EFFECT OF VACUUM IMPREGNATION PROCESS ON IRRADIATED BLUEBERRIES (*VACCINIUM CORYMBOSUM L.)*

A Thesis

by

HUIZHU TONG

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Elena Castell-Perez
Committee Members,	Rosana Moreira
	Stephen Talcott
Head of Department,	Stephen Searcy

May 2017

Major Subject: Biological & Agricultural Engineering

ABSTRACT

Blueberries have many health benefits such as high content of anthocyanins, phenols and other antioxidants, which could prevent cancer and cardiovascular diseases. However, the texture of blueberries can easily deteriorate during handling, processing and distribution, which leads to food loss. This is a particular problem when blueberries are irradiated as a treatment for food safety purposes.

This research evaluated the effects of vacuum impregnation on the texture and quality of irradiated blueberries. Moisture content, texture, color, total soluble solids, pH, total titratable acidity, and total phenol amount were evaluated on Day 0 (the day of the treatment), Day 7, and Day 14 of storage at 4.5-5ºC, 50-55%RH. A consumer test was also conducted.

Fresh blueberries were vacuum impregnated with calcium lactate solution at 4%, 5%, and 6% concentration (w/w). Results showed that under 160 mm Hg bar pressure and 8% solid-liquid ratio (blueberries-impregnation solution), the 4% calcium lactate solution with two-step vacuum impregnation process yielded blueberries with firmer ($P<0.05$) texture. Blueberries were initially exposed to vacuum for 5 mins followed by 5 mins at atmospheric conditions. A second step consisted of 10 mins vacuum followed by 10 mins at atmospheric conditions. Overall, vacuum impregnation treatment did not affect ($P>0.05$) moisture content, pH or other quality attributes and consumers equally liked vacuum impregnated blueberries and the fresh samples.

On a second study, blueberries impregnated with 4% calcium lactate solution were irradiated using a 1.35 MeV electron beam irradiator. The irradiation doses tested ranged from 0.0 kGy (control) to 2.0 kGy. Irradiation did not ($P>0.05$) affect the pH or total phenolic amount of blueberries. Although vacuum impregnated blueberries were firmer ($P<0.05$) than non-VI treated blueberries under the same irradiation dosage, fruit firmness was affected ($P<0.05$) by exposure to the electron beams. This negative effect was more significant at higher dose levels. On the other hand, when exposed to the maximum allowed dose for fresh produce (1.0 kGy), vacuum impregnation helped maintain the texture of the blueberries ($P>0.05$) throughout the refrigerated storage while the controls showed considerable softness.

DEDICATION

To my parents, Bo Tong and Meixia Sun, thanks for raising me up with all your love, patience and support. I feel so blessed to be your daughter.

To my boyfriend, Jianping Su, thanks for your support and company on my journey of graduate school.

To my academic advisor, Elena M. Castell, appreciate all the guidance and patience you provided. I would not get my degree without your guidance. You are the mentor who showed me food engineering is fun and exciting.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Castell, and my committee members, Dr. Moreira and Dr. Talcott, for their guidance and support throughout the course of this research. And my friend Basri Omac, who helped me conduct E-beam irradiation treatment on west campus.

Thanks also go to my lab friends, Paulo Da Silva, Mauricio Martinon, Silvia Lopez, Zeynep Yurttas, Brinda Joshi, Samar Almohaimeed and staff for making my time at Texas A&M University a great experience.

Finally, thanks for my internship mentor Karen Kane and my intern friends from Kellogg Company, especially Yuda Ou and Iva Na. They helped me correct my English while I was working on my thesis.

You guys are awesome!

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Professor Dr. Castell, and Professor Dr. Moreira from the Department of BAEN and Professor Dr. Talcott of Nutrition and Food Science Department. All work for the thesis was completed independently by the student.

Funding sources

There are no outside funding contributors to acknowledge related to the research and complication of the document.

NOMENCLATURE

VI	Vacuum impregnation
IR	Irradiation
TTA	Total titratable acidity
TPH	Total phenolic amount
TSS	Total soluble solids
Aw	Water activity

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

The US fresh fruits consumption market is expected to have a 9% increase in the next 5 years due to a high demand (Better Health Foundation 2015). Most fruits and vegetables are excellent sources of natural antioxidants, vitamins, and minerals, which have positive impacts on diseases such as cancer and cardiovascular diseases (Steinmetz and Potter 1996; Smith and others 2000). According to the US Highbush Blueberries Council (USHBC) report, in 2016, 43% of consumers say that blueberries make a menu item more attractive, and nearly 58% of consumers believe that menus containing blueberries are healthier. Overall, blueberry mentions on American menus have increased 97% from 2007 to 2013 – a stronger growth rate than that of strawberries, raspberries or blackberries. However, fresh blueberries have a short shelf life due to their high moisture content and sugars, which induces microbial survival and limited marketability. Furthermore, the thin fruit skin is easily ruptured and increasing permeability for microbial infection.

Food irradiation is an important non-thermal technology in terms of safety and shelf life prolonging. It could kill the microorganisms on food surface as well as within its irradiation penetration depth. However, food irradiation will deteriorate food texture and other possible quality attributes because irradiation could decompose sugar, water and other substance in food matrix. Currently, most of the studies focused on using food irradiation as a single methodology to prolong the shelf life and keep food safe. Studies

of combing this technology with others are rare. Thus, minimizing the irradiation undesirable effects becomes critical when applying this technology.

Vacuum impregnation is a technology applied in food systems to keep food texture and add supplemental nutritional values to food. It is a process which exchanges the internal gas or liquid with outside liquid because of the hydrodynamic mechanisms promoted by pressure and concentration changes. When an ideal solution (functional purpose based solution) migrated to the food samples, the food matrix could be modified and achieve the ideal functionality.

This project aimed to evaluate the effectiveness of vacuum impregnation as a pre-treatment to improve the texture of irradiated blueberries while maintaining their total phenolic content and extend fresh produce shelf life.

The main hypotheses of this study were tested by pursuing the following objectives:

1. Optimize the vacuum impregnation (VI) process in terms of product quality attributes, such as moisture content and water activity, firmness, color, pH and titratable acidity, sugar content, and total phenolic content.
2. Quantify the effects of the vacuum impregnation (VI) pre-treatment on the quality of fresh blueberries and irradiated blueberries.

1.1 Blueberries

In the market of blueberries, there are mainly two species. The smaller size fruit species are known as "lowbush blueberries" (*Vaccinium angustifolium Ait.*), which are usually found in wild. While the larger size fruit species are known as "highbush blueberries"(*Vaccinium corymbosum L.*), which are often being cultivated. The most common cultivars are “Bluecrop”, “Duke”, “Jersy”, “Elliott”, “Sharpblue” and “Liberty”. Both these two species of blueberries are nutritious and have high content of antioxidants, such as calcium (6mg/100g fresh fruit), vitamins (A&C, >10mg/100g fresh fruit) and phenolic contents (Appendix A), which include anthocyanins, flavonols, chloruretic and proanthocyanidins. Studies have shown that the consumption of blueberries could prevent several diseases due to the antioxidants blueberries have. Phenolic extracts from berries could inhibit producing low-density lipoprotein, which is considered bad for health (Rimando and others, 2005). Additionally, blueberry consumption has protective effects against cancer and vascular diseases (Neto, 2007) and enhances brain function in healthy elderly participants (Bowtell and others, 2015). Moreover, blueberries, blackberries, and raspberries, have the ability to scavenge free radicals in the human body, which could delay senescence (Wang and Lin, 2000; Koca and Karadeniz, 2009). However, fresh berries have a shorter shelf life compared with other fruits, such as apples and peaches. Hancock and others (2008) found that fresh highbush blueberries usually have a shelf life of 1–8 weeks depending on stage of fruit ripeness, method of harvest, presence of fruit disease, and storage conditions (temperature, relative humidity, and atmosphere). In this

project, when local blueberries were stored at 4.5 ± 0.5 ^{o}C and 50 - 55% relative humidity in refrigerator, samples could have a 15-day shelf life without showing mold.

1.2 Food irradiation

Ionizing radiation (IR) is an important non-thermal processing technology to keep food safety. It is also known as “cold pasteurization”. It can minimally affect the food quality comparing with some other non-thermal and thermal processing methods, such as canning, heating, and fermentation & germination. These processes destroy the food texture or change food image as they are “cooking” the food somehow. Moreover, these methods cause nutritional losses especially for heating sensitive nutrients, such as phenolic and vitamin C (Cleland 1983). Hence, food irradiation (IR) is an ideal treatment to keep food safety has been fast developed in the past several decades.

