Novel Food Gelling Agent

As A Coating Material

On Blueberries

A Dissertation

submitted in partial fulfilment

of the requirements for the Degree of

Master of Science in Food Innovation

at

Lincoln University

by

Hinal Gala

Lincoln University

2021

Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Master of Science in Food Innovation.

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The objective of this study was to see the efficacy of novel gel as a good coating material on blueberries. Blueberries are highly perishable and edible coating as an alternative technique is used as it reduces the transpiration and respiration rate, maintains colour and texture and delayed fruit maturation. The novel gel was made from disodium guanylate (DG) and citric acid (CA). The parameters that were investigated during this study included weight loss, surface colour, texture, pH and total soluble solids. Three treatments were followed: (A) Fruit washed with distilled water as a control, (B)1M citric acid+ 0.25M disodium guanylate+ 1% glycerol, (C) 1M citric acid+ 0.25M disodium guanylate. The results were observed on the 0th day, 7th day and 14th day.

The blueberry coated samples did not a show significant difference in weight loss, firmness, pH with the control group. The TSS showed a significant difference between coated blueberries and control. The TSS value of control berries was increasing with storage time than coated samples. The colour indexes showed few significant differences among treatments and storage time. However, the colour change was highly dependent on the storage time rather than the type of treatments. The novel gel as a coating material needs modification to be effective coating material.

Keywords: Novel gel, blueberry, citric acid, disodium guanylate, moisture barrier, gas barrier, weight loss, pH, soluble solids, firmness, colour.

Acknowledgements

I am grateful to Dr. Venkata Chelikani, Course co-ordinator, Department of Agriculture and Life Sciences for giving me an opportunity to do FOOD660 Research Dissertation.

I would like to take the privilege to extent my gratitude to Dr. Lokesh Kumar, Postdoctoral Research Fellow, Department of Agriculture and Life Sciences, for assisting me throughout the research dissertation.

I would like to thank Dr. Maneesha Mohan, for guiding me in the research dissertation.

Thanks to Ms. Letitia Stipkovitis, lab technician for assisting me in handling and conducting the experiments and all the lecturers and technicians for right advice and encouragement. I would like to thank Ms. Yamuna Gopan for helping me with the whole experiment.

Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

1.1 Background

Blueberries are a rich source of nutrients and have a lower shelf life. There are various techniques to increase the shelf life which include UV radiation, ozonation and edible coatings. Edible coatings can reduce human labour, are environmentally friendly and non-toxic. There are different types of edible coatings that are used to see the effectiveness and implement the application of edible coatings on fresh produce. However, the novel gel was developed from non-animal sources (Chelikani et al., 2020). The novel gel has unique physicochemical and antimicrobial properties, that extended shelf life for meat products when applied as a coating material. It was prepared using citric acid and disodium 5-guanylate.

Further application as a coating material on blueberries has been studied in this experiment. The efficacy of the novel gel as a coating material depends on analytical techniques and quality parameters. The parameters like weight loss, colour, texture, pH and soluble solids are analysed. There are different coating materials which are based on polysaccharides, proteins, lipids and composites. Different studies have performed experiments with different coating ingredients on different fresh produce. Thus, considering the low shelf life of blueberries, it is worth studying novel gel as a coating material and understanding the parameters.

1.2 Objective

In the present study, we observed a new gel developed from disodium guanylate (DG) and citric acid (CA). The experiment aims to investigate the effectiveness of the newly developed gel as a coating material by improving some qualitative characteristics of blueberry during refrigerated storage.

1.3 Experimental Design

Three treatments were followed: (A) Fruit washed with distilled water as a control, (B)1M citric acid+ 0.25M disodium guanylate+ 1% glycerol, (C) 1M citric acid+ 0.25M disodium guanylate. The parameters that were observed were weight loss, colour of the whole blueberry, the texture of the whole blueberry, pH, and total soluble solids. The results were observed on the 0th day, 7th day and 14th day. The results were analysed using analysis of variance (ANOVA) and comparison was done with the Tukey test.

Chapter 2

Literature Review

2.1 Shelf life of fruits

There has been an increase in demand for fresh fruits among the consumers. Fruits are rich in vitamins, minerals and fibers. They have high moisture content which makes them suitable for the growth of microorganisms, yeast and mould (Del Nobile, Conte, Cannarsi, & Sinigaglia, 2008). Fruits deteriorate the most after harvesting and it is approximated that in developed countries 20-25% of the harvested fruits are rotted by the microorganisms (Sharma, Singh, & Singh, 2009). The situation is worse in developing and underdeveloped countries due to a lack of preservation techniques. Fruits respire and metabolise proteins, fats, carbohydrates and organic acids after harvesting. The food quality is lowered regarding the nutritional quality, flavour, colour and weight (Nunes & Emond, 2007). Fruit spoilage can occur through a wide range of reactions like microbial, chemical, physical and enzymatic. Thus, to provide high-quality and safe products by controlling the growth of microorganisms it is necessary to follow sanitation practices after harvesting fruits.

2.1.1 Factors affecting the shelf life of fruits

Fruits begin to decay after harvesting resulting in loss of quality and quantity. Quality loss refers to soft tissue, imbalance levels of sugar and organic acid, increased synthesis of anthocyanins, loss of amino acid and phenolic content (Sharma & Singh, 2000). Quantity loss refers to a large amount of product loss. There are various factors like improper handling, ethylene exposure, light, temperature and humidity which affect the shelf life of the fruits.

Improper handling practices

The quality of fruits can be maintained by harvesting the fruit when it is perfectly matured. Improper handling is harvesting the fruit when it is unripe, it leads to bruises, spots, rots and decaying of fruit by providing a suitable environment for the growth of pathogens. Poor handling practices lead to mechanical injury which causes water loss and a decrease in the shelf life of the products and destroying the colour, flavour, and texture of fruit which is unacceptable to consumers to eat (Ahvenainen, 1996).

Microbial decay

The deterioration of fruits by microorganisms can occur through the soil, air, water, and storage conditions. The bacteria *Pseudomonas* and *Erwinia* are predominant microorganisms responsible for lowering the quality of the fruits (Ahvenainen, 1996). The pathogens attack the cell wall, produce lytic enzymes and produce an environment favourable for other pathogens. Different fruits have different spoilage patterns depending upon the characteristics like pH of the fruit, levels of acetic acid, lactic acid and carbon dioxide (Ahvenainen, 1996). During storage, the growth dominancy of pathogens will depend upon the temperature and carbon dioxide concentration. Thus, to prevent microbial contamination it is apparent to implement good manufacturing practices along with HACCP (hazard analysis and critical control points).

Environmental factors

The shelf life of fruits depends on light, temperature and humidity. High temperature speeds up the metabolic process of fruits and decreases the shelf life while low temperature slows down the metabolic process and increases the shelf life (Mutari & Debbie, 2011). The same study showed a reduction in the size of fruits is observed when there is low humidity while microbial growth is observed during high humidity. The amount of light determines the quality of the fruit.

Physical and biochemical changes

The enzyme which causes browning in fruits is polyphenol oxidase which involves four components: oxygen, oxidising enzyme, copper and substrate (Ahvenainen, 1996). Another enzyme is lipooxiadse which leads to the formation of aldehydes and ketones causing a bad odour. Ethylene is used for maturing fruit and causes physiological changes like soft texture. Ethylene reduces the quality of the fruit by accelerating the ripening and respiration process which leads to senescence and cellular disintegration, hydrolysis of the compounds, limiting the shelf life (Mohapatra, Mishra, Giri, & Kar, 2013). There are various factors responsible for affecting the shelf life which should be remarked, and possible solutions should be undertaken.

2.1.2 Techniques used to increase the shelf life of the fruits

Fruits are rich in nutrients and have a shorter shelf life, thus various minimally non-thermal technologies preserve fruits, maintaining the fresh characteristics. However, to enhance safety and extend shelf life there are various chemical and physical technologies with their advantages and limitations. Chemical methods include ozone treatment, use of chlorine and organic acids while physical methods include modified atmosphere packaging, irradiation and ultraviolet light. Physical methods involve a reduction of pathogens from the surface by shear force while chemical methods involve washing the surface with sanitizers and cleaning (Gil, Selma, López-Gálvez, & Allende, 2009).

Chlorine treatment

The chlorine concentrations used for cleansing fresh produce range from 50-200ppm with a contact time of less than 5 minutes for bacterial inactivation (Baur, Klaiber, Wei, Hammes, & Carle, 2005; Rico, Martin-Diana, Barat, & Barry-Ryan, 2007). It is available easily and it is of low cost. During the production of chlorinated by-products, there is the liberation of chlorine vapours which have bad effects on health. Also, this method depends on pH of the product, sensitive to temperature, light and air (Baur et al., 2005).

Ozone treatment

Ozone is an antimicrobial agent having high reactivity and penetrability. The concentration of aqueous ozone ranges from 0.03 to 20 ppm while gaseous ozone concentration has a higher dose up to 20,000 ppm (Rico et al., 2007). The advantages of this treatment are high antimicrobial activity, extend shelf life and has short contact time. However, it can induce various physical and chemical changes like soft tissue, loss of flavour and colour and bad odour.

Organic acids

Organic acids like lactic acid, citric acid, tartaric acid are strong antimicrobial acidulants due to pH reduction, disruption of membrane permeability and anion accretion (Parish et al., 2003). Citric acid when treated on papaya and watermelon showed a reduction in *Campylobacter jejuni* after six hours of treatment (Castillo & Escartin, 1994). Organic acids are easy to use with no toxicity however, it affects the sensorial property of the fresh produce.

Irradiations

The ionising radiations are gamma-rays, X-rays and electron beams. They produce ions, electronically charged atoms or molecules. The water is the target of ionising radiations which produce free radicals that react with food products which deactivate microbial growth and extend shelf life (Rico et al., 2007). It can be performed at room temperature, but it alters texture and quality is reduced when doses are high.

Ultraviolet light

Ultraviolet lights are classified based on different wavelengths: UV-A which ranges from 315-400nm, UV-B which ranges from 280-315nm and UV-C that ranges from 100-280nm. This treatment is inexpensive and have no residual toxicity. UV-C can damage bacterial DNA and induce resistance to pathogens (Ben-Yehoshua & Mercier, 2005). But it produces off-flavour and colour change.

