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Application of bacteriocins and *Aloe vera* gel as biopreservatives in edible coatings to extend the shelf life of fruits and vegetables: A review

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Abstract

The freshness of fruits and vegetables prevails for a very less time, as they continue to respire and occurrence of exchange od gases, solutes and other metabolic activities goes on after harvesting. So, the need of post-harvest is very much required to enhance their shelf life. Edible coating are defined as a very fine layer of substance made over the surface that can be eaten along, it works by modifying the environment surrounding the food commodity. It controls the exchange of gases and other metabolic processes thus helps the fruits and vegetable to perpetuates the freshness for extended period of time. The use of chemical preservatives is becoming less and less obvious with the increase in consumer awareness. So, bio preservation has gained a lot of attention. Bio preservation refers to the use of microorganism or plants or their substances to improve the shelf life. Bacteriocin produced by lactic acid bacteria are GRAS certified and have shown considerable antimicrobial effects against different food borne pathogens. *Aloe vera* gel is plant derived and do have antimicrobial activities and has shown a noticeable success for the extension of shelf life of fruits and vegetables.

Keywords: Edible coatings, *Aloe vera* gel, bacteriocins, shelf-life extension, antimicrobial, lactic acid bacteria, bio preservation

1. Introduction

Fruits and Vegetables (F&V) are imperative for good physical and mental health. They make crucial components of the balanced diet (Brookie, Best and Conner, 2018) ^[10]. F&V contain ample amount of all the vital nutrients such as vitamins, minerals, organic acids, antioxidants and polyphenols. People having F&V in diets in a considerable amount are known to be less prone to certain diseases such as diabetes, obesity, cardiovascular diseases, and some type of cancer (Volpe, Cavella & Torrieri, 2019)^[64]. Fruit and vegetable spoilage is affected by the number of external factors (Valdes et al., 2017)^[62]. Post-harvest contamination could originate from microbial, physical or physiological sources (Palou, Valencia-Chamorro & Perez-Gago, 2015) [54]. Fresh fruits are usually accounted as microbiologically safe for consumption. However, they get contaminated due to various reasons such as air, irrigation water, animals and during harvest and post-harvest (Iglesias *et al.*, 2018)^[29]. Intrinsic factors such as sugars, water activity, pH, enzymatic activity play critical role in microbial growth and degradation of fruits and vegetables (Fan and Song, 2013) ^[21]. With the passing time overall quality of the fruit start getting worse affecting the flavor, visual and nutritional attributes (Nunes & Emond, 2007) [48]. Fruits are easily spoiled because of its high-water content; loss of water affects the nutritional profile makes the fruit look mushy results in flabbiness and withering. Various Post harvest treatments such as Controlled atmosphere packaging (CAP), Modified atmosphere packaging (MAP) and edible coatings which works on the principle of suppressing the rate of the metabolic processes taking place in fruits and vegetable to enhance the shelf life. These treatments lower the rate of the respiration and transpiration, eventually resulting in the gradual decrease in the moisture loss (Dhall, 2013)^[18].

An Edible coating is widely used ongoing method for the extension and improvement of the shelf-life of the vegetables and fruits that work as an obstacle against the exchange of gases, moisture loss and microbial attacks by manipulating the environment inside the food stuff (Chiabrando and Giacalone, 2016; Puttalingamma, 2013) ^[14, 56]. An edible coating having antimicrobial characteristics are applied to fruits by using proteins, lipids or polysaccharides biopolymers (Valdes *et al.*, 2017). An optimal consumable coating is that which does not totally block exchange of gases but delay the degradation process with minimal effect on the

quality of the product. This edible coating controls the change in the aroma, water content, taste, exchange of oxygen and carbon dioxide from the eatables (Athmaselvi, Sumitha & Revathy, 2013)^[6]. The formation of an edible film could be foccused on fruit wax, fruit (apple puree), vegetable starch (pumpkin), gum Arabic, gum cordia, chitosan, pectin, carboxymethyl cellulose, whey protein isolate, alginate, and *Aloe vera* gel with antimicrobial properties (Mohapatra *et al.*, 2013)^[41].