1.2.1 Mechanism of killing pathogens

Irradiation energy can kill foodborne pathogens directly and indirectly. It can break microorganism’s DNA strains to kill bacteria straightforwardly. In a direct way, a photon or electron can strike the DNA’s strain and cause a lesion in DNA. However, striking one single strain of DNA is not lethal as DNA can repair itself. When the orientation of the DNA strain is appropriate, the energy can break both strands of DNA,

which is lethal as repairing the double-strand lesions is beyond the ability for all biological systems (Grecz and others 1983; Monk and others 1995).

In addition, electrons and photons can induce water ionization to damage microorganisms' genetic material to kill pathogens indirectly. The working mechanism of killing pathogens indirectly is by irradiating water. Water is the most common molecule adjacent to the genetic material. Exposed to irradiation, water molecules lose one electron and produce some reactive components, such as hydrogen (H^+), hydroxyl radicals (OH^-), molecule hydrogen (H) and oxygen (O), as well as hydrogen peroxide (H_2O_2) (Arena 1971). These hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2) can react with the nucleic acids which would cause DNA damages. Therefore, irradiation process kills pathogens.

1.2.2 Types of irradiation processes

There are three types of IR that can be applied in food products: electron beam IR, X-ray IR and gamma IR. Table 1 compares these three types of IR. Electron-beam (E-beam) IR is an ideal treatment as it can provide high efficiency and high throughput. It also has superior dose rate which is time saving for treatment process. Moreover, the cost of E-beam irradiation is less expensive than gamma ray irradiation because it is machine generated. However, the E-beam IR can sometimes produce X-rays when the electrons hit heavy metal materials (Cleland 1983).

Table 1 Differences among different types of irradiation processes (Adapted from Cleland 1983)

	E-beam	X-ray	Gamma
Source	Electrons	Electrons hit heavy metals	Co-60 neutron
Generate type	Machine	Machine	Nature
Basic substance	Electrons	Photons	Photons
Energy (MeV)	10	5 to 7	1.17 to 1.33
Throughput	+++	++	+
Dose uniformity	+	+++	++
Dose rate	+++	++	+
Treatment time	seconds	minutes	hours
Processing cost	+	++	+++

Note: Low (+); Medium (++); High (+++).

1.2.3 Irradiation dose

Absorbed dose is defined as the energy imparted by IR to the matter per mass unit. Its unit is Gray (Gy), which 1 Gy is equal to 1 joule (J) of energy absorbed by 1 kg of matter. Usually, kGy is also widely used where 1kGy equals to 1000 Gy. Different doses yield different effects. Low dose E-beam irradiation, in which the dose is less than 1 kGy, can inhibit vegetable sprout and insect infestation. For example, potatoes are usually being treated under IR to prohibit sprout. Medium dose, a dose between 1 to 10 kGy, can extend the shelf life of food as well as control foodborne pathogens. Dry seasoning spices can always being treated under medium dosage. Kirkin and others (2014) found that Gamma ray irradiated thyme and black pepper on a dose of 7kGy can effectively kill pathogens. When IR dose was higher than 12 kGy, microbial could been hardly seen. Finally, when the dose is higher than 10 kGy, the food is sterilized and could be used for space food. In addition, high dose irradiation is also being used in hospital facilities' sterilization (Molins 2001).

1.2.4 Historical notes on food irradiation

Food irradiation has been developed over one hundred years. In 1905, a British patent was issued for the use of ionizing radiation to kill bacteria in foods. Later in 1920s to 1930s, publications on the effects of ionizing radiation on enzymes first appeared, and studying the toxicology of irradiated foods was developed. Since many electron accelerator machines were developed over that time, food irradiation was researched more. During the World War II, U.S. Army started to use X-ray to process ground beef. U.S

government, industry, universities and private institutions began to get involved with food irradiation. Shortly after the war, there was widespread distribution of pasteurized fluid milk labeled irradiated in U.S market because it had been treated with infrared light to develop Vitamin D from the precursors. In 1958, U.S. Food Additives Amendment classified food irradiation as an "additive" and soon after in 1959, the Soviet Union approved irradiation of potatoes and grains. Because food irradiation was studied that it was effective on shelf-life extension and pathogen killing, Japan approved the irradiation of potatoes on an industrial scale in 1973. Later on the 1980s, World Health Organization (WHO) declared that "irradiation of any food commodity up to an overall average dose of 10 KGy presents on no toxicological hazards". In 1986, U.S. FDA approved irradiation to delay maturation, to inhibit growth, and to disinfect food, including vegetables and spices. In 1990, FDA approved irradiation of poultry to control salmonella contamination. In 1997, FDA's regulation permitted the irradiation to treat frozen uncooked meat and meat byproducts to control foodborne pathogens. Finally, in 2000, FDA announced that irradiation can be used for control of Salmonella in shell life stable food and for decontamination of seeds for sprouting (Molins 2001). Because of an increase in the demand for irradiation-sterilized foods brought to the military and hospital need, irradiation is being currently studied more intensively (Diehl 2002; Gould 2012; Feng and others, 2016)

1.2.5 Irradiation effects on blueberries

Eaton and others (1970) found that different cultivars had the different physiological response of high-bush blueberries to gamma irradiation at doses from 1.0 to 5.0 kGy. Generally, the higher the dose was applied, the less firm the blueberries presented. Other quality attributes, such as water activity, acidity was not obviously related to the irradiation dose. Later, Miller and others (1995) observed that "Sharpblue" blueberries could tolerate electron beam irradiation up to 0.75 kGy without changing the food image ($P>0.05$), such as no changing on soluble solids concentration, acidity, pH, and skin color. Besides, the firmness of blueberries was not affected in the first 3 days of storage at 1°C, however, samples became softer when stored after 7 days. They found that the firmness of blueberries is the most vulnerable quality attribute under irradiation. Hallman and Thomas (1999) reported that irradiation was effective in killing pathogens in blueberries when used for disinfestation purpose. When applying gamma rays to blueberries at 24 Gy level, the blueberry maggots were eliminated by 99%. The maggot is a kind of worms which are available in the field during blueberries harvest season and can cause decay. Thus, controlling blueberries disinfestation by IR could prolong the shelf life. Later, Moreno and others (2007) found that when the E-beam irradiation dose was less than 1.1 kGy, the texture (firmness) of blueberries was not affected for 7 days ($P>0.05$). At higher dose level (>1.1 kGy), blueberries became softer and less acceptable after the first 3 days. However, different dosage level up to 3.2 kGy irradiation did not ($P>0.05$) affect blueberries' density, pH, water activity, moisture content and acidity during the storage. Kong and others (2014) found that that low dose

(<0.75 kGy) of gamma irradiation increased the shelf-life of blueberries and control decay in fruits without influence on quality. Conversely, when Electron-beam irradiation was applied to fresh blueberries, significant decreases ($P<0.05$) in the antioxidant activity was found in both control after 7 days and irradiated blueberries after 14 days storage at 4 °C.

Whenever irradiation is used on a food product, a compromise must be achieved between the dose and food quality. The ideal method would be maximizing the dose to the pathogens while minimizing the loss of produce quality. Determination of the appropriate dose level for electron beam treatment of blueberries without detriment to their quality is therefore essential. Miller and others (1994) noticed that TSS, color, pH and acidity of "Climax" blueberries were not changed for 14 days at 1 ^{o}C under E-beam irradiation, which the dose was up to 1.25 kGy. Similarly, Golding and others (2014) observed that low dose gamma irradiation (<1 kGy) did not yield TSS change ($P>0.05$) of the "Brigitta" blueberries. They also noticed that overall quality, firmness, weight loss, acidity and pH were not affected either ($P>0.05$). Tong and others (2015) found that, in general, color, weight loss, and TSS was not different ($P>0.05$) after irradiation when the dose was up to 0.6 kGy. However, the firmness was different among different varieties as well as the irradiation dose.

Ehlermann (2016) found that production and commercialization of wholesome food by the application of electron beam irradiation can extend shelf-life and improve disinfestation treatments of meat and vegetables. He points out that irradiation is typically applied in combination with other treatments to improve food quality. To date, there is

lack of information about the effects of electron beam irradiation combing with another non-thermal technology on blueberries.