Modified Atmosphere Packaging (MAP)

Modified atmosphere packaging (MAP) involves the removal of oxygen (O_2) and replacing it with carbon dioxide or nitrogen (N_2) while packing (Rosa, Sapata, & Guerra, 2007). Thus, it reduces the production of ethylene, reducing the metabolic rate and respiration rate, extending shelf life. They are odourless convenient packages that acts as a barrier for microbial contamination and delay ripening. Conversely, it often produces carbon dioxide (CO_2) which leads to off-flavours and the growth of potential pathogens (Rosa et al., 2007). It needs a temperature control environment. There is an alternative to MAP is edible coatings, which can extend the shelf life by providing a barrier to various gases, moisture and different solutes. They can carry active ingredients like flavours, colours, nutrients and antimicrobial compounds with them and reduce synthetic packaging waste (Pranoto, Salokhe, & Rakshit, 2005).

2.2 Edible Coatings

One method for extending the shelf life of fruits is using edible films and coatings. The development of edible films and coating is increasing in demand, but industrial implementation is still emerging. It regulates the transfer of moisture, oxygen, carbon dioxide, aroma and flavour compounds. In general, edible coatings are thin layers made from edible materials that are applied on the surface of the food product while films are preformed thin layers that are kept on or between the food product (Otoni et al., 2017). In addition, edible coatings are directly applied on the surface of the food by dipping, spraying or brushing in liquid form and films are preformed solid sheets which are later applied on the surface or placed in between the food which is shown in figure 2-1 (McHugh & Senesi, 2000). According to the USA Code of Federal Regulations (Food & Administration, 2006) the amount of ingredients used in edible coatings must be in proper amount to have intended effect and it has to be GRAS and listed in Code. It also mentions that ingredients used must be food-graded.

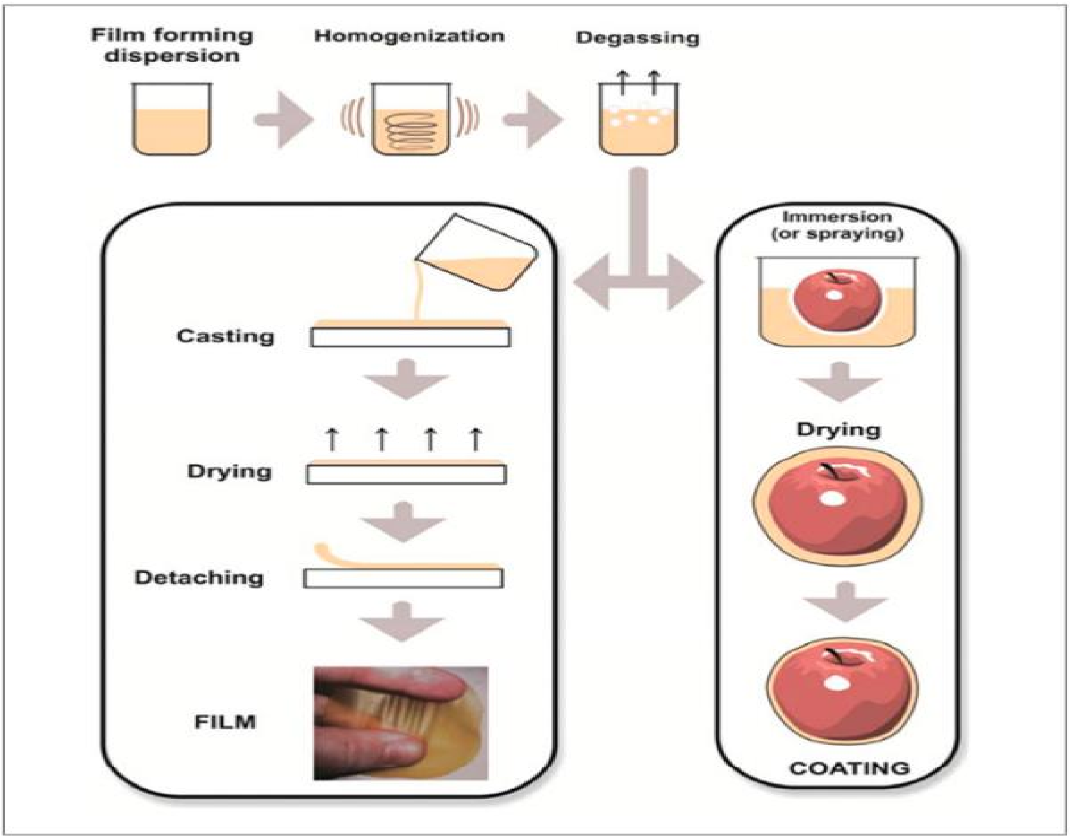

Figure 2.1Schematic representation of production of films and coatings(Otoni et al., 2017).

The mechanisms used for the formation of edible films and coating are as explained (Gontard, Guilbert, & CUQ, 1992):

Coacervation: In which two hydrocolloid solutions are combined with opposite electron charges which cause interaction and precipitation of the complex. Another coacervation is where hydrocolloid is dispersed in water and undergoes phase change after solvent drying.

Gelation: heating of the macromolecule leads to denaturation, followed by protein precipitation or cooling of dispersed hydrocolloid.

Films may be categorised into single or bilayer, dry or moist films and having single or multiple components (Guilbert, Gontard, & Gorris, 1996). Biopolymer coatings are made of biological materials such as proteins, polysaccharides, lipids and their derivatives. Coatings used for one type of fruit may not be suitable for other fruit since each fruit has different skin resistance, gas diffusion and fruit respiration rate (Olivas, Dávila-Aviña, Salas-Salazar, & Molina, 2008). The selection of coating depends on the transpiration and respiration rates of fruit and storage conditions. The application method of coating and the thickness of the coating has a great influence on the selection. The effect of coatings on fruits depends on temperature, pH, the thickness of the coating material, variety and condition of the fruit (Guilbert et al., 1996).

2.2.1 Properties of Edible Coatings

The properties of edible coating depend on molecular structure and not on the molecular size or chemical constitution. There are various properties that an edible coating or film should have (Guilbert et al., 1996):

Edible coating should not affect the sensory property of the food that it has been coated, coating should odourless, tasteless. It should have permeability to water vapour and solutes and selectively permeable to gases. The coating should be transparent and capable to hold slight pressure. It should have low viscosity and inexpensive. The drying performance of the coating should be effective and non-sticky. Edible coating's main purpose is to improve texture, improve mechanical handling properties, retain volatile compounds, extend shelf life, carry active ingredients like vitamins, antimicrobial agents. It should not build up carbon dioxide and deplete oxygen on the surface of the food as it will initiate the growth of anaerobic microorganisms. It should be water-resistant. The potential benefit of using edible coating is shown in figure 2-2.

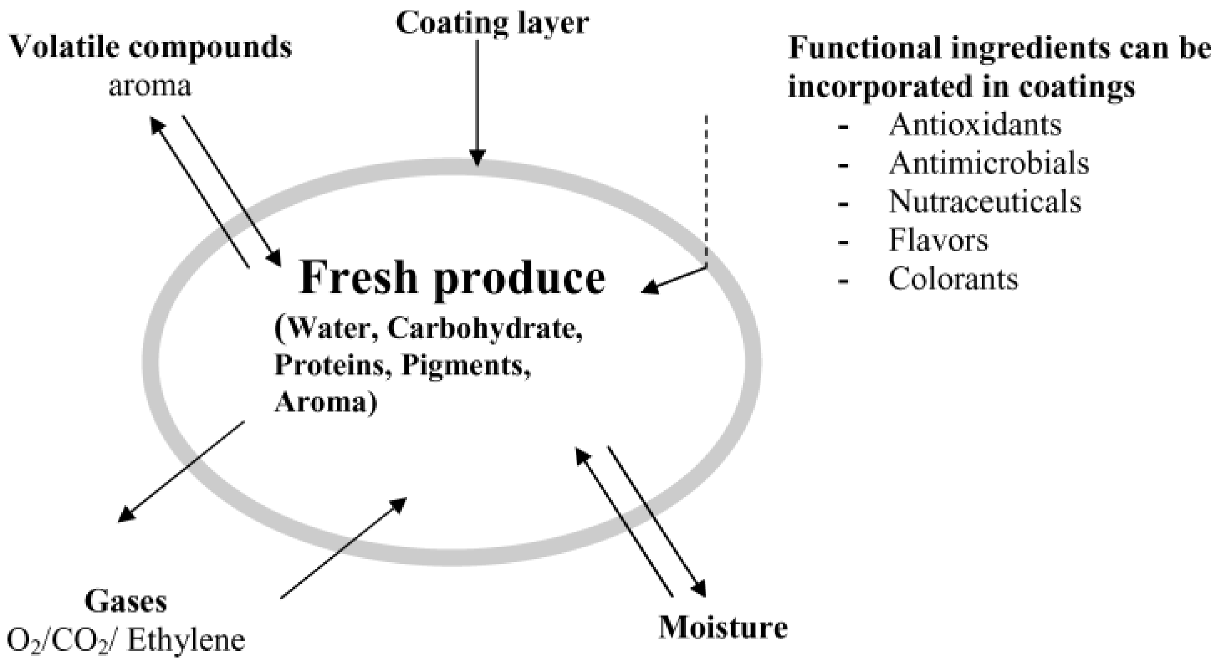

Figure 2.2Functional properties of an edible coating on fresh fruits and vegetables(Lin & Zhao, 2007).

2.2.2 Action of Edible Coating

After harvest, fruits and vegetables use oxygen for respiration and matures, while in edible coating there is an accumulation of CO_2 and uses less O_2. As carbon dioxide is not permeable to coating, it accumulates. It results in the fruit respiration shift from aerobic to anaerobic where it uses less oxygen (Guilbert et al., 1996; McHugh & Senesi, 2000). Ethylene which requires oxygen to ripe the fruits is slowed down when there is less oxygen and water loss is also reduced. Thus, fresh produce remains fresh, nutritious and has an intact texture for a long time and shelf life is increased. It acts as a natural barrier to fruits and vegetables. The type and amount of coating will determine the modification of internal CO_2 and O_2 levels and reduction in moisture loss.

