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Shelf life extension of fresh berries using essential oil based nanoemulsions

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Abstract

Berries already have a “health halo” that is in part due to the widespread acceptance of the health benefits of fruit and the fact that they are a delicious and popular way to enhance dietary intake of fruits and vegetables. However, due to their delicate skin, high moisture, and abundant nutrition, they are highly sensitive to decay and deterioration during the postharvest phase. Thus there is a need to preserve their postharvest attributes during storage. In this situation, essential oils have been researched for their potential to preserve berries instead of the customary synthetic preservatives. However, their application as natural preservatives is constrained by their high volatile nature, strong sensory qualities, low solubility in water and instability to heat, oxygen, and moisture. The development of nanoemulsion offers solution to this issue. By forming a strong barrier that increases antibacterial qualities, lowers water loss, slows respiration, and prevents loss of firmness, nanoemulsions increase the functional properties of edible coatings that integrate essential oils and thereby lengthen the shelf life of berries. This paper summarises how essential oil nanoemulsions used in edible coating matrix ameliorates decay and deterioration of berries during post-harvest period and suggests utilizing them to preserve berries.

Keywords: Berries, edible coatings, nanoemulsions

Introduction

Berries are characterized as fleshy fruits with a cartilage-like endocarp that contains seeds, and can be comprised up of either simple fruits (blueberries), or composite fruits (blackberries or raspberries) (Kumar *et al.*, 2018) ^[1]. Berries are exceptionally abundant in antioxidants thus imparts significant health advantages (Sobekova *et al.*, 2013; Okatan, 2020) ^[2, 3]. Berries contain a variety of antioxidants, colourants, tannins, vitamins, and minerals. Berries contain bioactive compounds such as phenolics, flavonoids, and anthocyanins (Skrovankova *et al.*, 2015) ^[4]. Some berries are uniquely coloured red, purple, or blue thanks to anthocyanins, which are flavonoid pigments (Echegaray *et al.*, 2020) ^[5]. Berries are high in dietary fibre, significant minerals, and vitamin C yet low in calories, fats, and salt. The sweetness of most berries is increased by the presence of carbohydrates like sucrose, glucose, and fructose (Ferremi *et al.*, 2016) ^[6]. One factor that has helped the markets for fresh berries grow is the realisation that berries are abundant in healthy phytochemicals that may improve human health and offer disease protection. By far, the most often consumed fresh berry is strawberry. By volume, the next-largest retail market is for fresh blueberries. Due to intense marketing of the fruit's beneficial health properties, blueberries have grown in popularity. Although fresh blackberries and raspberries are substantially smaller in size, their popularity has increased quite quickly (Sobekova *et al.*, 2011) ^[2].

Nevertheless, berries lack a protective peel and are extremely perishable in nature due to the physical, chemical and microbial damage of the postharvest produce. According to estimates, inefficient postharvest processing and storage results in a loss of 25–50% of the total yield of fruits and vegetables produced (Nunes, 2011) ^[7]. Microbial infections are the primary cause of fruit decay at the post-harvest stage. Inherent fruit characteristics including high nutritional value, acidity, and pH typically provide optimal conditions for microbial growth; nevertheless, because of their acidity, yeasts and moulds flourish more than bacteria. Food spoilage not only puts customers' health at risk but also wastes a significant sum of money owing to post-harvest losses. Different microorganisms that cause food to degrade have the capacity to create toxins that, when swallowed, cause food poisoning. The health of consumers and the economies of exporting countries are both impacted by mycotoxin contamination of fruits, according to Fernández-Cruz *et al.*, (2010) ^[8]. Foodborne illnesses caused by the release of toxins from invading microorganisms is an increasingly important public health issue. Each year, millions