A new and effective technique is gaining popularity to extend shelf life of fruits and vegetables i.e., bio preservation. It refers to the tactics that are executed to extend shelf life and improve food safety by friendly microorganisms or their products or any plant-based compounds such as mucilages, essential oils etc. It is also fairly accepted by consumers due to its total natural ingredients and research is rapidly engaged in the field of bio preservation (Narsaiah *et al.*, 2014; Sharma *et al.*, 2011)^[47, 59].

Bacteriocins are the antimicrobial compounds that are produced by lactic acid bacteria (Ghanbari et al., 2013)^[22]. Lactic acid bacteria (LAB) are Gram positive rods or cocci, non-aerobic but aerotolerant, they can ferment carbohydrates for energy and Lactic acid production (Prada et al., 2007)^[54]. LAB has the GRAS (Generally Regarded as Safe) status. Bacteriocins produced by LAB are rapidly degraded by human digestive enzymes due to which they have immense potential in food safety and preservation (Ghanbari et al., 2013) ^[22]. Most common genera that used for bacteriocins production are; Carnobacterium, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus and streptococcus (Altuntas, 2013) ^[5]. A variety of bacteriocins has been purified and found to show bacteriostatic and bacteriocidal activity against different food deteriorating and pathogenic microbes (Jeevaratnam, Jamuna and Bawa, 2005)^[30].

The antibacterial activity of acidocin B produced by *Lactobacillus acidophilus* and *Plantaracin*

Aloe vera (AV) gel contains huge number of bioactive compounds such as antioxidants, mannans, lectine, antron etc. It is also environment friendly (Ergun and Satici, 2012) ^[20]. AV gel is also found to be nutrient rich; it contains vitamins, amino acids, minerals, sugars and enzymes (Mohebbi *et al.*, 2012) ^[42] and has wide applications in fruits and vegetable preservation. AV gel has been used as coating material for sweet cherry (Martinez-Romero., 2006) ^[38], tomatoes (Chauhan *et al.*, 2015; Kumar *et al.*, 2018) ^[12, 33], and table grapes (Valverde *et al.*, 2005) ^[63] to extend their shelf life. AV gel has been used in combination with other components such as AV gel and ascorbic acid for strawberries and AV gel and shellac is used in synergism to extend shelf-life tomatoes (Chauhan *et al.*, 2015) ^[12].

Edible Coatings

An edible coating can be defined as a very fine semipermeable layer of substance that can be eaten along made over the surface of fruit and vegetable with purpose to maintain its quality for longer period of time. These days edible coating have gathered enough attention because of various disadvantages related to the synthetic coating (Delgani, Hosseeini and Regenstein, 2017) ^[17]. Edible coatings work on the same principle to that of modified atmosphere packaging (MAP). It modifies the internal environment of the fruit and vegetable by altering the rates of various metabolic processes and moisture loss. Edible coating functions as an obstacle against the movement of oxygen, carbon dioxide, solutes and moisture. It also prevents the fruits and vegetables from the direct contact with the microbes and works as a protective layer (Hasan *et al.*, 2020; Chaudhary *et al.*, 2020) ^[24, 11]. The primary focus of edible coating is to protect the product against any biological, physical and mechanical injury (Orgel Zamudio *et al.*, 2017) ^[52]. An optimal consumable coating is that which does not totally block exchange of gases but delay the degradation process with minimal effect on the quality of the product. The edible coating controls the change in the aroma, water content, taste, exchange of oxygen and carbon dioxide from the eatables (Athmaselvi *et al.*, 2012) ^[6].

The effectiveness and the stability of the edible will rely on its composition. The most common components of the edible coating are basically obtained from the natural sources, and can be categorized into polysaccharides, proteins, lipids and composites (blend of one or more of these biolpolymers) (Dhall, 2013; Mannozi et al., 2017) ^[18, 37]. Among polysaccharides cellulose, chitosan, starch, pectin, pullulan, and different gums have been used. There are enough number of researches where different proteins such as gelatin, corn zein, wheat gluten, whey protein and albumen have been used. Same applies for the lipids and studies are available for the application of various waxes, fatty acids, acetoglycerides are available. The application of edible coating is performed by spraying, dipping, brushing and extrusion method. Dipping and brushing remain the most favored methods while other two are used only at some special cases only (Raghav, Agarwal and Siani, 2016)^[57]. Edible coatings can be enriched with or composed of variety of antimicrobial compounds, antimicrobial agents, colorants, flavoring compounds, nutrients and essential oils from spices and herbs (Hassan et al., 2018; Tesfaye et al., 2017)^[25, 60].