2.2.3 Composition of Edible Coating

The materials required for edible coating should have film-forming properties. These materials are dissolved in solvents like water, alcohol or a mixture of solvents. To expediate proper dispersion for a particular polymer, pH is adjusted, and heating is provided. As per requirement, the process of films or coatings is done. Edible coatings are made from polysaccharides, proteins, lipids and composites (Guilbert et al., 1996). The mechanical and chemical barrier properties like transfer of gases, lipid and moisture transfer depend on the constituents of the edible coating. Polysaccharides, lipids, proteins none of these ingredients can give the best results for barrier properties, thus the combination of these ingredients are used (Guilbert et al., 1996; McHugh & Senesi, 2000).

Polysaccharides

The polysaccharides that are used in edible coatings are starch and its derivatives, cellulose and its derivatives, alginates, carrageenan, chitosan, and pectin. Polysaccharides are highly water-soluble (hydrophilic), they have high water vapour permeability and has good gas barrier properties (Kester & Fennema, 1986). The crystalline nature of the polysaccharides creates performance problems when applied to moist products. The resistant nature to fats and oils, toughness, flexibility and transparency are obtained in edible coatings because of the linear structure of few polysaccharides. They are obtained from plants and animals.

Cellulose is a natural and linear polymer of anhydroglucose. The higher the molecular weight of the cellulose, the better is the mechanical and barrier properties of the coatings (Bravin, Peressini, & Sensidoni, 2004). This creates a crystalline structure in water media. The derivatives of cellulose like carboxymethyl cellulose (CMC), methyl cellulose (MC), hydroxypropyl methyl cellulose (HPMC), or hydroxylpropyl cellulose (HPC) are obtained when cellulose is treated with alkali in water and later reacted with chloroacetic acid, methyl chloride or propylene oxide (Bravin et al., 2004). MC, HPMC, HPC and CMC are odorless, tasteless, flexible, good strength, transparent, resilient to oils and fats, hydrophilic and semi-permeable to water and oxygen (Bravin et al., 2004).

Starch is obtained from legumes and tubers, is a renewable and biodegradable raw material. There are two types of glucose polymers: amylase which is linear polymer and amylopectin which is a branched polymer (Rodríguez, Osés, Ziani, & Mate, 2006). Starch is inexpensive, ample in nature and easy to use. In presence of high humidity, starch-based coatings lose strength and barrier properties, become fragile in the dry atmosphere (Petersson & Stading, 2005). The addition of biodegradable plasticizers like glycerol and polyether overcomes the brittleness of starch (Rodríguez et al., 2006). Plasticizers reduce the water activity and limit microbial growth.

The polysaccharide chitosan is obtained from crab and shrimp shells and has a structure similar to cellulose (Hirano, 1999). Chitosan films and coatings are selectively permeable to $CO_2\ and\ O_2$ and have good mechanical properties. They have high water vapour permeability and have antifungal antibacterial properties because of their polycationic nature(Butler, Vergano, Testin, Bunn, & Wiles, 1996; Cuero, 1999). These properties of chitosan can help to reduce microbial growth, extend shelf life and reduce the oxidation process in food. Alginates and carrageenans are used to make edible coatings and films. Alginates are the salts of alginic acid and carrageenan are a complex mixture of five water soluble galactose polymers λ, κ, ι, μ, and ν-carrageenan. Alginates react with divalent and trivalent cations (calcium and magnesium) to form alginate coatings (Cha & Chinnan, 2004). Pectin is a complex anionic polysaccharide where uronic acid carboxyls are fully (HMP, high methoxy pectin) or partially (LMP, low methoxy pectin) methyl esterified to form films.

Proteins

Proteins are categorised as globular proteins and fibrous proteins. Fibrous proteins are insoluble in water and are obtained from animal tissues like collagen, gelatin, casein and whey. Globular proteins are soluble in water and are obtained from plants like corn-zein, soy protein (Kester & Fennema, 1986). As they are obtained from different sources there are different characteristics of the protein coating. Proteins coatings are hydrophilic in nature thus, humidity and temperature disrupt the coating features. The proteins have to be denatured by heat or solvents to form a coating structure. The dispersion of proteins in solvent leads to film formation as it is made of amino acids and side chains form bonds with other molecules. According to Kester & Fennema (1986), protein films are stronger because of chain-to-chain interaction but are less flexible and permeable to gases and liquids, thus they are good oxygen barriers at low relative humidity.

Collagen is obtained from an animal's skin, tendon, and connective tissues. It is a flexible polymer and insoluble in water. It forms the raw material for gelatin formation. Alkaline hydrolysis of collagen forms gelatin. Gelatin films are composed of 40-70% water, 20-30% gelatin, 10-30% plasticizers (Guilbert et al., 1996). They are used to encapsulate oil-based food ingredients as they are moisture sensitive. Casein and whey proteins are obtained from milk. They both have brittle structure, so plasticizers are added to give mechanical strength and form a tough structure. Corn zein is obtained from a corn plant and it is insoluble in water. The zein film is formed by dissolving the protein in ethanol and later drying the solution (Guilbert et al., 1996). As the film is formed it has hydrophobic, hydrogen and disulfide bonds therefore have a brittle structure. According to Guilbert (1986), zein films are good water vapour barriers. The study showed that zein coatings reduce moisture and firmness loss in tomatoes and delayed colour change (Park, Chinnan, & Shewfelt, 1994). Soy protein is obtained from soybeans. Soybeans are grinded with water and soymilk is extracted. Soy protein edible film is obtained by surface film formation on heated soymilk or soy protein isolate. The protein structures are disrupted and hydrophobic, disulphide and hydrogen bonds are formed while the film is formed (Gennadios, McHugh, Weller, & Krochta, 1994). Thus, the above few examples briefed about the formation of edible coatings from proteins.

Lipids

Lipid-based edible coatings are made from animal wax which includes beeswax and shellac wax, vegetable waxes include carnauba wax and palm wax, mineral and synthetic waxes include paraffin wax (Kester & Fennema, 1986). Lipids are hydrophobic in nature and thus lipid coatings are good moisture barriers (Morillon, Debeaufort, Blond, Capelle, & Voilley, 2002). They are brittle and thick coatings because of their hydrophobic nature. They lower the respiration rate and extend the shelf life of the food, improves texture by shiny layer. Lipids are made of fatty acids, more the number of fatty acids more effective as moisture barriers. Most lipids are in solid-state they can only be stretched to few percentages. Fatty acids are obtained from vegetable oils and monoglycerides are prepared by transesterification of glycerol. They are used as emulsifiers and dispersing agents in edible coatings. Such emulsifying property of fatty acids and monoglycerides can form moisture barrier and do not form a shiny layer. Shellac resin is not generally regarded as safe (GRAS) and it is added in coatings as a food additive. Rosins are oleoresins from pine trees and are included in edible coatings to have a glossy effect.

Composites

Composite coatings are made from different layers by deposing one layer over another. The design of composite coating is to blend ingredients of polysaccharides, lipids and proteins. These mixtures of ingredients can give good mechanical strength, water and gas barrier properties to the film. Lipids have good moisture barrier properties while hydrocolloids have good gas barrier properties with good mechanical strength (Krochta, 1996). Thus, in bilayer coating, the first layer is made of polysaccharide or protein and the second layer is of lipids thus method of application affects the barrier properties (Guilbert et al., 1996; Krochta, 1996). Another technique to obtain composite coating is dispersed the lipid in the hydrophilic phase of an emulsion (Shellhammer & Krochta, 1997). Hydrophilic and lipophilic property of composite films is the future trend in the edible coating.

2.2.4 Incorporation of Active Ingredients

The functionality of edible coatings can be improved by the addition of active ingredients like vitamins, minerals, antimicrobial agents and plasticizers. The quality, safety and shelf life of the fruits can be enhanced by the incorporation of active ingredients. The polysaccharide and protein-based coatings have brittle structure, low elasticity and flexibility with low toughness, the addition of plasticizers can overcome these drawbacks (Guilbert et al., 1996). Plasticizers are part of polymer macromolecules and are low molecular weight which is added to enhance the coating properties. Food-grade plasticizers like glycerol and sorbitol are added to edible coatings (Otoni et al., 2017). They reduce chain interactions by placing them between the polymer molecules and separating them. The review by Ontoni et al (2017), also mentions a study that glycerol has better plasticizing efficiency than sorbitol as sorbitol is a high molecular weight when compared to glycerol. Water acts as a plasticizer but when added to coating it depletes due to dehydration at a low relative humidity (Guilbert et al., 1996). Plasticizers absorb water molecules from the environment. They can decrease glass transition temperature and increase coating flexibility. Few plasticizers increase water vapour permeability and others resistance to permeate gases. High glycerol concentration in edible films has sticky films and forms a plasticizer layer on the surface of the film(Otoni et al., 2017). Thus, a proper amount of plasticizers can have a great effect on edible coatings.

Antimicrobial agents like organic acids, essential oils, esters, nitrites are used in the edible coating to inhibit pathogenic bacteria by effective concentrations of the active ingredients on the food surface (Dhall, 2013). Antimicrobial coatings are made to slow down the diffusion of active ingredients from the surface of the coated samples and maintaining the preservative activity (Krochta, 1996). Chitosan which has antimicrobial property promotes cell adhesion by interacting positively charged amines with negatively charged cell membranes (Vargas, Pastor, Chiralt, McClements, & Gonzalez-Martinez, 2008). They have prevented fungal growth and microbial growth when coated on strawberries, raspberries and grapes. Citric and ascorbic acid has an antimicrobial property which helps to reduce pathogen growth and increase shelf life.