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of people die from diarrhea caused by the consumption of contaminated food, thus the growth of microorganisms in food is an issue of global concern. However, because of low pH, berries are rarely linked to outbreaks of foodborne illness caused by bacterial species. Bacterial pathogens are occasionally present on berries. Berries are more susceptible to fungal spoilage because of their acidic nature and low pH. Several fungal species produce secondary metabolites known as mycotoxins. Ochratoxin A, aflatoxins, alternaria, and patulin toxins are among the mycotoxins most frequently produced by the fungi that can be found on berries and other fruits. The US Food and Drug Administration (FDA) considers aflatoxins, the Aspergillus toxins, to be the most common contamination of food during storage and transport. Aflatoxin is additionally categorized by the International Agency for Research on Cancer as a group 1 human carcinogen (WHO-IARC). Another potent carcinogen categorized as a 2B cancer compound is the phenylalanine dihydro isocoumarin derived ochratoxin A toxin, produced by many aspergillus and penicillium species. This toxin is known to cause kidney and urothelial cancer. In addition, it is also known to damage the human brain (Fernández-Cruz *et al.*, 2010) [8].

Therefore, it becomes imperative to preserve berry fruits against the microbial, physical and chemical degradation during the postharvest period. The plastic based packaging for the protection of berries is the widely used method to protect berries from the postharvest damage. However, due to rising consumer awareness about the harmful effects of plastics on environment, research has been shifted towards the production of environment friendly packaging. Since edible coatings are environmental friendly and biodegradable, they have become a potential alternative. With the incorporation of essential oils with antimicrobial components, microbial damage can be attenuated. Furthermore, the fabrication of nano-emulsified essential oil edible coatings offers desired advantages which enhance the shelf life of berries.

Physiological disorders of berries

Berries have a short shelf life due to a number of factors, including their delicate texture, which predisposes them to physical and microbial damage; a high rate of water loss, which causes skin and flesh to shrivel and shrink; a greater propensity to microbial degradation; and an accelerated metabolism rate, which enhances respiration as well as transpiration mechanisms. These elements cause significant postharvest losses, with up to 25–50% of the total yield going to waste (Nunes, 2011) [7]. Berries are more vulnerable to postharvest fungus because of higher water activity, favourable pH (3–4) and nutrient richness that serve as growth factors for microorganisms. Typical fungal species found on berries are Alternaria, Botrytis cinerea, Colletotrichum, Fusarium, Cladosporium, Rhizopus, and Penicillium (Bell *et al.*, 2021) [9]. Furthermore, the three most prevalent harmful bacteria discovered in berries are *Salmonella typhimurium*, *Listeria monocytogenes*, and *Escherichia coli*. Postharvest losses may be considerable when conditions are favourable for disease growth, reaching in some cases rates of 80–90%. Due to their big cells and fragile cell walls, berry fruits like strawberries are particularly sensitive to mechanical injury (abrasions, wounds, bruising, and juice leakage). The surface of the berries might suffer from mechanical damage which in turn causes the fruit to undergo degradation (Aliasgarian *et*

al., 2015) [10].

Postharvest decay of fruits is the biggest reason of economic losses even in areas where advanced and more sophisticated postharvest conservation strategies are implemented (Barkai-Golan, 2001) [11].

Strategies to avoid berries from deteriorating

Before storage, postharvest berries are subjected to washing and quick cooling to maintain fruit quality and decay. Currently, the postharvest berry preservation strategies implemented include storage at low temperatures, use of chemical fungicides and preservation under modified environment such as storage with high CO₂ concentrations. These use of these methods however is not encouraged because of several drawbacks. Washing of berries before selling is not advisable as it may render the berries skin dry, delay precooling and increase the chances of microbial attack. Likewise, the use of synthetic fungicides is additionally discouraged due to negative effects on the environment and food safety (Horvitz *et al.*, 2017) [12]. The berries can be stored at low temperatures under ideal storage circumstances to extend their shelf life because they are resistant to chilling damage. Despite the significant impact that low temperature storage can have on the shelf life of berries, conflicting findings can be found in the literature. Following storage in CO₂ enriched environments, several authors found altered chemical properties such pH change, variations in total soluble solids, altered organic acid composition, titratable acidity, and fermentative metabolites (Holcroft *et al.*, 1999; Sanz *et al.*, 1999; Gil *et al.*, 1997) [13, 14, 15]. Therefore, research and focus have been shifted towards the production of more environment friendly packaging. Edible packaging appears as a viable solution to this problem since they are biodegradable, non-toxic, renewable, and sustainable. Numerous edible materials can be used to create edible packaging, which is safe for human consumption. Biopolymers, which can be made from microorganisms, bio derivative monomers, and biomass, are the renewable basic materials utilized in edible packaging. For the creation of edible packaging, polysaccharides, proteins, lipids, and composites are the biopolymers that are most frequently employed.