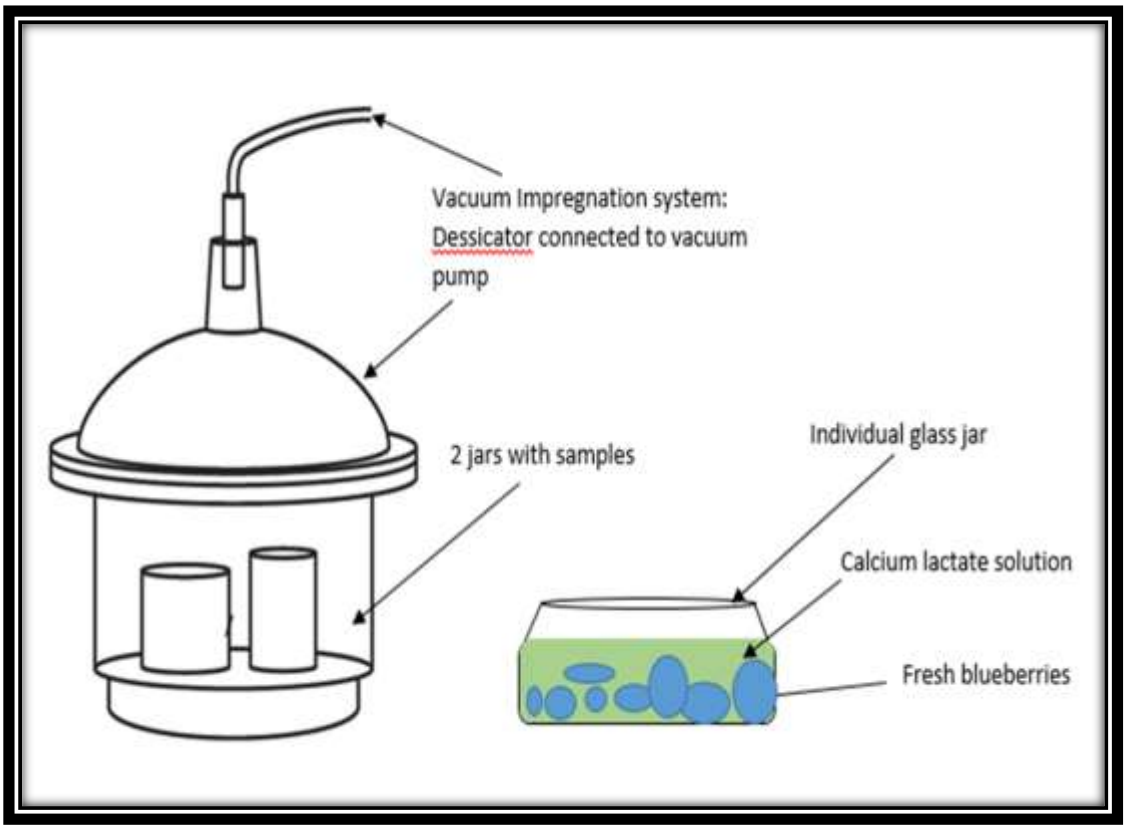
1.3 Food vacuum impregnation

1.3.1 Vacuum impregnation process

Vacuum impregnation is a technology that allows an ideal solution to penetrate into the product pores as a gentle non-thermal treatment, where an ideal solution always has some specific functions, such as texture enhancer and color preservative. Vacuum impregnation could modify the composition of the food matrix through partial water removal and also new solution adding. The process was driven by both osmotic gradient between the sample and solution and pressure gradient. Also, vacuum impregnation could save energy by removing some moisture from the product. In other words, if the product needs a post dry processing step, it would require less energy to remove water (Zhao and Xie 2004).

First, the product is immersed into an ideal solution (functional purpose based solution) and put it into a sealed container (Figure 1). Then, using a vacuum pump to reduce the pressure inside, the gas is allowed to flow out from the product. Second, the pressure inside the container is increased to atmospheric pressure, where the pressure difference would become a driven force for the ideal solution to go into the product structure, the blueberries (Fito and others 2001).

Figure 1 Schematic of a vacuum impregnation system.



1.3.2 Vacuum impregnating solution

Fruit tissue has a structure in which the intercellular spaces may contain a gas or liquid phase and are susceptible to impregnation with an external solution. Hence, vacuum impregnation processing for developing high quality fruits is a feasible technology (Zhao and Xie 2004). Calcium lactate is generally recognized as safe (GRAS) because it is colorless, tasteless, physio-chemical and microscopically stable, and is a texture enhancer element (Gras and others 2003; Anino and others 2006; Moraga and others 2009). Calcium can preserve texture because the calcium ions form cross-links of the pectin chains, which the pectin chains are the support of the cell wall. Thus, increasing the calcium amount could strengthen the cell wall of fruits and improve the structural integrity (Sams, C.E, 1999). Additionally, calcium element is positive charged where most of the texture deteriorated enzymes in the fruits are negative charged. Hence, adding calcium into the fruit tissue could consume the enzymes in some degree and prevent the firmness loss (Poovaiah and others, 2011).

Calcium has been proven to be effective as a firmness enhancer on peach, apple, strawberries, fig fruit and other fresh fruits by either pre-harvest methods, such as spraying and irrigation; or by post-harvest methods, such as vacuum impregnation and dipping treatment (Martin and others 2007; Valero and others 2010).

Calcium lactate has some advantages such as tasteless in comparison with calcium chloride, which is widely used in the food industry as a firming agent in fresh cut commodities. Luna-Guzman and Barrett DM (2000) noticed that both calcium lactate and calcium chloride solutions improved the firmness of fresh cut cantaloupe. However,

calcium chloride induced bitterness. Similarly, Rico and others (2007) noticed that calcium chloride induced some bitterness and off-flavors on carrots due to the chlorine residue on the surface of the product. Hence, calcium lactate is seen more suitable for treating fruits and vegetables.

1.3.3 Vacuum impregnation calcium solution effects on fruits

Gras and others (2003) found that vacuum impregnation of calcium lactate solution on eggplant and carrot did improve the food texture, however, the oyster mushroom was not affected. The authors also noticed that this texture improvement was induced by calcium ions promotes formation of bonds in middle lamellae and cell walls, thus increasing the stiffness and rigidity of cell tissue. Similarly, Rico and others (2006) found that calcium lactate did have a texture improvement effect on carrots. Washing fresh cut carrots with calcium lactate (15 g L−1) at 25 °C and 50 °C (heat-shock) maintained the texture significantly ($p < 0.05$) than the non-treated samples. Irfan and others (2013) studied using 2% and 4% calcium solution to treat dip fig fruit. The 4% calcium chloride was the more effective in maintaining texture, color, titratable acidity, ascorbic acid content and soluble contents during the storage for 14 days, when fruits were stored at 1 ± 0.5 °C, 95–98% RH. The storage life of these treated fig fruits was extended to 14 days as compared to untreated control fruit. Gong and others (2010) found that vacuum impregnation carrots and blueberries with nano-calcium carbonate solution, the solution could go into the inner parts of these two fruits, but for corn and strawberries, the solution remained in the outer part. Yurttas and others (2014) observed that vacuum impregnation

of mushroom slices with a calcium lactate–ascorbic acid solution helped maintain the texture of the irradiated produce.

1.4 Quality attributes

1.4.1 Moisture content and water activity

Water is the most important and basic substance for all biological materials. Water is referred to as bound water and free water. Bound water is always unfreeze and it has high energy hydrogen bond which makes it unavailable as a solvent. Conversely, free water is a good solvent and can be easily removed by drying process. Water activity (Aw) measures how much percentage of free water in the system and it indicates how tightly water is "bound". Scott in 1957 first shaped this idea and later Salwin in 1959 defined water activity as a minimum moisture contents for foods. Water activity is relevant for quality and safety issues as the chemically bound water is not available for microbes. For example, mold could not survive when water activity is below 0.94. In addition, when Aw is lower than 0.6, there is no microbial proliferation. The food system had less unwanted fermentation and undesirable biochemical changes when water activity is low. Additionally, a food product with Aw is less than 0.6 water activity is considered a low moisture product (Fennema, 2000). Usually, the food with a high water activity is render and chewy and low water activity food is dry, rough and hard (Rockland and Stewart 2013).

Moisture content is another important parameter to quantify the water in food. It

described how much water is removed by evaporation (Fennema, 2000). Fruits usually have a moisture content ranging from 80% to 90% and their water activity is above 90%. For fresh blueberries, the moisture differs among different cultivars. In general, the moisture content of the blueberries is approximately about 84% and the water activity is around 96% (USDA 2004).