2.2.5 Physicochemical properties

Edible coating effectiveness is evaluated by quality parameters of coated samples. These parameters include water loss, respiration rate, texture, colour and pH. Colour indicates the ripening stage of the fruit and determines the quality and consumer acceptance. Anthocyanins and lycopene tend to increase during maturation. Edible coatings slow the ripening process and colour change. The weight loss of the fruit happens due to the water pressure gradient and respiration. Coatings reduce moisture loss and maintain the relative humidity in the tissue environment (Olivas et al., 2008). Respiration rate is decreased when edible coating provides a barrier to gases by an internal modified atmosphere. TSS increases during storage as it breakdown the starch into soluble sugars. Coatings slow down the respiration rate and conversion into simple sugars. Organic acids release free hydrogen ions and there is an increase in pH. All these mechanisms are related to the texture which can be improved by the inclusion of texture enhancers in the coating.

This study investigates the novel gel as a coating material on blueberries. Blueberries are a rich source of phenolic acids, anthocyanins and flavonoids (Mannozzi et al., 2017). As it is rich with nutrients there are chances of microbial growth. During storage, there are various factors that causes deterioration of blueberries like moisture loss, texture and colour loss. The shelf life of blueberries is from 10-40 days and depends on several factors like a method of harvest, stage of fruit ripeness, temperature, humidity and atmosphere of storage. There are different methods like modified atmosphere packaging, UV radiation, cold storage and ozonation which can increase the shelf life and retain the nutritional properties of blueberries. However, edible coatings can extend shelf life by giving a protective layer against pathogens and gases. Edible coatings can replace plastic packaging as they are bio-degradable and pollution-free. They are non-toxic so edible coated blueberries can be used as ready-to-eat food. Only food-grade ingredients have to be used while making the edible coating.

Chapter 3
Methods

3.1 Experimental Design

3.1.1 Materials

New Zealand grown fresh blueberries by Pams were purchased once from New World, Lincoln. Fresh berries were kept in the refrigerator at 4°C until they were used. Blueberries with the same colour and no damages were selected for the experiment. Disodium 5-guanylate (DG) and citric acid (CA) were purchased from Sigma-Aldrich.

3.1.2 Preparation of coating solutions

The concentration and preparation of Novel Gel (NG) were performed as previously described (Chelikani et al., 2020). DG was dissolved in RO water and 0.25M was prepared. CA was dissolved in RO water and 1M was prepared. Later, both the solutions were mixed and dissolved with the help of a vortex for few seconds. The mixture was kept in the water bath until the solution became transparent and kept at room temperature. NG was prepared to perform experimental analysis.

3.1.3 Blueberry preparation and coating treatments

The blueberry fruit was rinsed with distilled water and air-dried at room temperature for 30 min before starting the experiment. After drying, the fruits were divided randomly into three treatments. For each treatment or group 40 blueberries were used. Coated blueberries were air-dried for 1 hour at room temperature. Later, they were packaged in white trays covered with plastic film to maintain relative humidity and stored in the refrigerator at 4°C. Forty blueberries were sampled each day and their quality parameters were analysed on the 0^{th} day, 7^{th} day and 14^{th} day. The treatments or groups that were prepared: (A) Fruit washed with distilled water as control (G 1), (B) 19% (w/v) CA + 10% (w/v) DG + 1% (v/v) glycerol (G 2) and (C) 19% (w/v) CA + 10% (w/v) DG (G 3).

3.2 Methods

3.2.1 Weight loss

Weight loss was calculated by equation 1 by weighting all the samples with a weighing balance at the beginning of the storage and at all sampling days.

Weight loss (%) = $\frac{W_i - W_f}{W_i} \times 100$ (1)

3.2.2 Colour

A chroma meter (CR-400, Minolta, Tokyo, Japan) was used to evaluate the surface colour of coated and uncoated blueberries. Colour was recorded using the CIE L*a*b* uniform colour space. A white standard plate (Y= 94, x=0.3158, y=0.3322) was used for calibration. The colour was evaluated on 20 berries per treatment and sampling day. Colour change was analysed through changes in lightness (L*).

3.2.3 Firmness

Texture analysis of coated and uncoated blueberries was done with TA.XT plus Texture Analyzer (Stable Micro Systems Ltd.) Penetration test was performed on twenty blueberries for each treatment. It was equipped with 5kg load cell with a stainless-steel cylindrical probe of 3mm diameter. Texture analyzer was calibrated with force and height first before starting with the sample. The speed of the probe was set to a constant speed of 1mm/s to a distance of 5mm for a single compression test. Blueberry is placed on the bottom in the centre of the texture analyzer. The probe proceeds to penetrate the blueberry to a specific distance as the force is attained. Firmness is the force that measures the skin strength when the penetration probe enters the sample which was evaluated using the exponent software.

3.2.4 pH

Blueberry juice was prepared with Kenwood centrifugal juice extractor. The pH of the blueberry juice was measured using Eutech digital pH meter. The pH meter has an electrode which measures the pH. The meter was calibrated using pH 4 and pH 7 buffers at room temperature and probes were cleaned with distilled water. pH was measured on the 0^{th} day, 7^{th} day and 14^{th} day.

3.2.5 Total Soluble Solids

Total Soluble Solids (TSS) was performed at room temperature by measuring the Brix of blueberry juice with KEG KING portable refractometer having 0-30% brix scale having 0-1.120 SG scale. Refractometer was cleaned with distilled water and wiped after every analysis. TSS was evaluated on 0^{th} day, 7^{th} day, and 14^{th} day.

3.3 Statistical Analysis

The evaluate difference in the physicochemical properties of the blueberries among the three treatments and three days, collected data were analysed by analysis of variance (ANOVA) using General Linear Model (GLM) and comparisons were done by Posthoc Tukey test ($p < 0.05$) by Minitab 18. The graphs were made in excel.

Chapter 4

Results

4.1 Weight Loss

The weight loss percentage of the treatments or groups on the interval of the seven days is shown in figure 4.1. The G 1 control group weight loss percentage is highest on the 14th day, followed by G 3. It is observed that group G 2 increases and then weight loss decreases drastically on the 14th day. There was no significant difference observed in weight loss percentage between the treatments and storage period of seven days.

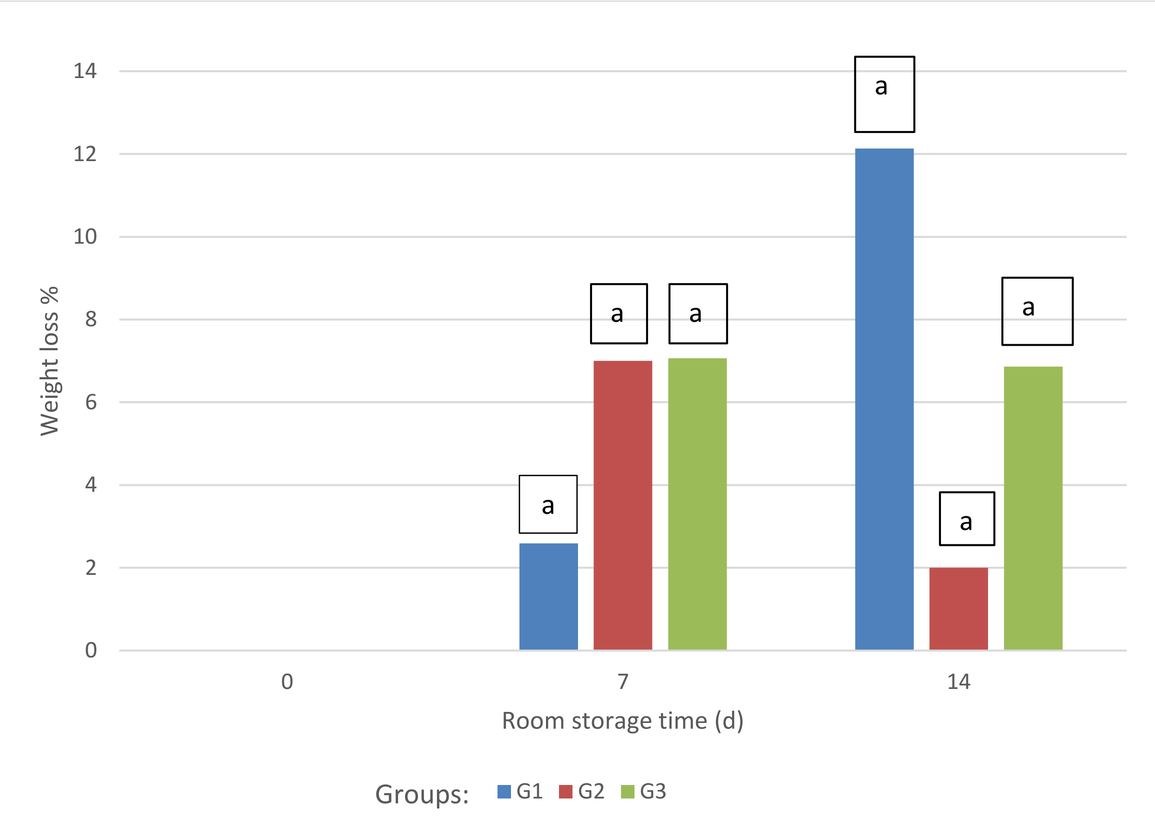

Figure 4.1Weight loss percentage of coated and uncoated blueberries during 14 days of storage.

*Means that do not share a same letter are significantly different (P < 0.05).

4.2 Colour Analysis

The colour indexes value of L*, a* and b* of three groups G 1, G 2 and G 3 on the 0th day, 7th day and 14th day are represented as mean ± standard in table 4.1. The colour was observed through changes in lightness (L*), red to green (a*) and blue to yellow (b*) chromatic values. The L* represents colour lightness (0 = black and 100 = white). The a* scale ranges from maximum the red (+a*) and minimum to green colour (-a*) while the b* axis ranged from yellow (+b*) to blue (−b*). The G 1 control group had a lightness (L*) chromatic value maximum on 0th day, which dropped off severely on 7th day and increased slightly on the 14th day. On the 14th day, the L* values of group G 2 showed highest value followed by G 1 and G 3. The a* values of the G 2 were greatest during 7 days of storage when compared with G 1 and G 3. The values of redness raised gradually for all the groups as the number of days of storage increased. Group G2 showed maximum values on the 14th day, followed by G1. The blueness of G 1, G 2 and G 3 had negative readings on 0th day while it showed positive values on the 7th and 14th day. The most high values of b* were seen on 14th day of G 2, followed by G 1 and G 3. The chromatic values of L*, a* and b* decreased on the 7th day and increased on the 14th day. There was a significant difference in treatment G 3 with G 2 and G 1 on all 7 days of storage and between all L*, a* and b* values. There was a significant difference seen on all three days (0th, 7th and 14th).