The necessary components in edible coatings

Polysaccharides

The widely used polysaccharide biopolymers include chitosan, alginate, starch derivatives and gums. These biopolymers are frequently employed because they offer resilient and stable coatings (Chen *et al.*, 2016) [16]. The primary advantage of coating strawberries with a polysaccharide based edible coating is the prevention against oxidative damage. Oxidative damage can be prevented since coatings shield the membrane and therefore helps in the retention of antioxidant enzymes. Additionally, it preserves the nutritional value of the strawberries by preserving their ascorbic acid and total phenolic content.

Proteins: Proteins extracted from whey, wheat, gelatin, casein, seen and soy are commonly employed as edible coatings in food packaging sector. Globular proteins are in particular advantageous as a coating material because they have high solubility in water as well as in saline solutions (Hassan *et al.*, 2018) [17]. Protein bio polymers provide strong barrier properties against oxygen, can generate stable gels and thus

improve the transportation and availability of active components from coating matrix to fruit (Sahraee, Milani, Regenstein, and Kafil, 2019) [18].

Composites: In most cases, coatings composed of just one biopolymer are insufficient to offer adequate protection against deterioration. A combination of different biopolymers as well as the addition of other elements are required to boost product protection and market value. Therefore, composite polymers are created to overcome the limits of a single biopolymer. For instance, polysaccharides lack effective moisture barrier qualities yet provide good barrier capabilities against oxygen and carbon dioxide. Lipid-based materials, on the other hand, have poor mechanical properties despite being great moisture barriers since they are hydrophobic. Proteins, on the other hand, are hydrophilic by nature yet have high mechanical properties (A. Pascall, 2012; Nei *et al.*, 2019) [19, 20]. To achieve sufficient mechanical strength and selective permeability for edible coatings, the mixed biopolymers' amounts must be controlled (Zikmanis *et al.*, 2021) [21].

These can be converted into various kinds of films and coatings by altering the biopolymer's thickness. Because they are sprayed directly to the food's surface, coatings are a crucial component of packaged foods (Trajkovska Petkoska *et al.*, 2021) [22]. According to Ju *et al.*, (2019) [23], the edible coating is described as the application of a thin liquid layer of edible material over the food surface. Food businesses frequently use fluidized bed processing, panning, dipping, and spraying as coating techniques (Andrade *et al.*, 2012) [24].

Methods of application of edible coatings

Dipping: The steps that make up the dipping method of coating are as follows: The food to be coated is first immersed steadily in a coating slurry, and then given time to dwell. Dwelling makes sure that the coating mixture is completely wetted and interacts with the surface to be coated. (Valdés *et al.*, 2017) [25] As a result, the combination deposits itself in a thin layer on the food's surface. During the evaporation

process, extra liquid sinks and is cleared from the surface. The covered food must then be dried, either naturally or using a dryer, to complete the process. (Andrade *et al.*, 2012) [24]

Spraying method

Spray coating allows different aqueous solutions or suspensions to be deposited over the surface of food in thick or thin layers. The growing interest in using this approach in various industries is related to how inexpensive it is to produce a finished good of excellent quality. The liquid coating material is applied to the food surface in the form of droplets using a set of nozzles. The capacity to produce coatings that blend hydrophobic and hydrophilic elements is one advantage of spray coating. (Martin-polo *et al.*, 1992; Bosquez- Molina *et al.*, 2003) [26, 27].