1.4.2 Color

Pigments in fruits affect their color. There are three types of primary pigments, which are carotenoids, flavonoids, and betalains (Steyn 2012). (1) Carotenoids are long-chain terpenoid hydrocarbons with numerous double bonds, which produce hues from yellow to red. (2) Flavonoids, for example, anthocyanins, which is a precursor for red, blue and purple color depending on fruit pH. Usually high concentrations of anthocyanins will give food a black color. (3) Batalains is a kind of alkaloid which could produce a red to violet color (Willson and others 1990). In blueberries, according to the USDA database (Appendix A), there is a considerable amount of carotenoids (13%) and flavonoids (34%) compared to batalains (<1%).

Color is an important factor that indicates food quality and maturity. It is usually characterized in three terms, lightness (L*), redness to blueness (a*) and yellowness to greenness (b*). For blueberries, different cultivars may display different colors from light blue to deep black (Nunes and others 2004). Also, a higher storage temperature induced blueberries' color changing from blue to red (MatIaCeVIC and Silv 2012). Hernandez and

others (2006) found that calcium dip had delayed strawberries' color changing from green to red. However, very few studies had been focused on if calcium treatment would induce the change of color of blueberries.

1.4.3 Firmness

Appearance and texture are two fundamental characteristics determining the shelf life of fresh fruit and vegetables. The shelf life of blueberries is mainly determined by their firmness, which varies by cultivars and storage conditions, such as temperature, relative humidity, and atmosphere (Hancock and others 2008). The authors observed that the texture of blueberries could remain at most 8 weeks unchanged under storage at 5 ^{o}C when optimizing O_2 and CO_2 concentrations in an appropriate ratio of modifies atmosphere packaging. Duan and others (2011) studied that the calcium caseinate coating did improve ($P<0.05$) the firmness of 'Duke' blueberries under room temperature and extend the shelf life, however, calcium did not affect the "Elliott" blueberries.

Fruits firmness is determined largely by the physical anatomy of the tissue, particularly cell size and shape, cell wall thickness and strength, and the extent of cell-to-cell adhesion, together with turgor status. Many of these factors are inter-related, for example, tissues with smaller cells tend to have a greater content of cell walls and a greater area of cell-to-cell contact. However, it will also induce less intercellular air spaces and cause a stronger cell-to-cell adhesion. Thus, the food tissue is firmer (Toivonen and others 2008).

Fresh produces become softer during storage due to the primary cell wall change and middle lamella change. There are several mechanisms behind, such as enzymatic activity and physical change (Redgwell and Fischer 2002). For example, the endo-polygalacturonase was responsible for tomato softening, which leaded to a lamella integrity loss. Additionally, peaches' softening process was mainly due to depolymerization of hemicellulose and pectin degradation (Toivonen and others 2008).

As texture is a critical quality of fresh blueberries, identifying the best measurement method to quantify texture becomes essential. Texture could be quantified using chewiness, firmness, hardness and other descriptive attributes. For blueberries, the firmness is the most valuable quality attributes as it presents consumer's mouthfeel (Rohrbach and others 1982). Many objective tests were available to evaluate the firmness of fruits, including the uniaxial tension and compression test. In this particular case, tension test was not suitable as blueberries are crispy but not cohesive and do not perform well under tension. Thus, a compression test was considered. TPA test is a kind of compression test, which would conduct double compression to mimic two bites of human beings. However, it was not necessary as blueberries' properties such as springiness, and resilience are not seen as very important. The Kramer shear cell was either not considered because it was more suitable used for meat, fish products and snacks like potato chips, which requires a larger amount of samples instead of single blueberry. Hence, the firmness of blueberries was conducted by a simple compression test (Bourne 2002).

1.4.4 Total soluble solids

Total soluble solids (TSS) can be used as a fruit ripening indicator as well as taste predictor. Sugars, such as glucose, sucrose, fructose and xylose, are the major soluble solids in fruit juice. Additionally, acids, such as organic acid and amino acids are also important part of TSS. Sturm and others (2003) pointed out that the taste of the strawberry became sweeter along fruit maturity, which the TSS value was increasing. Usually, the more mature the fruit is, the higher TSS in the food and the sweeter the food tasted.

TSS concentration (TSS %) can be expressed by ^{o}Brix, which is measured by a refractometer. Temperature of storage and different cultivars of food are two critical factors affecting the TSS. For a sugar solution, the change is about 0.5% ^{o}Brix, for every 10^{o}F change (Constenla and others 1989). TSS of fresh blueberries ranged from 8.3-14.3 ^{o}Brix, depending on different cultivars. Yang and others (2008) found that "Bluecrop" blueberries had the lowest TSS among "Elliott" and "Liberty" blueberries. Since samples included different cultivars, and cultivars' variety was the main factor of blueberries quality, TSS were different among sample groups. Chiabrando and others (2009) found **similar result that "duke" blueberries had a significant higher ($P<0.05$) TSS than the** "Bluecrop" blueberries.

1.4.5 Titratable acidity and pH

Liberated H+ ions in fruit juice are measured and expressed in terms of pH. The pH is a measure of active acidity in the fruit, while the titratable acid measured the bond

acidic components. Titratable acidity and pH are related. The acidity level influences a wide range of factors including microbial stability (spoilage), physical stability (protein, tartrate), oxidation level, SO_2 activity, color and flavor, especially sourness (Fennema, 2000).

pH is defined as the decimal logarithm of the hydrogen ion concentration in a solution, where pH = - Log_{10} (the concentration of H^+).

Moreno and others (2007) found that E-beam irradiation, which was up to 3.2 kGy, did not affect ($P>0.05$) pH and total acidity of the blueberries over 14 days. Similarly, Perkins and others (2008) found that total titratable acidity and pH were not affected by the UV treatment but by different varieties. “Collins” blueberries had higher pH and less malic acid equivalent (both $P<0.05$) than “Bluecrop”.

1.4.6 Total phenolic content

Phenolic compounds are always referred as polyphenols. They comprise an aromatic ring with one or more hydroxyl substituent and can be varied from a simple phenolic molecule to highly polymerized compounds (Blanda and others 2009). Phenolic acids, flavonoids and tannins are main dietary phenolic compounds in fruits. Phenolic compounds are being focused to study recent years because of their health benefits, such as scavenging free radicals and chelate metal cations (Balasundram and others 2006).

Blueberries has been reported with a wide variety of total phenolic content, which are ranged from 171 to 961 Gallic acid equivalents/ 100g fresh weight depending on

varieties and growing conditions. Even the same variety could present different phenolic compounds. Usually, the total phenolic content decreases with maturation process (Sellappan and others 2002; Ribera and others 2010). Castrejon and others (2008) found that total phenolic compounds were decreasing ($P<0.05$) of all these four cultivars, which are including "Bluecrop, Peru, Berkeley and Reka" along with blueberries' ripening process.

Zheng and others (2008) found that high oxygen environment (>60% oxygen) significantly increased ($P<0.05$) the total phenolic amount of the blueberries. However, Lohachoompol and others (2004) observed that there was no differences ($P>0.05$) of total phenolic amount among fresh, dried, and frozen blueberries. Additionally, Perkins and others (2007) found that UV treatment did not affect ($P>0.05$) total phenolic content of blueberries. There are few studies of VI effects of total phenolic contents, this thesis would fill this gap.

1.5 Sensory analysis

Sensory analysis is critical to show consumers' acceptability of new food products. It reduces variations in judgment among individuals and also be a bridge among researchers, industry and consumers (Olivas and Barbosa-Canovas 2005). Basically, sensory evaluations could inspect three aspects of food properties, such as optical properties (appearance and color), mechanical properties (texture), and chemical properties (flavor, taste and aroma). Instruments can simulate human judgments by

imitating the way people test the product or by measuring fundamental properties, which gives objective results. However, consumers' feeling about the food is even more important, due to it can provide the subjective responses. It can tell the consumers' preference as well as predict the market direction (Abbott 1999).

CHAPTER II

METHODOLOGY

2.1 Fruits

Highbush blueberries *(Vaccinium corymbosum)* were purchased at a local market (HEB, College Station, TX, USA). Blueberries that were not damaged or bruised were selected by visual inspection, placed into plastic containers (Rubbermaid, 580g, square box), and stored in a refrigerator at 4.5-5^{o}C and 50-55% relative humidity for 14 days.

2.2 Sample preparation

Sixty of fruits were placed in plastic containers (Rubbermaid, 580g, square box), and placed in refrigerator at 4.5-5^{o}C and 50-55% relative humidity up to 14 days for the shelf life study.

2.3 Chemicals for vacuum impregnation

Calcium lactate (Modernist Pantry, LLC, Portsmouth, NH, USA) was dissolved in sterile distilled water at room temperature by weight/weight percentage and used at three different concentrations of 4, 5 and 6% (w/w).