Table 4.1Colorimetric analysis of coated and uncoated blueberries during storage of 14 days.

Indexes	Treatments	Storage time (days)		
		0	7	14
L*	Control-(G 1)	27.74±1.75^{Aa}	6.81±1.18^{Ac}	8.23±1.23^{Ab}
	19% (w/v) CA + 10% (w/v) DG + 1% (v/v) glycerol-(G 2)	25.33±2.95^{Aa}	7.10±1.57^{Ac}	8.85±1.29^{Ab}
	19% (w/v) CA + 10% (w/v) DG-(G 3)	25.71±2.95^{Ba}	5.64±1.51^{Bc}	7.29±1.89^{Bb}
a*	Control-(G 1)	0.16±0.44^{Ac}	6.95±1.22^{Ab}	8.39±1.26^{Aa}
	19% (w/v) CA + 10% (w/v) DG + 1% (v/v) glycerol-(G 2)	0.23±0.99 ^{Ac}	7.18±1.61^{Ab}	8.90±1.35^{Aa}
	19% (w/v) CA + 10% (w/v) DG-(G 3)	0.24±0.56^{Bc}	5.73±1.52^{Bb}	7.26±2.03^{Ba}
b*	Control-(G 1)	-1.62±0.90^{Ac}	9.05±1.79^{Ab}	10.64±1.69^{Ba}
	19% (w/v) CA + 10% (w/v) DG + 1% (v/v) glycerol-(G 2)	-1.54±1.29^{Ac}	9.12±2.12^{Ab}	11.09±1.64^{Ba}
	19% (w/v) CA + 10% (w/v) DG-(G 3)	-1.61±1.31^{Bc}	7.30±1.85^{Bb}	9.11±2.88^{Ba}

*Data are means ± standard deviations; Means that do not share a same letter are significantly different ($P < 0.05$); Capital letters are indicated for any difference between the treatments and small letters indicate differences in days during storage.

4.3 Firmness

Figure 4.2 shows the firmness of the blueberries of G1, G2 and G3 during the storage period up to 14 days. The G1 group was marked highest in firmness on the 0th day while it decreased gradually until

14th day. On the 0th day, the firmness values of G2 and G3 were almost same. The graphs show a difference in values of G1 with the values of G2 and G3. While there was increase in firmness values on the 7th day of G2 and again dropped down. The firmness increased gradually from the 0th day to the 14th day for G3. There was no significant difference observed in firmness between the treatments and storage days.

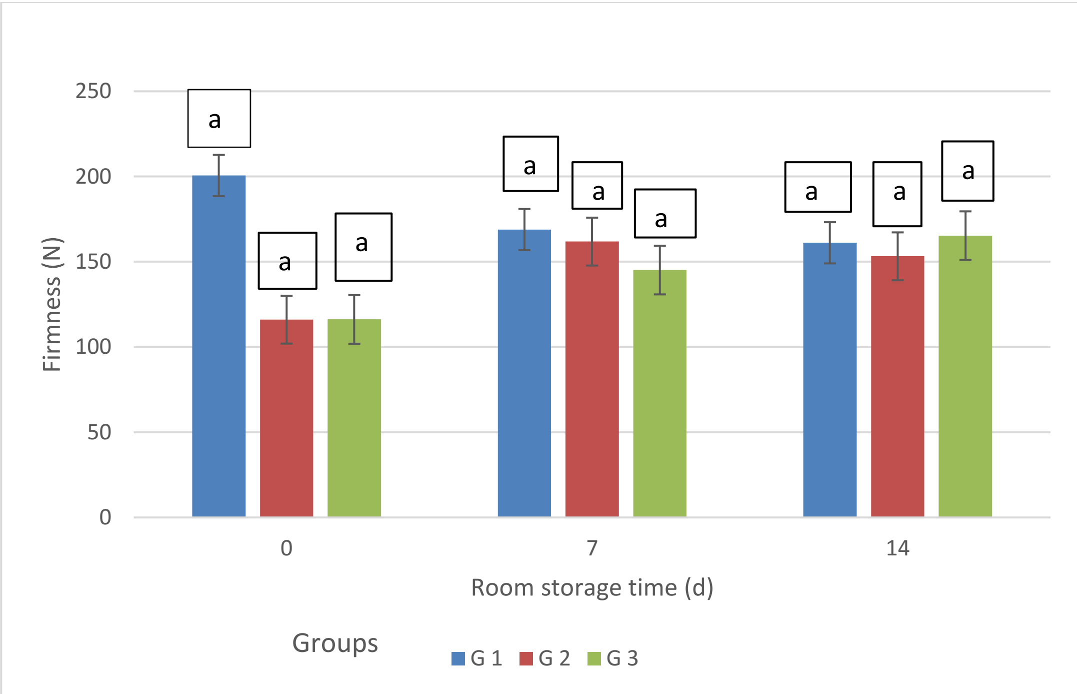

Figure 4.2Firmness of coated and uncoated blueberries during 14 days of storage.

*Means that do not share a same letter are significantly different (P < 0.05).

4.4 pH

The pH values of the blueberries juice uncoated (G1) and coated with gel (G2) and (G3) during storage from 0th day to 14th day is shown in table 4.2. The pH values of group G1 has been increased during storage, while on the 0th day G3 had the highest value among all three treatments. On the 7th day group G2 had a high value of pH. On the 14th day G1 showed high pH. The pH value of G2 increased on the 7th day while lowered on the 14th day. Likewise, pH value of G3 increased from 0th day to 7th day and decreased from the 7th day to the 14th day. There was no significant difference among the three treatments while there was a significant difference seen on the 0th day and 7th day and 14th day.

Table 4.2 pH of coated and uncoated blueberries during storage of 14 days.

Treatments or Groups	Storage time (days)		
	0	7	14
Control-(G 1)	2.75 ± 0.13^{Ab}	3.02 ± 0.06^{Aa}	3.19 ± 0.09^{Aa}
19% (w/v) CA + 10% (w/v) DG + 1% (v/v) glycerol-(G 2)	2.68 ± 0.38^{Ab}	3.15 ± 0.07^{Aa}	3.05 ± 0.07^{Aa}
19% (w/v) CA + 10% (w/v) DG-(G 3)	2.91 ± 0.06^{Ab}	3.14 ± 0.04^{Aa}	3.2 ± 0.05^{Aa}

*Data are means ± standard deviations; Means that do not share a same letter are significantly different ($P < 0.05$); Capital letters are indicated for any difference between the treatments and small letters indicate differences in days during storage.

4.5 Total Soluble Solids (TSS)

Table 4.3 shows the TSS of G1, G2 and G3 on the 0th, 7th and 14th day. The control group had a maximum value on the 0th day when compared with other treatments. Likewise, it was observed on the 7th and the 14th day as well. However, the TSS value of the control group decreased after seven days and increased after another seven days. The same scenario was seen with glycerol containing group and only the gel coated group. The values of the control and G3 group were almost similar during storage. There was significant difference between the control group with G2 and G3 in respect to treatments on all days. There was a significant difference observed with 14th day with 7th day and the 0th day. There was no significant difference between G2 and G3. Likewise, no significant difference was seen between the 0th day and the 7th day.

Table 4.3 TSS of coated and uncoated blueberries during storage of 14 days.

Treatments or Groups	Storage time (days)		
	0	7	14
Control-(G 1)	13.15 ± 0.07^{Ab}	11.35 ± 0.07^{Ab}	14.25 ± 0.07^{Aa}
19% (w/v) CA + 10% (w/v) DG + 1% (v/v) glycerol-(G 2)	10.45 ± 0.07^{Bb}	10.5 ± 0.14^{Bb}	12.95 ± 0.07^{Ba}
19% (w/v) CA + 10% (w/v) DG-z(G 3)	10.05 ± 0.07^{Bb}	11.2 ± 0.14^{Bb}	14.1 ± 0.14^{Ba}

*Data are means ± standard deviations; Means that do not share a same letter are significantly different ($P < 0.05$); Capital letters are indicated for any difference between the treatments and small letters indicate differences in days during storage.

Chapter 5

Discussion

The results of the current experiment analysed the variance among the treated and untreated blueberries by physicochemical parameters. The physicochemical parameters include weight loss, firmness, colour, pH and TSS. The reason behind the variances among different treatments and on different days are discussed further.

5.1 Weight loss

According to figure 4.1, coated samples did not show any significant difference in weight loss when compared with control. However, all the treatments underwent a slight weight loss during 14 days of storage. The weight loss in fruits during storage is caused by the migration of water from fruit to the environment (Duan, Wu, Strik, & Zhao, 2011). The weight loss increases during fruit storage after harvest and represents the freshness of the fruit (Antunes, Dandlen, Cavaco, & Miguel, 2010). The reason behind the moisture loss in fresh produce is the gradient of water vapour pressure occurring from different locations in the cell tissues (Yaman & Bayoındırlı, 2002). According to mass transfer rate, the barrier thickness and moisture permeability are important to note. This study also mentions that the effect on vapour pressure difference between fruit and environment is due to temperature and relative humidity of the coating medium. The respiration rate causes weight reduction as the fruit donates carbon atom in each cycle.

A study showed similar results where there was no significant difference observed between coated and uncoated blueberries (Mannozzi et al., 2017). They used sodium alginate, pectin and a combination of both the ingredients to see the variance in weight loss. It mentions that cold storage conditions might affect in the difference of vapour pressure of the fruit and atmosphere. Edible coating act as a barrier to control the gas and water vapour exchange to delay weight loss, however, the novel gel as a coating material delayed weight loss after the 7^{th} day. As reported the weight loss of up to 4-5% does not significantly affect the freshness of the fruit (do Nascimento Nunes, 2015). The difference in the ability to lower weight loss is fitted to different water vapour permeability used while formulating edible coating (Vargas et al., 2008). The novel gel to be a good coating material should have good water vapour permeability. However, this study, it shows that the addition of glycerol drastically lowers the weight loss in group 2, although there is no significant difference observed. According to some authors, the addition of glycerol as a plasticizer helps to reduce weight loss (Moldao-Martins, Beirao-da-Costa, & Beirao-da-Costa, 2003; Serrano et al., 2008). The addition of glycerol can change the polymer network and create more interchain distances and increases the permeability (Cerqueira, Souza, Teixeira, & Vicente, 2012).