Panning method

A sizable pan that rotates automatically is filled with the object to be coated. To guarantee a uniform coating solution distribution, the solution for the coating is either applied by pouring or spraying into the revolving pan in which the sample to be coated is tumbling. The coating is dried using forced air, either at room temperature or at a higher temperature (Dangaran *et al.*, 2009; Pandey *et al.*, 2006) [28, 29]. By adding cold air, the heat generated during the panning process is reduced.

Fluidized bed processing

The coating solution is typically applied to the fluidized powder surface using a number of nozzles in a fluidized bed panning process to create a shell-like structure (Andrade *et al.*, 2012) [24]. The particle size of 100µm is generally used for coating in the fluidized bed coating since smaller particles either forms agglomerates or are highly unstable in conventional fluidized bed. (Dewettinck *et al.*, 1998; Guignon *et al.*, 2002) [30, 31].

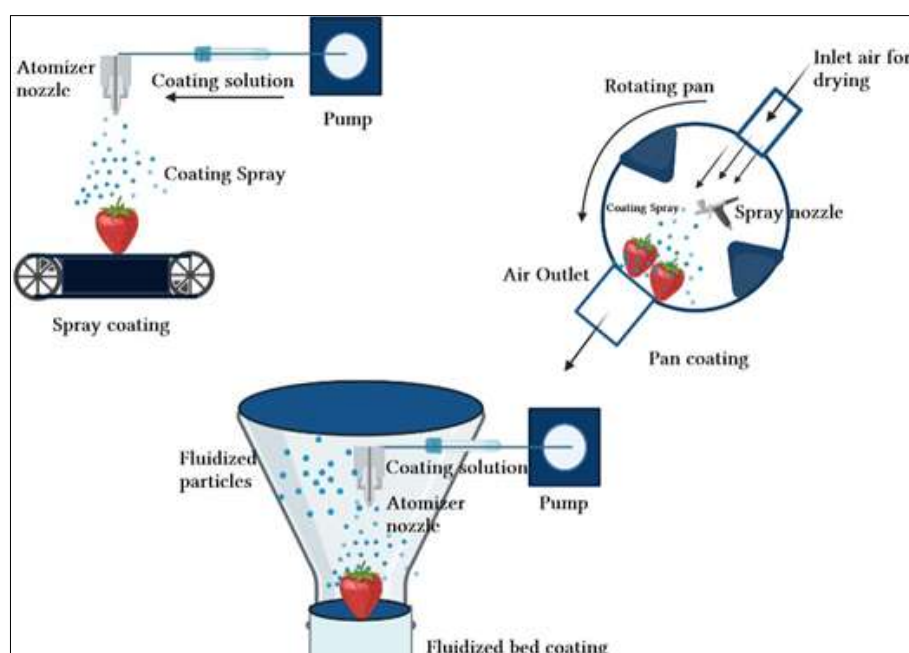


Fig 1: Application of edible coating- methods (Spraying, pan coating and fluidized bed processing coating).

Edible coating: By permitting the controlled exchange of gases during respiration, providing water resistance,

controlled enzymatic characteristics, and suppressing microbiological activity, edible coating deters the post-harvest

decay and deterioration of fruits. As a result, it maintains the texture and stiffness of packaged food (Jafarzadeh *et al.*, 2021) [32]. By altering the gas exchange between the interior of the fruit and the outside environment, edible coatings function. It lowers the concentration of oxygen while raising the concentration of carbon dioxide. The formation of ethylene is hindered by low oxygen levels, which slows down microbial decomposition, slows down transpiration, and prevents loss of firmness and weight by limiting water loss.

