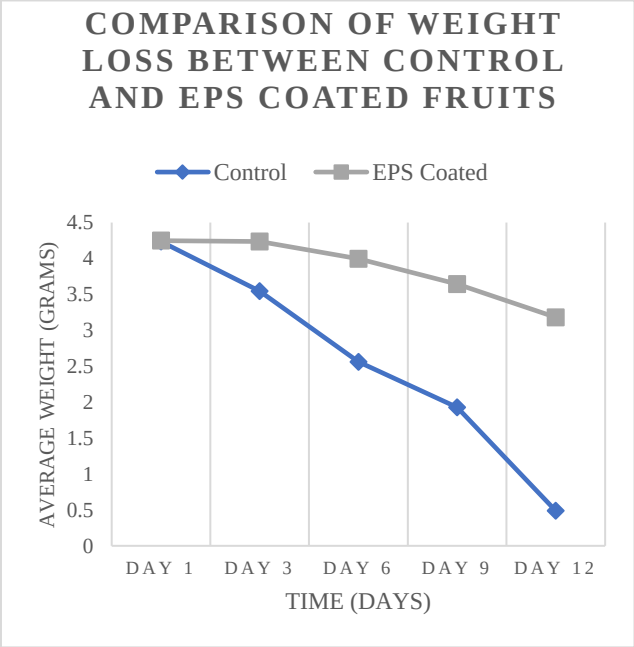
Grape 1	0.721g	2.944g
Grape 2	0.273g	2.978g
Grape 3	0.538g	3.150g
Grape 4	0.419g	3.602g

(e)

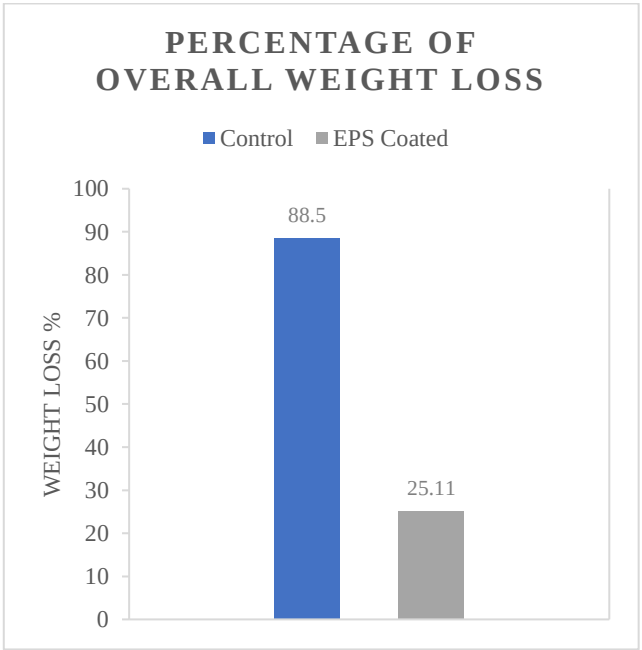
	Day 1	**Day 3**	**Day 6**	**Day 9**	**Day 12**
Control	4.235g	3.545g	2.563g	1.927g	0.487g
EPS Coated	4.248g	4.236g	3.995g	3.645g	3.181g

(f)

Table 2: (a) Weights observed on day 1. (b) Weights observed on day 3. (c) Weights observed on day 6. (d) Weights observed on day 9. (e) Weights observed on day 12. (f) Average weights of the grapes over the span of 12 days.



(a)



(b)

sFig 5: (a) Comparison between the weight loss that occurred in the control grapes and the EPS coated grapes. (b) From the data available on the weights, the percentage of weight loss was calculated and represented graphically.

The graphical representation in Fig 5(a) shows a clear distinction between the weight loss that occurred in the fruit samples over the span of 12 days. There is a significant drop in the weight of the fruits that were not coated, i.e., the control samples, whereas the samples coated with EPS had much lesser weight loss. Fig 5(b) showed an immense difference in the percentage of overall weight loss that occurred in the day 12 readings as compared to the day 1 readings. This indicated that the weight loss (which is mostly caused due to the moisture evaporation) is largely reduced by the application of EPS coating. This proves that the

application of EPS coating on fruits and vegetables helped in improving its moisture retention and other environmental effects that could further increase the rate of spoilage. EPS/ dextran films have great barrier properties which prevent the movement of water from the grapes to the atmosphere.

4. Conclusion

Exopolysaccharides (EPS) are molecules that are released by most bacteria which act as a protection for the bacteria, but these molecules when extracted can be of great value in the field of food processing. EPS based food packaging can serve as a great alternative to the current problems in the field of food processing and packaging. It allows the enhancement of shelf-life as well as add some prebiotic nutrition to the food item. This study included the production of EPS by culturing Lactic Acid Bacteria, identification, and analysis of the EPS and finally preparation of an edible film. The presented results indicated that the fruits coated with dissolved EPS (mixed with 30% glycerol) showed the best resistance to environmental factors that lead to spoilage. Amongst the fruits and vegetables used for testing, bananas showed the best results and showed the most firmness, least weight loss and the least colour change/rotting. Another batch made with lyophilized EPS showed better results than the dissolved EPS, by maintaining the samples (blue grapes) at regular environmental conditions.

The use of EPS as a coating also increased the moisture retention by about 63.4% and therefore prevented shrinkage and spoilage of the fruits and vegetables.

These results support our objectives with the efforts to create an edible film which can serve as a shelf-life extender with extra nutritional values in the form of prebiotics.

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