In similar to our study, weight loss was not reduced in coated blueberries and an insignificant difference was observed (Guerreiro, Gago, Faleiro, Miguel, & Antunes, 2015). However, chitosan coating applied on strawberry and raspberry showed a reduction in weight loss as the coating lowered the water vapour permeability. The novel gel may be used as a coating material in the future if there is a higher percentage of glycerol added, or the concentration of the ingredients in the gel is varied and might be an addition of some lipophilic compounds which will help to reduce the water loss. Also, there is a correlation of weight loss with the visual quality of fruits. As the storage time increases, the weight loss increases, the fruits shrink and firmness is decreased, the more it becomes unacceptable for sale. The images of the blubbery of all treatments during storage up to 14 days are represented in A.1. Thus, novel gel with further modification can show a significant difference when coated on samples.

5.2 Colour

The colour indexes of coated and uncoated blueberries are shown in table 4.1 during 14 days of storage. Colour is an important parameter to determine the freshness of the blueberry. Changes in the external colour of the blueberries were monitored by L*, a* and b* values. Colours change in fruits indicates the ripeness or maturation. The results of this experiment showed that lightness decreased during storage and on the 14^{th} day it increased slightly. While the redness and blue colour of the blueberries increased during storage. In a previous study, the novel gel showed higher L* values and had colour neutral property (Chelikani et al., 2020). Thus, coating with novel gel lowered the L* values and intensified the dark colour of a blueberry. The modification in the surface reflection properties can reduce the L* values of the coated samples (Hoagland & Parris, 1996). In this study, colour indexes were more affected by storage time rather than the type of the treatment. Loss of moisture and storage in cold temperatures tend to decrease the lightness values (Moreno, Castell-Perez, Gomes, Da Silva, & Moreira, 2007; Yaman & Bayoındırlı, 2002). There was no significant difference in any indexes observed between control samples and samples coated with novel gel and glycerol. While there was a significant difference observed in novel gel coated blueberries, may be glycerol has some effect on colour indexes. In a similar study after 7 days of storage it showed increased in lightness values but there is no specific reason mentioned (Chiabrando & Giacalone, 2015).

The a*(redness) and b*(blue colour) of the coated samples in this study were increasing throughout the storage. Anthocyanin pigment can change colour in blueberries (Mannozzi et al., 2017). Ideally, the coated samples showed have stable a* and b* values as it indicates the delaying of the ripening process and senescence of the fruit. In numerous studies, it has shown that coated samples have lower a* and b* values during storage (Mannozzi et al., 2017; Tahir et al., 2020). However, in this study, during storage, there was no significant difference observed between coated and uncoated blueberries in terms of red and blue colour. The values of the a* and b* increased with time, thus it shows that ripening was not delayed by the novel gel coating. The control samples and novel gel treated samples showed maturation of blueberries with time. The contrast in our study and other studies showed that novel gel did not have any effect with colour delaying. The anthocyanin values are affected by the oxidation reactions of polyphenol compounds which delay the colour change (Forney, 2008). During ripening, the formation of polymers and synthesis of anthocyanins occurs which is due to the co-pigmentation phenomenon which causes an increase in colour indexes (Jiang, Sun, Jia, Wang, & Huang, 2016). Edible coatings prevent the synthesis of anthocyanins which is related to the ripening process, prevent the release of cellular fluids containing enzymes and substrates related to browning and cause oxygen distress, which can delay the colour changes (Thakur et al., 2018).

During this study, decolouration of blueberries was observed in the initial stage of storage and it increased with the storage time. The strong concentration of novel gel might have caused the decolouration which was observed in a previous study of novel gel with minced beef (Chelikani et al., 2020). The visual appearance of uncoated samples was better than coated samples.

5.3 Firmness

Firmness is one of the quality parameters that indicate the shelf life of the fruit and its quality. Lower firmness results in the unacceptability of the fruit to consumers and low market value. The delayed deterioration of insoluble protopectins to soluble pectic acid and pectin, retains firmness (Yaman & Bayoındırlı, 2002). When fruit is in the ripening stage, depolymerization of pectin substances occurs with an increase in pectinestarse and polygalactronase activities. The activity of these enzymes is lowered when there are low oxygen and high carbon dioxide and allows retention of firmness of fruits. Edible coatings provide structural rigidity in fruits. In various studies, it has been shown that edible coatings have higher firmness of the blueberries, having a significant difference with control samples (Duan et al., 2011; Mannozzi et al., 2017). In contrast, the novel gel when coated on blueberries had an insignificant difference from the control samples during storage. At cold temperatures, the firmness of the fruits is retained for a longer period, the coated samples in this study showed no difference with control samples.

Water loss and firmness are correlated, water loss leads to increase firmness during storage (Mannozzi et al., 2017). The softening of the fruit is occurred due to cell turgor loss stimulated by transpiration and enzymatic hydrolysis of the cell wall. The study showed that citric acid coated samples had a high water loss and caused partial dehydration of the tissue (Rocculi et al., 2007). Similarly, it might have affected the outer cell wall structure of the blueberries. Figure 4.2 shows the lower firmness in coated samples than control samples on the 0th day itself. While another study showed that calcium chloride along with citric acid in polysaccharides showed good firmness during storage (Ribeiro, Vicente, Teixeira, & Miranda, 2007). The concentration of novel gel has high water permeability which caused changes in the firmness of the fruit. The addition of glycerol also had an insignificant difference with control samples. Usually, the addition of plasticizers helps in the retention of firmness. The results from this study suggest that a higher concentration of plasticizers might help with moisture barrier property. The visual appearance of the blueberries coated with novel gel showed shrinkage of the berries under cold storage and uncoated berries had a good firm structure. On the 14th day, the firmness values of three treatments were almost similar and showed no significant difference. Thus, novel gel showed an insignificant difference in firmness during storage and is not suitable as a coating material in terms of firmness.

5.4 pH

The pH in the blueberry defines the acidity present in them. Blueberries are a rich source of vitamin C. The acids in the fruit release hydrogen ions and have a sour flavour to them. Table 4.2 represents the pH of the novel gel treated and control blueberries. There was no significant difference between the control and gel treated samples. As the fruit matures the pH increases with time. Edible coatings delay the maturation of the fruit and thus lower values of pH are expected over storage. The study showed that edible film coated blueberries had lower pH which delayed the fruit ripening process (Abugoch et al., 2016; Tahir et al., 2020). However, the novel gel had no significant difference between the control sample and coated samples, which shows that the novel gel had timely fruit maturation.

Similarly, polysaccharide coating on blueberries had no significant difference observed between control and coated samples (Mannozzi et al., 2017). The pH increases due to metabolic reactions, converts starch and acids to sugars (Tahir et al., 2020). As the pH increases, there is the formation of alkaline autolysis compounds and deteriorates the blueberry (Vieira et al., 2016). A significant difference was observed in terms of days where the pH value was increasing. The increment in pH causes microbial growth and leads to spoilage. Thus, a novel gel is not effective in controlling the pH of the blueberry as citric acid in the gel increases the acidity of the blueberry juice. Citric acid is an anti-browning agent that inhibits polyphenol oxidase (PPO) by controlling pH and binds copper ions to an active site of PPO to form an inactive complex (Shahkoomahally & Ramezanian, 2014). However, the present study had contrasting results.

5.5 Total Soluble Solids

TSS represents the sugar content in the fruit which is measured by Brix. As the fruit ripens, the metabolic activity of the fruit increases, resulting in high pH and TSS over time. TSS results in less acidic and sweeter flavour in the fruit. Table 4.3 represents the TSS of the coated and uncoated samples. There was a significant difference observed between control samples and coated berries. Control samples have high TSS value than coated samples. The results obtained for TSS is unreasonable as there was no difference in pH values during storage, it was increasing, showing fruit maturation. While TSS shows the difference with control samples, which also represents the fruit maturation. As there is water loss, the concentration of sugar increases (Vieira et al., 2016). It was observed with our study that uncoated berries showed high TSS. The study showed no difference in control and coated berries in TSS and pH as they both are interlinked (Medina-Jaramillo, Quintero-Pimiento, Díaz-Díaz, Goyanes, & López-Córdoba, 2020). In similar to our study, TSS of control samples were higher than coated samples during storage (Eldib, Khojah, Elhakem, Benajiba, & Helal, 2020). Thus, the novel gel can help in lowering the metabolic activity of fruit.

Chapter 6

Conclusion

In this study, we investigated the physiochemical parameters of the blueberry when it was coated with novel gel. The parameters assessed for analysing the effectiveness of the novel gel were weight loss, colour, texture, pH, and total soluble solids. There was no significant difference observed in weight loss when coated berries were compared with control. Also, firmness and pH had no difference between control and coated samples. The control samples showed a higher TSS value than coated berries. The colour parameters differed on the 0^{th} day, 7^{th} day, and 14^{th} day. The control group and novel gel added with glycerol group had no difference while analysing the colour parameter. The L*, a*, and b* values differed in terms of days.

The effective gel should have good water vapour permeability and should have a barrier to water and gases. It should delay the ripening and senescence of fruit. The addition of glycerol did show slight variation and needs to be studied further. Citric acid plays a great role in coating material as it has antimicrobial and anti-browning qualities. The citric acid in coating material can change the pH of the fruit and TSS. Temperature and atmospheric conditions play a major role during the storage of coated samples. The addition of lipophilic or hydrophilic components in novel gel would make it effective. Overall, the novel gel was ineffective as a coating material in blueberries and need further modification to be effective.