2.3.1 Vacuum impregnation process and design of experiment

Measurements of selected fruit quality attributes were carried out to determine the effect (if any) of the vacuum impregnation pre-treatment on the texture and other quality characteristics of blueberries. Fruits were removed from the refrigerated storage (4.5-5^{o}C and 50-55% RH) at days 0 (day of vacuum impregnation), 7 and 14 for evaluation of the different product quality attributes. Based on preliminary results, vacuum impregnation (VI) treatments were conducted at 160 mm mercury bar vacuum pressure and 8% solid-liquid ratio (blueberries-impregnation solution). Treatments differ in terms of vacuum impregnation solution concentration and vacuum impregnation time combinations (Table 2). Samples not exposed to vacuum impregnation served as controls.

Table 2 Experimental design of vacuum impregnation process.

Treatment	Calcium lactate solution (w/w %)	Vacuum time *(mins)	Atmospheric time (mins)
Control	0	0	0
T1	4%	15	15
T2	4%	First step VI: 5 Second step VI:10	First step VI: 5 Second step VI:10
T3	5%	15	15
T4	5%	First step VI: 5 Second step VI:10	First step VI: 5 Second step VI:10
T5	6%	15	15
T6	6%	First step VI: 5 Second step VI:10	First step VI: 5 Second step VI:10

Note: T1- T6 refers to treatment #1 to #6.
Non-VI treatment served as non-vacuum impregnated control.
*Vacuum impregnation is applied in two steps.

2.4 Product quality attributes

Quality attributes of control (non-VI) and vacuum impregnated fruits were evaluated to determine the best VI treatment on Day 0 (the day of the treatment), Day 7 and Day 14. All measurements were conducted at room temperature (20^{o}C) in triplicate.

2.4.1 Moisture content and water activity

Moisture content of the blueberries was determined by blueberries' weight loss after drying in a vacuum oven (Squared Lab Line Instruments, Melrose Park, IL) at 70^{o}C until constant weight (about 6 hours) (AOAC 2002). Before drying, the weight of samples and canisters were recorded. Approximately five grams of samples were placed in a canister with the cap open to dry; after drying, the canister cap was closed, placed in a desiccator to cool down and then weighed (Appendix D). The moisture content in percentage wet basis was calculated as:

$$\text{Moisture content (\% wet basis)} = \frac{\text{Weight}_{wet} - \text{Weight}_{dry}}{\text{Weight}_{wet}} x100 \quad \text{(Equation 1)}$$

With Weight_{wet} and Weight_{dry} being the weight of the berries before and after drying, respectively. The moisture content of the blueberries was the average of three replications.

Water activity of the berries was determined using a Rotronic Hydrometer (Rotronic Instrument Corp., Huntington, NY) at room temperature. Approximately five grams of pulp were placed in an air-tight chamber and water activity and temperature were recorded until equilibrium was reached. Three replications were conducted (Appendix E).

2.4.2 Color

Samples' color of VI treated and non-treated controls were evaluated by using the Universal Version 3.73 software with a Lab Scan XE 16437 colorimeter (Hunter Lab, Inc., Reston, VA, USA). The measuring aperture diameter was 30mm, and the illuminant geometry was D65/10. Before loading samples, the parameter was calibrated with standard white and black plates. 30g of blueberries was loaded to cover the measured aperture and then measurements were taken. L* (lightness), a*(redness to greenness) and b* (yellowness to blueness) were recorded as parameters of each group of samples (Appendix K). Six replications were conducted for each group at room temperature.

Chroma (C*), color differences (ΔE*) and hue angle* (^{o}) were three visional parameters referring to the color of samples and were calculated as follows. Using the standard calculation for hue* [Arc tan (b/a)], positive signed results are generated for the first quadrant [+a, +b] only. Hence,

First quadrant: Hue*(^{0}) = [Arc tan (b*/a*)]

Second quadrant [-a, +b]: Hue = 180+Arc tan (b*/a*)

Third quadrant [−a, −b]: Hue = 180+Arc tan (b*/a*)

Fourth quadrant [+a, −b]: Hue = 360+ Arc tan (b*/a*)

(Equation 2)

Color differences (ΔE*):

$\Delta E^* = (\Delta L^* \Delta L + \Delta a^* \Delta a + \Delta b^* \Delta b)^{1/2}$ (Equation 3)

Δa* = a*- a* standard

Δb* = b*-b* standard

ΔL* = ΔL* - ΔL* standard

Chroma (C*):

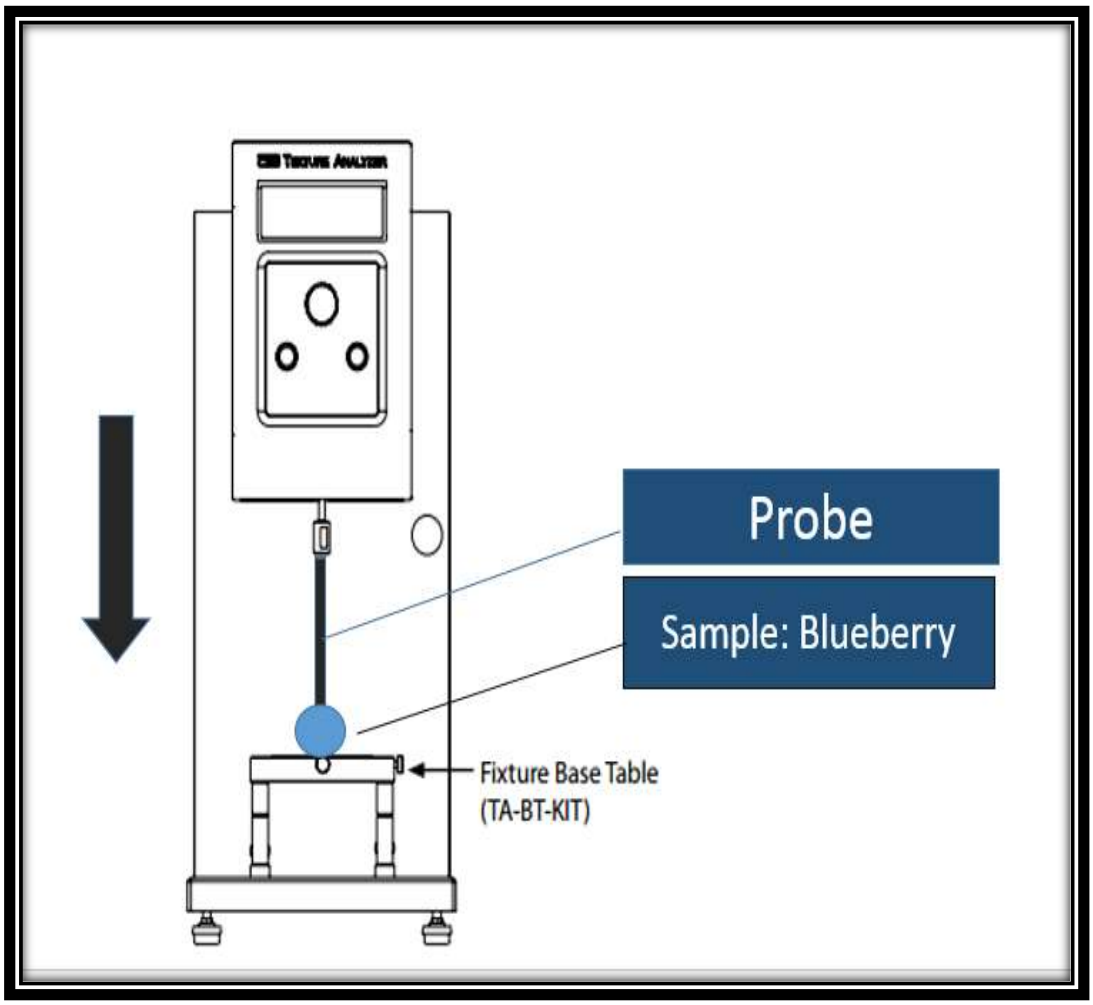
$C^* = \sqrt{a^{*2} + b^{*2}}$ (Equation 4)

2.4.3 Texture (Firmness)

The firmness was characterized by the value of the maximum force to compress the fruit by a CT3 Brookfield Texture Analyzer (Brookfield, MA, USA). The test probe was a 4mm diameter cylindrical probe, TA-44 (Figure 2). The probe moved downward at

a speed of 0.5mm/s to a distance of 9 mm, about 70% strain, to the sample, and the maximum force in Newton (N) was recorded. Nine blueberries were randomly picked to be tested and tests were conducted at room temperature.

Figure 2 Compression test of blueberries using a CT3, Brookfield.