Biopreservation

The term bio preservation refers to the techniques that are executed to extend shelf life and improve food safety by friendly microorganisms or their products. Increasing awareness regarding food safety has elevated concern among consumers. The implementation of biological methods to preserve the food is more acceptable approach to reduce the risk of food born ailments arise due to deterioration of food by microorganisms and to satisfy the consumers (Aluntas, 2013)^[5].

Lactic Acid Bacteria

Lactic acid bacteria are a large group of bacteria that belongs to phylum firmicutes. These are Gram positive cocci or rod shaped strictly fermentative but aero tolerant microorganisms (de Macedo et al., 2013)^[15]. Based on metabolic pathway categorized into homofermentative LABs are and heterofermentative types. The homofermentative LABs comprises Lactobacillus, Lactococcus, Pediococcus and Streptococcus species that produce two molecules of lactate for each molecule of glucose consumed via EMP pathway while heterofermentative LABs produce variety of products where carbon dioxide, ethanol and lactate are the major products. Species of Leuconostoc and Weisella are included in this category and majorly they metabolize sugars via 6phosphogluconate/phosphoketolase pathway (Hitendra et al., 2016) ^[27]. Lactic acid bacteria are known to produce bacteriostatic and bactericidal agents that have different modes of actions. Lactic acid and acetic acid production lower the pH of the food that restricts the growth of many

mesophilic bacteria. LABs have antagonist character because they synthesize bacteriocins and non-proteinaceous low molecular mass compounds (LMMC) which have antimicrobial properties (Hitendra *et al.*, 2016)^[27].

Bacteriocins

Bacteriocins are ribosomally synthesized antimicrobial peptides that vary in structure and are released extracellularly. Bacteriocins are rapidly hydrolyzed by proteases in the human digestive tract thus they are in generally regarded as safe (GRAS) by US FDA and can be used in food preservation (Naidu et al., 2006; O'Connor et al., 2020) [46, 50]. Most commonly used genera for the production of bacteriocins are Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus and Propionibacterium. For the preservation of food products different bacteriocins are employed individually or in combination of two or more. Amongst many bacteriocins only few are tested for preservation of fruits and vegetables such as bificin, bovicin, enterocin, nisin and pediocin (de Souza Barbosa et al., 2015) ^[16]. Adequate amount of data is available for the use of bacteriocins as food additives but little amount of data is available regarding the edible coating of bacteriocins (Narsaiah et al., 2015)^[47].

Bacteriocins produced by LABs are mostly cationic, hydrophobic or amphiphilic and heat stable peptides having 20 to 60 amino acid residues (Naidu et al., 2006) [46]. The bacteriocins are classified into four classes. Class I (Lantibiotics) they act by creating pores in bacterial membranes or by inhibiting some specific enzymes, Nisin, ancovenin, cinnamycin and duramycin are are the few examples of this class (O'Connor et al., 2020; Naidu et al., 2006)^[50, 46]. Class II (Non-lantibiotics) also known as small heat stable peptides. Potent anti-listerial activity is main feature of class II bacteriocins. Class II bacteriocins are further divided into class IIa to IIe. Pediocin, enterocin, diversin V41, plantaracin, sakacins, carnobacteriocin A and enterocin B all belong to this class (Naidu et al., 2006). Class III (Large Heat Labile Proteins) have size greater than 30kDa and are heat labile. Not much information is available regarding the characteristics of these bacteriocins. Helvetin and enteroysin that are produced by Lactobacillus helveticus and Enterococcus faecium respectively (Kumariya et al., 2019; Naidu et al., 2006) [35, 46].

Class IV (Complex Proteins) consists of lipoproteins or glycoproteins which require nonprotein compounds for antimicrobial activity (Kumariya *et al.*, 2019; Naidu *et al.*, 2006) ^[35,46].