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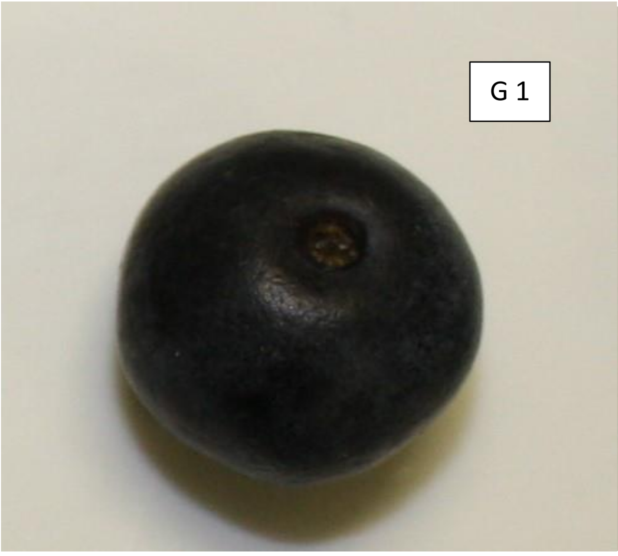
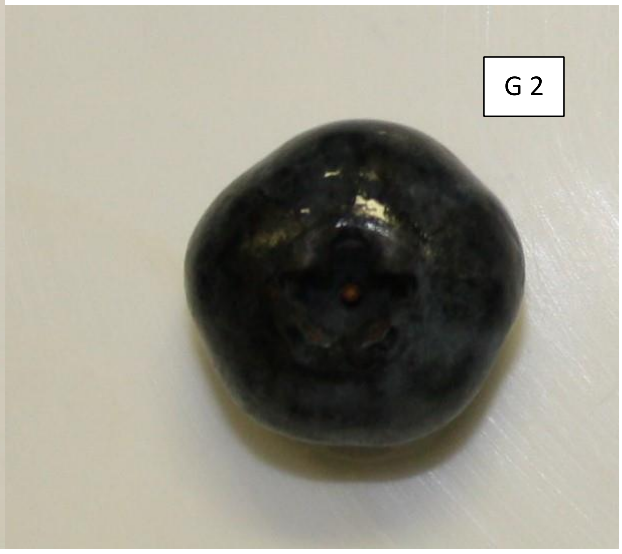
Appendix A

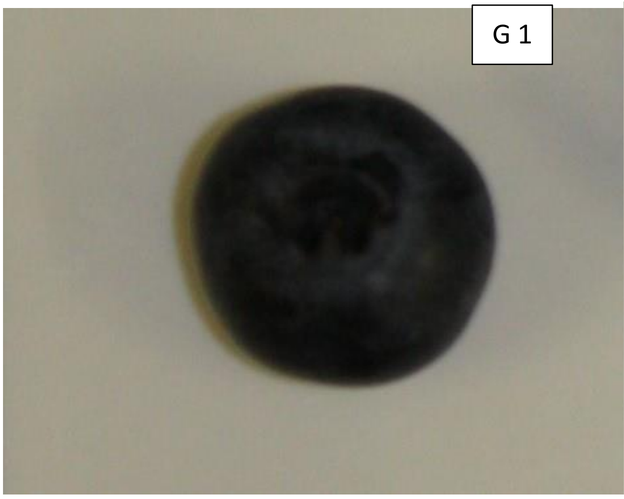
Pictures of blueberries during storage

A.1 Blueberries on the 0th day

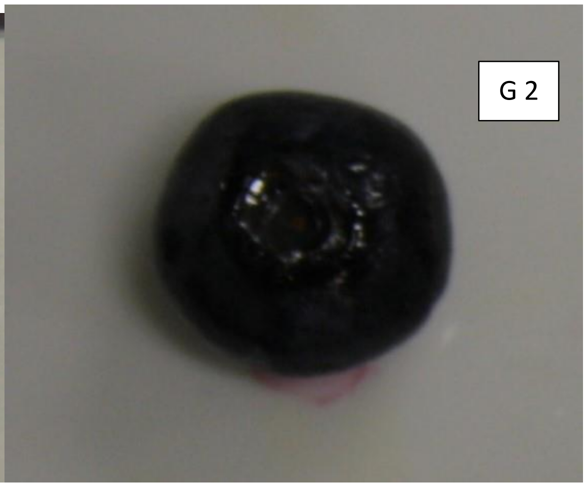




A.2 Blueberries on the 7th day



A.3 Blueberries on the 14th day



Appendix B

Raw Data of different parameters

B.1 Weight loss

Treatments	Day	Sample	Mean Weight loss
G 1	0	1	0.783
G 1	0	2	0.994
G 2	0	1	1.313
G 2	0	2	1.26
G 3	0	1	1.358
G 3	0	2	1.246
G 1	7	1	0.76
G 1	7	2	0.97
G 2	7	1	1.191
G 2	7	2	1.2
G 3	7	1	1.185
G 3	7	2	1.235
G 1	14	1	0.64
G 1	14	2	0.88
G 2	14	1	1.145
G 2	14	2	1.197

G 3	14	1	1.133
G 3	14	2	1.122

B.2 Colour

Treatments	Day	Sample	L*	a*	b*
G 1	0	1	27.34	0.09	-1.48
G 1	0	2	31.4	-0.06	-0.68
G 1	0	3	27.66	-0.02	-1.98
G 1	0	4	29.22	-0.26	-3.02
G 1	0	5	26.58	0.32	-0.74
G 1	0	6	26.98	-0.1	-1.52
G 1	0	7	26.2	0.56	-1.14
G 1	0	8	27.02	-0.07	-1.53
G 1	0	9	28.21	0.37	-2.35
G 1	0	10	29.02	0.68	-2.3
G 1	0	11	31.4	-0.35	-1.86
G 1	0	12	25.37	-0.22	-0.77
G 1	0	13	24.61	0.93	-0.85
G 1	0	14	26.62	0.01	-1.25
G 1	0	15	27.86	-0.48	-3.64
G 1	0	16	27.67	1.02	-1.35

G 1	0	17	28.18	0.45	-0.22
G 1	0	18	25.89	0.65	-0.72
G 1	0	19	28.65	0.19	-2.99
G 1	0	20	28.94	-0.42	-2.14
G 1	7	1	8.62	8.84	12.01
G 1	7	2	5.11	5.21	6.1
G 1	7	3	7.24	7.41	9.6
G 1	7	4	6.66	6.84	8.99
G 1	7	5	5.97	5.99	7.68
G 1	7	6	6.34	6.49	8.46
G 1	7	7	6.71	6.79	8.58
G 1	7	8	8.43	8.67	11.86
G 1	7	9	8.04	8.28	11.22
G 1	7	10	6.29	6.25	7.72
G 1	7	11	8.73	8.87	11.17
G 1	7	12	5.77	5.9	7.34
G 1	7	13	5.51	5.67	7.79
G 1	7	14	6	6.17	7.88
G 1	7	15	6.38	6.56	8.54
G 1	7	16	4.55	4.64	6
G 1	7	17	7.7	7.92	10.8
G 1	7	18	8.03	8.2	10.71

G 1	7	19	7.11	7.19	8.88
G 1	7	20	7.01	7.2	9.8
G 1	14	1	7.49	7.69	9.96
G 1	14	2	11.38	11.58	14.66
G 1	14	3	7.46	7.63	9.56
G 1	14	4	7.99	8.19	10.36
G 1	14	5	7.4	7.59	9.8
G 1	14	6	8.9	9.02	11.22
G 1	14	7	7.84	7.9	9.82
G 1	14	8	6.32	6.46	8.19
G 1	14	9	7.31	7.46	9.3
G 1	14	10	7.4	7.56	9.13
G 1	14	11	7.19	7.2	9.13
G 1	14	12	9.31	9.49	12.72
G 1	14	13	10.48	10.73	13.41
G 1	14	14	8.91	9.06	11.73
G 1	14	15	8.22	8.44	11.05
G 1	14	16	7.71	7.73	9.61
G 1	14	17	7.42	7.6	9.55
G 1	14	18	9.87	10.12	13.26
G 1	14	19	8.04	8.22	10.02
G 1	14	20	8.01	8.2	10.43

G 2	0	1	22.57	1.01	0.08
G 2	0	2	26.26	-0.49	-1.78
G 2	0	3	22.45	2.4	1.63
G 2	0	4	25.97	-0.23	-1.57
G 2	0	5	26.99	-0.26	-1.41
G 2	0	6	23.77	-0.35	-1.94
G 2	0	7	27.88	-0.2	-3.08
G 2	0	8	28.36	-0.48	-3.66
G 2	0	9	29.79	0.3	-3.7
G 2	0	10	21.81	-0.1	-0.67
G 2	0	11	24.29	-0.1	-0.69
G 2	0	12	27.24	0.16	-1.31
G 2	0	13	27.5	0.85	-1.74
G 2	0	14	29.58	-0.64	-3.15
G 2	0	15	20.1	0.35	-2.31
G 2	0	16	24.43	0.09	-1.23
G 2	0	17	25.4	-0.27	-1.07
G 2	0	18	26.88	-0.28	-1.47
G 2	0	19	19.37	3.27	-0.05
G 2	0	20	26.06	-0.25	-1.74
G 2	7	1	7.55	7.71	10.03
G 2	7	2	6.01	6.15	7.83

G 2	7	3	6.54	6.69	8.46
G 2	7	4	8.17	8.37	11.14
G 2	7	5	6.49	6.65	8.6
G 2	7	6	6.36	6.44	7.36
G 2	7	7	6.99	7.16	8.99
G 2	7	8	9.45	9.73	13.18
G 2	7	9	6.4	6.52	7.91
G 2	7	10	11.49	11.75	13.97
G 2	7	11	5.92	6.06	8.45
G 2	7	12	7.57	7.73	9.63
G 2	7	13	4.19	4.19	5.12
G 2	7	14	7.43	7.58	10.02
G 2	7	15	8.16	7.38	9.58
G 2	7	16	7.02	7.19	8.93
G 2	7	17	5.25	5.34	6.54
G 2	7	18	5.7	5.57	6.54
G 2	7	19	7.33	7.25	9.32
G 2	7	20	8.12	8.3	10.89
G 2	14	1	9.64	9.78	12.74
G 2	14	2	9.38	9.55	12.02
G 2	14	3	8.97	8.7	10.22
G 2	14	4	8.87	8.92	11.6