2.4.4 Total soluble solids (TSS)

Generally, sugar levels are expressed in degree of Brix (a scale to measure total soluble solids) which represents grams of sugars per 100g of juice. Soluble solids concentration in the samples were measured using a refractometer and expressed in °Brix scale (ABBE ATAGO model 3T, Bellevue). Six samples, about 10g, were squeezed by a plastic syringe (Bomien 100ml Syringe) in a beaker and then a glass bar was used to stir the mixed flesh and juice. A pipette was used to drop one or two drops into the refractometer. The soluble solids content was determined by correlating the refraction angles and refractive index value established by the refractometer. Three replications were conducted at room temperature.

2.4.5 Total titratable acidity (TTA) and pH

The pH was measured using a digital pH meter (Cole Parmer, pH 500 series, Model #59003-20, Singapore). Before measurement, the pH meter was calibrated with standard solutions with pH levels 4, 7 and 10. About 10grams of blueberries were squeezed in a small beaker and then put electrode immersed into it, reading the number directly when a constant number was obtained.

Titratable acidity was measured using AOAC method 22.060 (AOAC, 2002). About 10g pulp of blueberries were diluted to 250 ml with R.O water (deionized water) and then 0.3ml phenolphalein added as pH indicator (Fisher Scientific, Fair Fawn, NJ). Titratable acidity was determined by titration sample with 0.1N NaOH to a turning point

of pink color, which the pink color should persist for at least 30 seconds. Results were expressed in terms of g /100 mL of citric acid, which is the dominant acid in blueberries (Appendix E).

2.4.6 Total phenolic amount (TPH)

Approximately 5g of blueberries were weighed then mixed with 30ml deionized water and 70ml acetone in a blender. Blender was set up to mix for 3 mins at high speed. Next, this extraction sample solution was filtered and then put in a rotatory evaporator (K-4/R LAUDA, Brinkmann Instrument, USA) for distillation with a rotate speed of 90 RPM for 45 mins. The temperature was set up at 47 ºC. Thus, the acetone could come out of the system due to the boiling point of acetone was below the set up temperature (about 45ºC), which would leave the water with phenolic extraction in the sample. Next, collect the extraction sample and record the volume of sample solution (Ju and others 2003; Alothman and Karim 2009).

Total phenolic content was measured using the Folin-Ciocalteau method (Koca and others 2009). Results were expressed as milligrams of Gallic acid equivalents (GAE) per 100g of fresh weight. 0.02 ml blueberry extraction was added to 1.58 ml deionized water in tube, while the blank test tube was not adding any blueberry extraction. Then 0.1ml Folin & Ciocalteu's phenol reagent was added (2N, Sigma, USA) to each tube, stirring mix for 8 mins. Next, 0.3 ml (0.1 N) NaOH solution was added to each tube to neutralize the solution. The tubes were allowed to stand for two and a half hours in a dark

room. Finally a spectrophotometer (UV-1601, Shimadzu scientific instruments. INC. USA) was used to measure the absorbance at 765 nm (Kita and others, 2015). Three replicates were conduct at room temperature.

A standard curve was made by the same procedures except for replacing the sample solution with the Gallic acid. Ten different concentrations ranging from 0 to 500 mg/L Gallic acid were used for making the standard curve (Figure 3). Thus, final fruit total phenolic amount was expressed as mg Gallic acid / 100 g of fresh fruit. From the standard curve, x is the Gallic acid concentration and, y is the absorbance.

Thus*:* $y = 0.002\ x + 0.0394$, $R^2 = 0.9963$

Total phenolic concentration was calculated as follows:

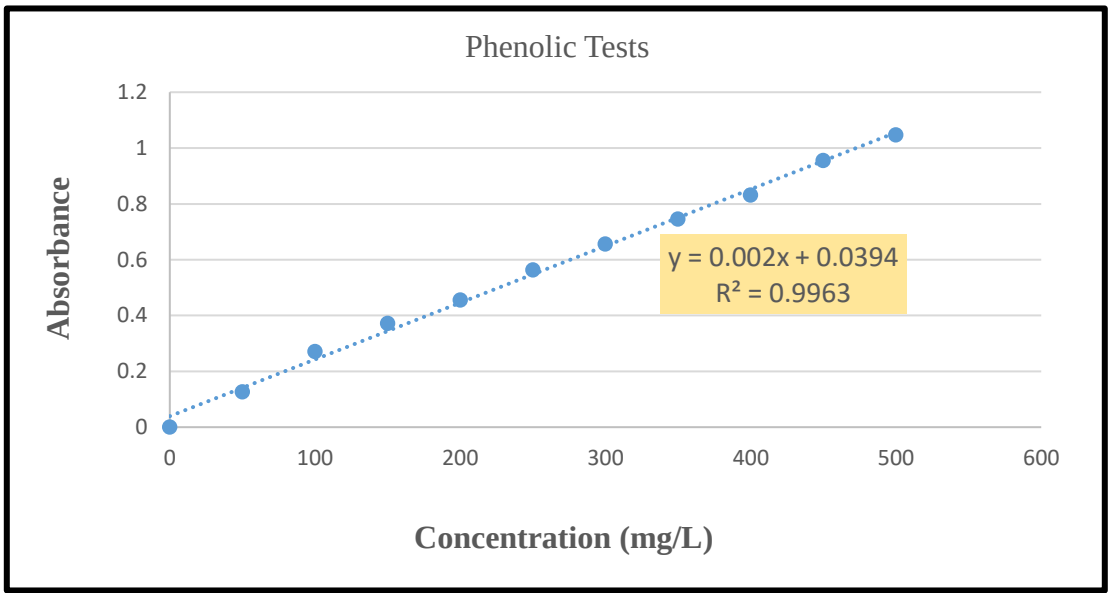
GAE concentration = (Reading absorbance value - 0.0394) /0.002; (mg/L)

Sample TPH = GAE concentration * sample volume / weight of the sample * 0.1;

(Equation 5)

The unit of sample total phenolic amount (TPH) is: mg/100g (w.b) GAE

Figure 3 Standard curve of Absorbance (wavelength at 765nm) vs. TPH.



Note: values are means of two readings.

2.5 Sensory Evaluation

The control (non-VI) and vacuum impregnated samples ware equilibrated to room temperature (4 samples from each group) and presented to the panelists. The four samples were placed on top of white paper plates identified by number and randomly placed in the trays. Fifty panelists were randomly picked from students and professors at Texas A&M University. Panelists were asked to evaluate the samples of three aspects which were appearances, color and texture for consumer's liking preference. For evaluating appearance and color of samples, panelists were asked to visually inspect the

samples; for the texture, they were asked to use their fingers to touch and slightly squeeze the samples to feel whether the samples were hard or not. Next, panelists were asked to grade the samples by a 7- point scale where a score of 1 represented the "most liked" and a score of 7 represented the attributes "most disliked" (Appendix G; Table 3) (Meilgaard and others, 2005).

.

Table 3 Sensory evaluation form template.

Sample No.	**xxx**						
Appearance	1	2	3	4	5	6	7
Color	1	2	3	4	5	6	7
Texture	1	2	3	4	5	6	7
Comments							

Note: The score scale refers as 1 most liked, 2 very good, 3 good, 4 fair, 5 poor, 6 very poor, 7 most disliked.

2.6 Electron beam (E-beam) irradiation

2.6.1 Experimental design

Based on previous results from vacuum impregnation (VI) and sensory tests, the best VI condition was selected as a pre-treatment before E-beam irradiation. Hence, fresh blueberries were vacuum impregnated with the 4% calcium lactate solution and subjected to two steps of VI process. The first step was 5 mins vacuum followed by 5 mins atmospheric conditions; the second step was 10 mins vacuum followed by 10 mins atmospheric conditions. The VI process was conducted under 160 mm mercury bar with 8% liquid/solids (solution/blueberries) ratio.

Both VI treated and control (Non-VI) samples were irradiated on both sides with a 1.35 MeV Van de Graaff accelerator (Appendix B). Samples were irradiated with 0.0 kGy, 0.5 kGy, 1.0 kGy, 1.5 kGy and 2.0 kGy doses. After irradiation, fruits were kept at 4.5-5°C, 50-55% RH for 14 days and tested for quality attributes on Day 0 (the day of irradiation), Day 7 and Day 14 (Table 4).

Table 4 Experimental design of E-beam irradiation process.

Storage time	**Control (Non-VI) blueberries**					**Pre-VI treated blueberries**				
Day	**Dose level (kGy)**									
	0.0	0.5	1.0	1.5	2.0	0.0	0.5	1.0	1.5	2.0
0	Record firmness, total phenolic content, pH.					Record firmness, total phenolic content, pH.				
7										
14										

Note: Forty grams of samples were irradiated at each dose level.