Mode of Action of Bacteriocins

Antibacterial peptides produced by bacteria provide immunity and defense against pathogenic bacteria. Although mechanism of bacteriostatic and bactericidal activity of every class differs from each other but all the bacteriocins are more effective against Gram positive bacteria than Gram negative bacteria due to the presence of external membrane which inhibit the action (Kumariya *et al.*, 2019; Parada *et al.*, 2007) ^[35, 54].

Different mechanisms include pore formation, inhibition of spore germination and altering enzymatic activity. It has been observed that for many bacteriocins the primary target is cytoplasmic membrane of the sensitive cells where they perform the disruption of the protein motif force (PMF) through the short lived, non-selective transmembrane pores that leads to the release of intracellular fluid and results in the death of the cell (Xu *et al.*, 2020; Ibarra-Sanchez *et al.*, 2020) [65, 28].

Bacteriocin as bio preservative

Antimicrobial properties of the lactic acid bacteria (LAB) allied to their safe use in traditional fermented foods; this makes them very attractive to be used as bio preservative (O'Connor *et al.*, 2020)^[50]. Bacteriocins produced by LAB have potent industrial applications, since majority of bacteriocins producing LAB are natural food isolates, so they are well suited for food applications (Gong *et al.*, 2010)^[23]. Bacteriocin-producing LAB are found to be highly potent in the bio preservation of foods of plant origin, especially minimally processed foods such as prepackaged mixed salads and fermented vegetables. Among various bacteriocins of LAB nisin A and its natural variant nisin Z has been effective against microorganisms causing spoilage (Zacharof & Lovitt, 2012)^[66].

Shelf life of fresh papayas has increased by 2-21 days when pediocin produced by Pediococcus pentosaceus was used in combination with 2% alginate for coating (Narsaiah *et al.*, 2015)^[47].

The effect of enterocin AS-48 on *Listeria monocytogenes* CECT 4032 was studied on various fruits stored at temperatures -20, 6, 15, 22° C. Enterocin AS-48 fully inactivated *L. monocytogenes* in the blackberries, raspberries and strawberries for 2 days at temperature 15 and 22 °C and for 7 days in blackberries and strawberries stored at 6 °C. It also resulted in the reduction of viable count of sliced melon, water melon, kiwi and pears (Molinos *et al.*, 2008a) ^[44].

Minimally processed Mangoes were tested against *Listeria monocytogenes* and *Staphylococcus aureus* using Nisin incorporated cellulose film. After 4 days *L. monocytogenes* count decreased below the detection level and reduction of 6 log units was observed for *S.aureus*. However, both the samples were initially inoculated with 107 CFU/g of viable cells of *S.aureus* and *L.monocytogenes* (Barbosa *et al.*, 2013)

Enterocin AS-48 tested for the decontamination of *Bacillus cereus* and *Bacillus weihenstephanesis* on various raw vegetables such as Alfalfa, green asparagus, and soybean sprouts. After washing vegetables with enteriocin AS-48 solution, the microbial count was decreased by 1.0-1.5 and 1.5-2.38 log units respectively for *B.cereus* and *B. weihenstephanesis*. During further storage at 6° C the bacteriocin was able to reduce the viable population below the detection level (Molinos *et al.*, 2008b) ^[45].

Tumbarski *et al.*, (2020) ^[61] studied the effectiveness of Bacillus methylotrophicus BM47 bacteriocin in the pectin based edible coating for the shelf life extension of fresh blueberries. Fruits were analyzed for the change in Titratable acidity, total soluble sugars, pH, decay percentage, antioxidant and total phenols over the storage of 16 days at 4° C and 75% RH. Coating was found to be effective in reducing the decay percentage, weight loss and total soluble sugars. However, no effect was noted down on the pH, antioxidant and total phenols due to pectin + bacteriocin coating.