G 2	14	5	12.05	12.26	14.45
G 2	14	6	9.79	9.84	12.67
G 2	14	7	8.98	9.03	11.43
G 2	14	8	9.45	9.36	11.72
G 2	14	9	7.2	7.19	8.73
G 2	14	10	8.72	8.65	10.63
G 2	14	11	8.47	8.66	11.28
G 2	14	12	8.53	8.71	11.08
G 2	14	13	11.65	11.91	14.29
G 2	14	14	7.82	7.73	10.12
G 2	14	15	8.9	9.06	11.69
G 2	14	16	7.35	7.35	8.97
G 2	14	17	7.31	7.26	8.91
G 2	14	18	8.55	8.54	10.08
G 2	14	19	7.82	7.98	9.94
G 2	14	20	7.55	7.62	9.28
G 3	0	1	28.68	0.48	-2.29
G 3	0	2	25.88	0.22	-0.4
G 3	0	3	25.42	0.34	0.2
G 3	0	4	25.55	-0.06	-3.26
G 3	0	5	22.74	0.12	-1.47
G 3	0	6	23.2	1.77	-0.34

G 3	0	7	24.56	0.41	-0.96
G 3	0	8	30.85	-0.09	-2.35
G 3	0	9	26.97	1.2	0.09
G 3	0	10	21.53	0.2	-1.93
G 3	0	11	27.18	1.14	0
G 3	0	12	28.61	-0.28	-1.68
G 3	0	13	28.51	-0.5	-2.04
G 3	0	14	25.82	-0.08	-0.5
G 3	0	15	28.52	-0.16	-1.69
G 3	0	16	27.72	-0.35	-5.27
G 3	0	17	22.11	-0.14	-1.54
G 3	0	18	28.23	-0.08	-2.67
G 3	0	19	20.08	0.31	-2.45
G 3	0	20	22.08	0.52	-1.66
G 3	7	1	4.63	4.74	6.19
G 3	7	2	6.29	6.43	8.44
G 3	7	3	4.43	4.46	5.54
G 3	7	4	6.24	6.35	8.1
G 3	7	5	3.55	3.6	4.46
G 3	7	6	5.36	5.5	7.18
G 3	7	7	6.97	7.15	9.74
G 3	7	8	3.59	3.67	5.02

G 3	7	9	3.91	3.95	5.02
G 3	7	10	3.85	3.92	5.2
G 3	7	11	7.72	7.85	9.82
G 3	7	12	5.28	5.35	6.72
G 3	7	13	4.34	4.42	5.49
G 3	7	14	5.15	5.24	7.41
G 3	7	15	7.95	7.92	9.33
G 3	7	16	5.19	5.26	6.58
G 3	7	17	5.97	6.12	7.99
G 3	7	18	6.41	6.47	7.89
G 3	7	19	7.85	7.89	9.84
G 3	7	20	8.19	8.32	10.23
G 3	14	1	8.7	8.83	10.58
G 3	14	2	8.44	8.49	10.88
G 3	14	3	6.68	6.56	7.78
G 3	14	4	5.91	5.7	7.71
G 3	14	5	5.45	4.81	5.68
G 3	14	6	7.11	7.06	8.8
G 3	14	7	10.91	11.2	15.31
G 3	14	8	5.82	5.38	6.2
G 3	14	9	9.09	9.21	11.2
G 3	14	10	5.33	5.42	6.57

G 3	14	11	8.56	8.77	11.79
G 3	14	12	6.6	6.3	7.69
G 3	14	13	6.43	6.47	8.1
G 3	14	14	6.64	6.62	7.91
G 3	14	15	9.19	9.37	11.59
G 3	14	16	4.77	4.76	5.74
G 3	14	17	8.81	9	11.5
G 3	14	18	5.54	5.57	7.1
G 3	14	19	10.95	10.89	14.48
G 3	14	20	5.06	4.85	5.68

B.3 Firmness

Treatments	**Day**	**Sample**	**Firmness**
G 1	0	1	113.937
G 1	0	2	87.942
G 1	0	3	127.726
G 1	0	4	176.765
G 1	0	5	166.024
G 1	0	6	271.905
G 1	0	7	233.816
G 1	0	8	262.022

G 1	0	9	120.859
G 1	0	10	156.097
G 1	0	11	209.263
G 1	0	12	168.181
G 1	0	13	224.572
G 1	0	14	251.765
G 1	0	15	345.331
G 1	0	16	220.643
G 1	0	17	238.438
G 1	0	18	177.436
G 1	0	19	312.734
G 1	0	20	146.578
G 1	7	1	210.204
G 1	7	2	246.689
G 1	7	3	168.777
G 1	7	4	270.418
G 1	7	5	67.753
G 1	7	6	206.803
G 1	7	7	302.742
G 1	7	8	214.243
G 1	7	9	74.566
G 1	7	10	208.905

G 1	7	11	200.628
G 1	7	12	51.904
G 1	7	13	235.848
G 1	7	14	68.766
G 1	7	15	191.394
G 1	7	16	91.438
G 1	7	17	190.338
G 1	7	18	51.112
G 1	7	19	148.130
G 1	7	20	176.833
G 1	14	1	211.295
G 1	14	2	247.162
G 1	14	3	201.852
G 1	14	4	252.544
G 1	14	5	94.703
G 1	14	6	45.552
G 1	14	7	51.814
G 1	14	8	236.201
G 1	14	9	146.737
G 1	14	10	44.121
G 1	14	11	68.245
G 1	14	12	139.880

G 1	14	13	134.157
G 1	14	14	195.832
G 1	14	15	163.993
G 1	14	16	213.881
G 1	14	17	212.099
G 1	14	18	283.546
G 1	14	19	229.983
G 1	14	20	48.942
G 2	0	1	151.112
G 2	0	2	242.609
G 2	0	3	79.281
G 2	0	4	130.521
G 2	0	5	45.473
G 2	0	6	125.558
G 2	0	7	101.666
G 2	0	8	111.339
G 2	0	9	122.257
G 2	0	10	238.262
G 2	0	11	80.547
G 2	0	12	55.807
G 2	0	13	77.025
G 2	0	14	47.784

G 2	0	15	75.187
G 2	0	16	68.419
G 2	0	17	184.645
G 2	0	18	138.885
G 2	0	19	29.945
G 2	0	20	214.590
G 2	7	1	88.169
G 2	7	2	288.445
G 2	7	3	98.063
G 2	7	4	206.319
G 2	7	5	46.489
G 2	7	6	59.961
G 2	7	7	86.936
G 2	7	8	186.343
G 2	7	9	89.182
G 2	7	10	23.520
G 2	7	11	243.299
G 2	7	12	123.454
G 2	7	13	285.000
G 2	7	14	229.332
G 2	7	15	196.226
G 2	7	16	253.479

G 2	7	17	229.552
G 2	7	18	92.120
G 2	7	19	175.557
G 2	7	20	235.308
G 2	14	1	276.613
G 2	14	2	105.389
G 2	14	3	282.050
G 2	14	4	124.495
G 2	14	5	71.349
G 2	14	6	308.870
G 2	14	7	216.809
G 2	14	8	138.373
G 2	14	9	223.886
G 2	14	10	145.592
G 2	14	11	180.403
G 2	14	12	345.431
G 2	14	13	282.952
G 2	14	14	224.073
G 2	14	15	76.764
G 2	14	16	195.513
G 2	14	17	295.190
G 2	14	18	138.450

G 2	14	19	104.156
G 2	14	20	67.805
G 3	0	1	17.146
G 3	0	2	121.706
G 3	0	3	150.650
G 3	0	4	114.861
G 3	0	5	31.937
G 3	0	6	126.042
G 3	0	7	104.483
G 3	0	8	149.890
G 3	0	9	143.519
G 3	0	10	231.428
G 3	0	11	145.995
G 3	0	12	48.852
G 3	0	13	117.898
G 3	0	14	81.823
G 3	0	15	74.483
G 3	0	16	121.596
G 3	0	17	108.379
G 3	0	18	168.379
G 3	0	19	207.690
G 3	0	20	57.029

G 3	7	1	120.769
G 3	7	2	262.482
G 3	7	3	199.506
G 3	7	4	175.392
G 3	7	5	225.722
G 3	7	6	78.935
G 3	7	7	162.922
G 3	7	8	49.461
G 3	7	9	70.306
G 3	7	10	26.668
G 3	7	11	247.459
G 3	7	12	150.771
G 3	7	13	99.351
G 3	7	14	82.512
G 3	7	15	155.119
G 3	7	16	162.129
G 3	7	17	215.674
G 3	7	18	113.406
G 3	7	19	166.389
G 3	7	20	137.465
G 3	14	1	341.028
G 3	14	2	196.592

G 3	14	3	233.262
G 3	14	4	468.197
G 3	14	5	113.401
G 3	14	6	51.000
G 3	14	7	45.145
G 3	14	8	191.122
G 3	14	9	334.260
G 3	14	10	45.101
G 3	14	11	140.486
G 3	14	12	260.886
G 3	14	13	130.834
G 3	14	14	49.283
G 3	14	15	165.468
G 3	14	16	38.255
G 3	14	17	35.449
G 3	14	18	223.709
G 3	14	19	88.495
G 3	14	20	154.441

B.4 pH

Treatments	**Day**	**pH**

G 1	0	2.85
G 1	0	2.66
G 2	0	2.95
G 2	0	2.41
G 3	0	2.87
G 3	0	2.96
G 1	7	3.07
G 1	7	2.98
G 2	7	3.2
G 2	7	3.1
G 3	7	3.18
G 3	7	3.11
G 1	14	3.26
G 1	14	3.13
G 2	14	3.11
G 2	14	3
G 3	14	3.24
G 3	14	3.16

B.5 TSS

Treatments	**Day**	**TSS**

G 1	0	13.2
G 1	0	13.1
G 2	0	10.5
G 2	0	10.4
G 3	0	10
G 3	0	10.1
G 1	7	11.3
G 1	7	11.4
G 2	7	10.4
G 2	7	10.6
G 3	7	11.1
G 3	7	11.3
G 1	14	14.2
G 1	14	14.3
G 2	14	12.9
G 2	14	13
G 3	14	14
G 3	14	14.2