2.6.2 E-beam irradiation process

2.6.2.1 Finding the hot spot

The hot spot is defined as the area with highest dose collected, and it is where the irradiation samples should be placed. An ion chamber was placed at different points along the Cartesian coordinates to collect dose counts and the hot spot area and average counts per kilo Grey (electron counts/ kGy) were identified.

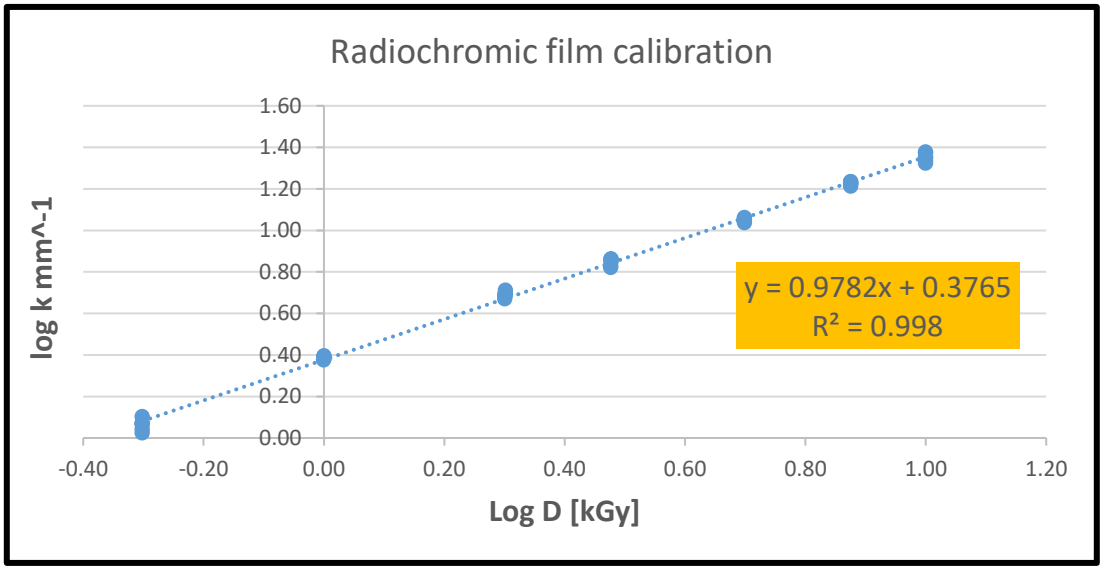
2.6.2.2 Determining actual dose by radiochromic film calibration

A radiochromic film (RF, Far West Technology, Batch 1086, 42.5 μm) was placed at the hot spot and irradiated with the needed dose by using dose count numbers that were

obtained from Step 1. Average counts / kGy were 419923 in this study. After irradiation, the optical density of the RF was read by the digital radiochromic reader (Model FWT-92D, Far West Technologies, Goleta, CA). The linear relationship of optical density of the RF and the dose was calculated and used for further actual dose calculation and calibration (Figure 4). After this step, the RF was placed in front, middle and back of the blueberries to check on the actual absorbed dose and determine whether the samples would need to be irradiated on one side or both sides.

From the calculations shown below, only the front side of blueberries absorbed the target dose; thus, samples should be irradiated on both sides since the middle and back sides did not reach the 1.0 kGy dosage (Table 5).

Figure 4 Radiochromic film calibration curve.



Actual Dose calculation:

From figure 4: Log K = 0.9782* Log D (Dose) + 0.3765, (Equation 6)

Where K= (OD2-OD1)/ thickness of the RF;

Thus, Actual dose = 10^ ((Log (k)-0.3765)/0.9782).

Table 5 Calculation of actual dose in irradiated blueberries by using radiochromic film.

Position	Counts	OD1	OD2	K	Target Dose, kGy	Actual dose, kGy
Back	419819	0.164	0.192	0.658	0.999	0.269
Middle	419611	0.157	0.199	0.988	0.999	0.407
Front	420048	0.164	0.260	2.258	0.999	0.948

Note: Target dose = Counts/Average counts per dose = Counts/ 419923

2.6.2.3 Sample preparation

Approximately 10 blueberries (about 13 grams) were wrapped in a small 5 x 5cm plastic bag (Appendix H) and irradiated on both sides with a 1.35 MeV Van de Graaff

accelerator at different dose level, 0.5 kGy, 1 kGy, 1.5 kGy, 2.0 kGy, at room temperature. Non-irradiated samples served as controls.

2.7 Statistical analysis

The impact of VI treatment on overall quality of fresh berries was established by comparison with the control (non-VI) blueberries and the VI treated samples. The effect of VI pre-treatment on enhancing the texture of irradiated berries was established by comparison with the control (non-VI) and the VI pre-treated samples under 5 different irradiation dose levels (0-2 kGy).

Data analysis was performed using JMP software for MAC (JMP @Pro 12.0.1(64 bit)). The effect of different treatments and irradiation doses were evaluated by one way ANOVA and Tukey's multiple means comparison test. Significant difference is presented by the P-value < 0.05 ($P<0.05$).

CHAPTER III

RESULTS AND DISCUSSION

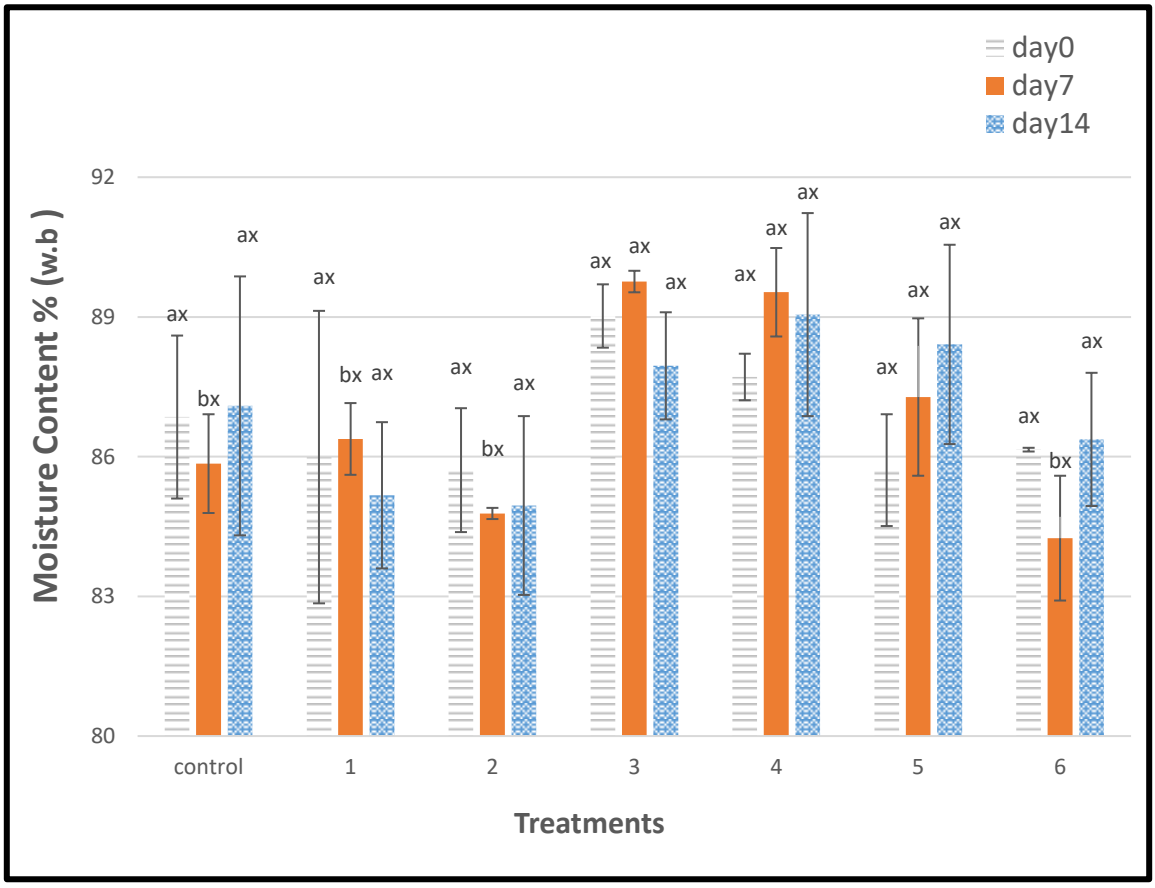
3.1 Effect of vacuum impregnation treatment on the quality of fresh blueberries

3.1.1 Moisture content and water activity

Moisture content of blueberries ranged from 84.25% to 89.76% w.b. (Appendix I). On Day 0 and Day 14, no difference ($P>0.05$) was observed among all the different groups ($P>0.05$). However, on Day 7, samples from treatments #3, #4 and #5 had more ($P<0.05$) moisture content than others. This could be a result of the differences in the ripening process of the blueberries. In the early stage of the mature process, berries would have an increasing moisture content as it ripens. After a certain time, the moisture content of the blueberries will reach an equilibrium, indicating that the berries have reached maturity (Paniagua and others 2013). Samples from treatments #3, #4 and #5 possibly reached the mature stage earlier than the others. During storage, all samples remained unchanged ($P<0.05$) in moisture content over 14 days (Figure 5). Hence, VI process did not induce any moisture content change of blueberries.