S. No.	Bacteriocin	Source microbe	Fruits & Vegetable	Storage temp. (° C)	Observations & Results	References
1.	Enterocin	Enterococcus	Blackberries, Raspberries,	-20,6,	Inactivation of Listeria	Molinos et
	AS- 48	faecalis	Strawberries, Melons	15, 22	monocytogenes, increase in shelf life	al. (2008a)
2.	Enterocin AS48	Enterococcus faecalis	Alfalfa, green asparagus, soybean sprouts	6	Reduction in the count of <i>B.cereus</i> and <i>B.weihenstephanesis</i>	Molinos <i>et</i> <i>al.</i> (2008b)
3.	Nisin	Lactococcus lactis	Mango	5	Reduced the count of <i>L.monocytogenes</i> and <i>S.aureus</i>	Barbosa <i>et</i> <i>al.</i> (2013)
4.	Pediocin	Pediococcus spp.	Рарауа	4	Increased shelf life from 2 to 21 days	Narsaiah <i>et al.</i> (2015)
5.	Bacteriocin	Lactobacillus acidophilus	Apple	25	Inhibition of microbial spoilage, maintained physiochemical properties	Bhatia <i>et al.</i> (2016)
6.	Nisin	Lactobacillus Lactis	Tomato	4, 10 and 25	Inhibited the growth of <i>Listeria</i> monocytogenes	Oladunjoye et al. (2017)

Table 1: Application of bacteriocin on different fruits and vegetables

Aloe vera Gel

Plant based edible coatings are contemplated as safe and compelling alternative for enhancement of the shelf life and quality preservance. These coatings successfully obstruct the loss of weight and exchange of gases (Mahajan *et al.*, 2018)^[36].

Aloe is a tropical or subtropical plant. That is usually characterized by lance shaped leaves with jagged edges and sharp points. Aloe vera gel is resinous material obtained from aloe plant (Aloe barbadensis Miller). Aloe vera gel has multidimensional applications. Aloe vera gel is gifted a vast resource of carbohydrates in form of glucomannans, antioxidants and secondary metabolites with biopreservative functionality. The Aloe vera gel has moisture conditioning and biopreservative properties which are required for formulating edible surface coating (Chauhan, Gupta & Agarwal, 2014)^[13]. Aloe vera gel coatings are also known to impede the activity of various enzymes such as polyphenol oxidases and peroxidases, it also reduces the chances of phenolic oxidation and browning. Aloe vera gel is made up of number of components such as polysaccharides, salicyclic acid, amino acids, glycoproteins, vitamins, phenolic compounds and enzymes. It exhibits antifungal, antibacterial and anti-inflammatory properties (Kumar and Bhatnagar, 2014) [34].

Parenchyma cells of leaves include mucilaginous clear gel, which is applied as Aloe vera gel. Aloe vera gel contains number of nutrients such as vitamins, fatty acids, sugars, minerals and various enzymes; therefore, it is used as functional ingredient for health benefits. The gel acts as barrier to oxygen and carbon dioxide, and moisture, thus decreases the consequences of weight loss, browning, softening, and growth of yeast and mold. The presence of number of antimicrobial compounds in the gel makes it prevent the decay (Valverde et al., 2005)^[63]. The preservative action of Aloe vera gel coating has been reported for Mango (Shah and Hashmi, 2020) [58], Litchi (Ali et al., 2019) [2], Sapodilla fruit (Khaliq, Ramzan & Balouch, 2019)^[32], Lotus root slices (Ali et al., 2019)^[3] and Peach (Hazrati et al., 2017) ^[26] alone or in combination with other additives. "Granny Smith" apples coated with Aloe vera gel (5% and 10%) has shown effective results by reducing weight loss, delaying the reduction in soluble solids and titratable acidity and impending change in their appearance (Ergun and Satici, 2012) ^[20]. Different concentrations Aloe vera gels were used for sweet cherry, tomatoes, and table grapes to extend their shelf life and maintain their quality parameters (Martinez-Romero., 2006; Chauhan et al., 2015; Valverde et al., 2005) [38, 12, 63]

Parven *et al.*, (2020) ^[55], studied the effect of *Aloe vera* gel as an edible coating to prolong the shelf life of papaya with and without polythene bags stored at temperature of $25 \pm 2^{\circ}$ C and 80-85% of relative humidity. Results depicted that *Aloe vera* gel alone without the polythene bag was found to be most effective in prolonging the shelf life of the papaya up to 14 days. *Aloe vera* gel coating not only delayed the process of color development but also there was less loss in the weight loss and moisture content by 13.2% and 28% after 12 days of the storage. There was reduction in total soluble solids by 3% and disease severity got reduced by 29%. Fruits coated with *Aloe vera* gel and stored in the polyethene bags experienced the drastic quality loss, so it should be avoided.