Active edible coating

An effective way to eradicate microbial decay, physiological decay, and mechanical decay is by formulating edible packaging with active biological properties. Such packaging that incorporates an active component is referred to as active edible packaging (Das *et al.*, 2020) [33]. The main antioxidant and antimicrobial compounds used in edible packaging are the phenolic compounds obtained from herbs, spices, and essential oils. Numerous techniques have been proposed for the preservation of food products in an effort to reduce the usage of synthetic preservatives in the food business. In this regard, essential oils with desired properties that do not have any negative effects on human health have been implemented in order to preserve food.

Essential oil

According to Husnū *et al.* (2007) [34], essential oil-also known as ethereal oil, etheric oil, or atheroleum-is an odorous, volatile liquid that results from secondary metabolism in aromatic plants. The unique qualities for which these plants are used in the food, medicinal, and fragrance sectors are conferred by the essential oil, which makes up a very small portion of a plant's overall makeup. Essential oils are complex lipophilic substances that are extracted from a variety of plant parts, including flowers, flower buds, leaves, rhizomes, seeds, fruits, and bark.

These oils are multifunctional and are mainly recognized for their aromatic, medicinal, and antimicrobial properties. Most significantly, they offer a feasible approach to avoid adding chemical preservatives to food. Essential oils are recognized by FDA as GRAS (Atarés and Chiralt, 2016) [35]. The broad spectrum of activity shown by essential oils is primarily due to the complex composition of more than 200 chemical compounds, divided into a volatile fraction and a non-volatile fraction.

However, direct use of these essential oils has some limitations such as low bioavailability and low solubility in aqueous solutions. In addition, during coating and storage, significant losses of volatile essential oils are reported. Nanoemulsions appear to be a successful strategy for the preservation of the bioactive components of essential oils and enhanced stability in aqueous solutions. The use of nanoemulsions, which produce stable systems, can be used to deliver nanodroplets with sizes in the nanoscale range to boost the stability of essential oils.

Nanoemulsions

According to Kumar *et al.*, (2019) [36], a surfactant is an amphiphilic molecule that stabilises heterogeneous colloidal dispersions comprising droplets of one phase distributed in another phase. Nanoemulsions are optically transparent or translucent with a faintly bluish tint because the particles in them are submicron in size (50–200 nm) (Tadros *et al.*, 2004)

[37]. Aqueous phase, oil phase, surfactant, and an external energy source are the four fundamental components of nanoemulsions. This process allows the initial components to be transformed into a colloidal dispersion. Nanoemulsions increase stability and make it easier to regulate the exchange of respiratory gases and moisture. The bioactive compounds in edible coated fruit are shielded from oxygen and humidity by adding nano-emulsified essential oils to the biopolymer matrix. This also avoids interactions between the compound and food or biopolymer.

Since nano emulsification enables the use of smaller amounts of essential oils for coating, unpleasant tastes, colours, and odours can be avoided. The controlled and gradual release of the bioactive ingredient from the nanoemulsion prolongs the shelf life of the food by preventing food deterioration over a longer period of time. When Salvia-Trujillo *et al.*, (2015) [38] compared the effects of conventional nanoemulsion and lemongrass-incorporated nanoemulsion on the postharvest quality characteristics of fresh-cut Fuji apples, they found that the latter showed quicker and greater inhibition of *Escherichia coli* than the former. The usefulness of nanoemulsions as delivery vehicles for active chemicals in edible coatings is thus highlighted by these results.

There are two possible approaches to provide energy to the system during the creation of nanoemulsions: high energy method or low energy method. While the majority of the free energy used to prepare the nanoemulsion is lost as heat, it is provided by the mechanical forces that are applied to the system in high-energy processes, such as shear, turbulence, or cavitation. A considerable portion of the free energy associated with emulsion formation in low-energy formation is accounted for by physicochemical reactions rather than the application of mechanical forces (Komaiko *et al.*, 2016) [39].