Mohammadi *et al.*, (2020) ^[40], investigated the effect of *Aloe vera* gel coating solely and in conjugation with two concentrations of basil oil at 500 μ L/L and 1000 μ L/L on the shelf life of strawberries stored at temperature of 4°C and relative humidity of 85% for up to 12 days. All the coatings successfully reduced the rate of weight loss, fungal growth and rate of softening of the fruit. Other attributes such as hue color, sensory attributes, flavor index and total acidity remain maintained. The effectiveness of the coating was maximum for 1000 basil oil concentration followed by 500 and then *Aloe vera* gel.

The study was done to investigate the effectiveness of *Aloe vera* gel coating to extend the shelf life of fig fruit that were stored at 5°C for 10 days in the macro perforated bags. The factors considered to evaluate the effectiveness were firmness of the fruit, weight loss and total soluble solids. Results showed that coating maintained and improved the quality of fruit during the period of 7 days storage. The weight and the firmness of fruit were maintained during the storage period (Allegra *et al.*, 2020)^[4].

Khaliq *et al.* (2019)^[31], studied the effectiveness of *Aloe vera* gel enriched with garlic essential oil coating to control the fungal disease anthracnose and the maintenance of quality of banana after harvesting. Fungus *Collectotrichum muase* is the main causative agent of this disease. Two concentrations of garlic oil (0.05% and 0.1%) were used in combination with *Aloe vera* gel, and in one case only *Aloe vera* gel is used as coated. Coating with 0.1% garlic oil + *Aloe vera* gel was found to the most effective in controlling the fungal disease, there was decrease in the germination of spores by 91.2% and mycelial growth by 87.7% in the 15 days of the storage of banana. Coating resulted in the delay in any changes in Total soluble solids, weight loss, titratable acidity and the firmness loss. However, the total phenolic content and total antioxidant activity witnessed the enhancement in their values.

S. No.	Fruits & vegetable	Storage Temp. (° C)	Observation and Results	References
1.	Table grapes	1	Reduction in weight loss, moisture loss, firmness loss	Valverde <i>et al.</i> (2005) ^[63]
2.	Sweet Cherry	10	Reduction in microbial population, delay in color change, softening	Martinez- Romero et al. (2006) ^[38]
3.	Button mushroom	10	Increase in shelf life and maintained hardness.	Mohebbi et al. (2012) ^[42]
4.	Apples	2	Delay in quality losses and increased shelf life	Ergun and Satici. (2012) ^[20]
5.	Kiwifruit	4±1	Retention of quality and sensory attributes	Benitez et al. (2013) [8]
6.	Mango	31	Prolonged shelf life by delaying ripening	Ajeethan and Mikunthan, (2016) ^[1]
7.	Cucumber	34	Increase in shelf life and consumer acceptance	Nwosu et al. (2016) ^[49]
8.	Plum	20	Delay in ethylene production and respiration rate reduction	Martinez- Romero et al., (2017) ^[39]
9.	Tomato	25	Prevention in fungal growth, increased shelf life	Kumar <i>et al.</i> , 2018

Table 2: Application of Aloe vera gel in different edible coatings

Conclusion

Edible coatings are renewable, natural and cheap. There are vast variety of substance still unexplored for the fabrication of edible coatings. Keeping in mind about bio preservation, bacteriocins obtained from different microbial strains are not only ecofriendly but have also shown promising results in maintaining the quality and shelf life for prolonged period of time. *Aloe vera* gel still being the thrust area and lot of studies are carrying out there, but there are still possibilities of exploring the *Aloe vera* gel with other bio preservation techniques. So there is need to be paid greater attention to bio preservation and development of herbal coatings to avoid the consequences of the chemical preservatives and synthetic edible coatings.

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