Characterization of nanoemulsions

Polydispersity index (PDI)

After preparation, nanoemulsions must be analysed in order to assess whether they are suitable for inclusion in the edible coating. A metric utilized to characterize the size range of the nano-carrier systems is the poly-dispersity index. Polydispersity describes the degree of non-uniformity in a particle size distribution. Using Malvern Zetasizer, photon correlation spectroscopy (PCS), which records changes in light scattering caused by the Brownian motion of particles over time, is used to investigate the properties of nanoemulsions (Particle size and PDI) (Kaur *et al.*, 2018) [40].

Zeta potential

An essential technique for evaluating nanoemulsions is the assessment of their zeta potential. Zeta potential value can be utilised to understand the surface charge on the particles as well as the physical stability of nanoemulsions. Zeta potentials between 0 and 30 mV indicate instability, while values greater than 30 mV indicate stability. The zeta potential of nanoscaled particles may be influenced by a number of factors, including particle source, processing with different surfactants, particle form and size, electrolyte concentration (ionic strength), state of hydration, and pH of the solution (Silva *et al.*, 2011) [41].

SEM

The morphological study of the nanoparticles is done using scanning electron microscopy (SEM). SEM allows for the

creation of high-resolution 3D pictures of sample surfaces (Luykx *et al.*, 2008) [42].

FTIR: An established method for molecular level material characterization is infrared spectroscopy. During the infrared

active transitions, the vibrating functional groups' electric dipole moments change as a result of their interactions with (absorption of) the incoming infrared light. FTIR data is used to study the functional groups present in the molecules and the interactions between them (Silva *et al.*, 2011) [41].

Table 1: Essential oil based nanoemulsion incorporated edible coatings for berries

Fruit	Coating solution	Process		
Strawberry	Cinnamon essential oil + pullulan	Ultrasonic homogenization	Coatings maintained chemical properties (Total soluble solids, Titratable acidity), reduced loss of firmness and showed strong antimicrobial activity against bacteria and molds.	Chu <i>et al.</i> , 2019 [43].
	Peppermint essential oil + carboxymethyl cellulose, Thyme essential oil 0 + CMC	Spontaneous emulsification	Carboxymethylcellulose + thyme essential oil showed lowest decay % value compared to Carboxymethylcellulose + peppermint essential oil and control.	Zahra Javanmardi <i>et al.</i> , 2023) [44].
	Thymol nanoemulsion + quinoa protein/ chitosan coating	Spontaneous emulsification	Coatings showed effective antifungal action, Maintained sensory properties, delayed weight loss, and increased shelf life.	Robledo <i>et al.</i> , 2018 [45].
	Chitosan + nutmeg seed oil	Homogenization	Nanoemulsion coated strawberries successfully minimized the bacterial and fungal growth.	Horison <i>et al.</i> , 2019 [46].
Grapeberry	Chitosan + lemongrass oil	Dynamic high pressure processing (DHP)	DHP emulsion coating was effective against total mesophilic aerobes, molds and yeasts	Oh <i>et al.</i> , 2017 [47]
	Carnauba wax + Lemongrass oil	Dynamic Pressxre processing High	Coating with 3% concentration of lemongrass oil greatly suppressed the growth of both <i>S. typhimurium</i> and <i>E. Coli</i> .	Kim <i>et al.</i> , 2014 [48]

Conclusion

A guaranteeing post-harvest management approach that has the ability to significantly lengthen the shelf life of berries is edible coating. The antimicrobial effectiveness of the coatings can be improved by adding essential oil nanoemulsions. Edible coverings with essential oil nanoemulsions provide a practical response to the rising demand for a green alternative to conventional way of berry preservation. Since the creation of an edible covering is more practical and affordable, this postharvest management strategy may prove to be an effective way to preserve small fruits in a developing nation (Like India) with scarce resources. Recent developments in nanotechnology have made it possible to use essential oils for berry preservation in a wider range of applications.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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