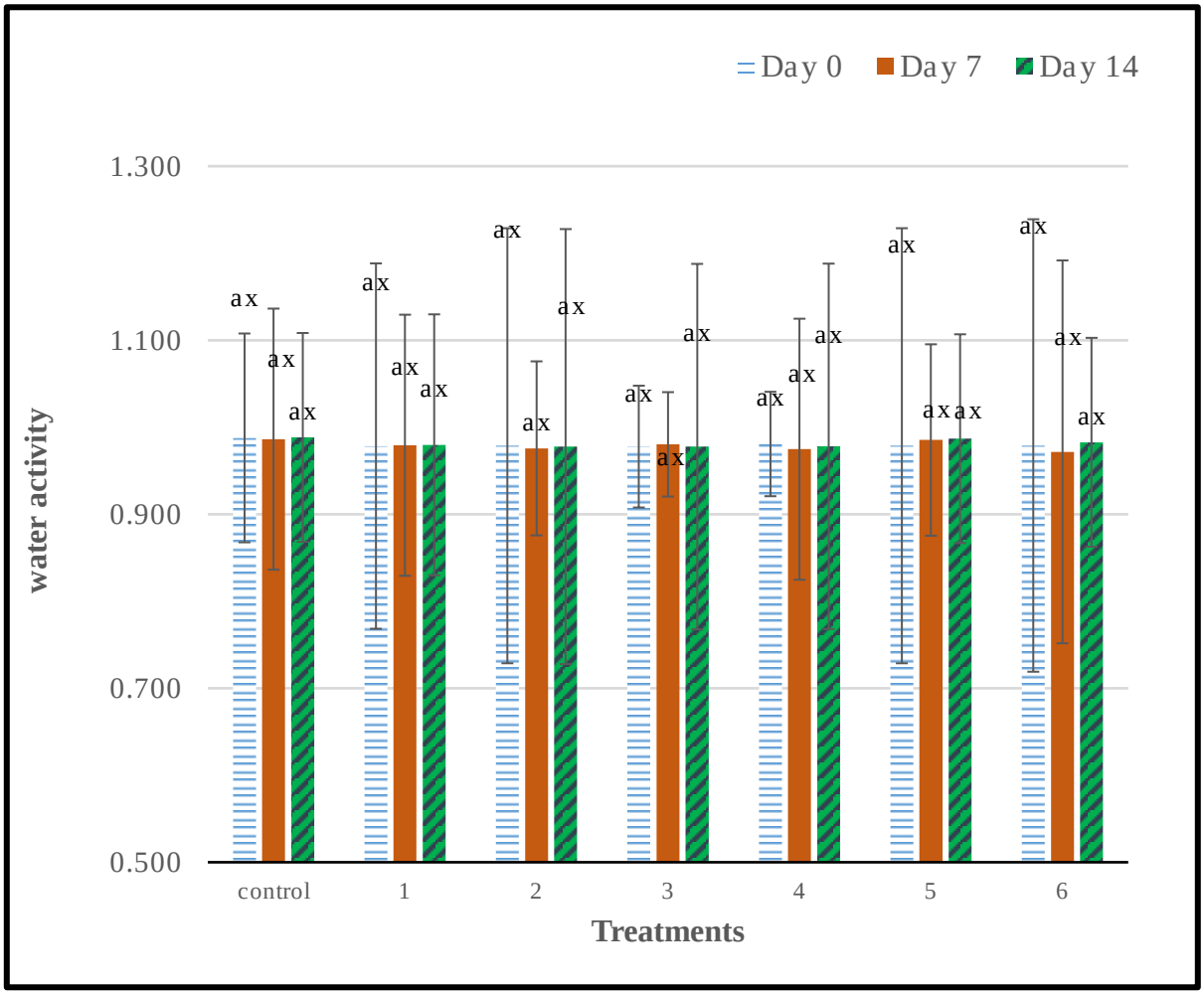
Water activity (Aw) of the blueberries ranged from 0.9717 to 0.9883 (Figure 6). No water activity differences ($P>0.05$) were shown over time for each group of blueberries during storage. Additionally, there was no difference ($P>0.05$) of water activity among the different treatments (Appendix J). Therefore, vacuum impregnation treatment did not affect the water activity of blueberries.

Figure 5 Effect of vacuum impregnation on moisture content (MC %) of blueberries. Samples were stored up to 14 days at 4.5-5ºC, 50-55%RH.



Note: Control: Non-VI samples served as control.
1 to 6 refers to vacuum impregnation treatment #1 to #6 (experimental design).
a-b Subscript letters within a column which are not the same shows a significant difference between treatments (P<0.05) and vice versa.
x-y Subscript letters within a row which are not the same shows a significant difference between days (P<0.05) and vice versa.

Figure 6 Effect of vacuum impregnation on water activity of blueberries. Samples were stored up to 14 days at 4.5-5ºC, 50-55%RH.



Note: Control: Non-VI samples served as control.
1 to 6 refers to vacuum impregnation treatment #1 to #6 (experimental design).
a-b Subscript letters within a column which are not the same shows a significant difference between treatments (P<0.05) and vice versa.
x-y Subscript letters within a row which are not the same shows a significant difference between days (P<0.05) and vice versa.

3.1.2 Color

Chroma (C*, color saturation) of the control group was higher than all VI groups ($P<0.05$, Table 6). This illustrated that control samples looks more saturated than the VI treated samples.). All the vacuum impregnated samples had less L* ($P<0.05$, Appendix K) because of some calcium lactate residue on the blueberries' surface. Vacuum impregnation process caused the calcium lactate solids attaching to the surface of the fruit (Appendix Q). During a 14-day storage, chroma of the control and treatment #4 samples remained unchanged ($P>0.05$) while the rest had a slight decrease ($P<0.05$) over time.

Total color differences (ΔE*) did not change ($P>0.05$) over time for all the samples (Table 7). Comparing different treatments, the control (Non-VI) showed significant larger ($P<0.05$) color differences than the vacuum impregnated blueberries. The control group were more red ($P<0.05$) than others as a* increased at the end of the storage. This could be caused by the variety in sample maturation process. Fresh blueberries' colors were different among different varieties and samples. (Sapers and others 1984; Saftner and others 2008). In general, blueberries' color changed from green to red along with ripening (Moreno, 2007; MatIaCeVIC, et al, 2012

There was no difference in Hue* angle among control and all vacuum impregnated blueberries ($P>0.05$, Table 8). Throughout the storage, all the samples were stable in Hue* over time ($P>0.05$). This clarified that all the samples' colors were very close to each other. Hence, vacuum impregnation treatment did not yield color change of blueberries in this study.

Table 6 Effects of vacuum impregnation on Chroma (C*) of blueberries. Samples were stored up to 14 days at 4.5-5ºC, 50-55%RH.

Treatment	Day 0	Day 7	Day 14
Control	2.18 ± 0.04^{ax}	2.14 ± 0.07^{ax}	2.41 ± 0.06^{ax}
T1	0.98 ± 0.08^{cxy}	0.65 ± 0.14^{cy}	1.18 ± 0.06^{bcx}
T2	2.09 ± 0.02^{abx}	1.35 ± 0.02^{bcz}	1.79 ± 0.09^{bcy}
T3	1.74 ± 0.03^{bx}	1.26 ± 0.04^{bcy}	1.56 ± 0.02^{bcxy}
T4	1.26 ± 0.07^{cx}	1.22 ± 0.05^{bcx}	1.34 ± 0.12^{cx}
T5	1.93 ± 0.01^{bx}	1.59 ± 0.07^{bcx}	1.29 ± 0.03^{cy}
T6	1.58 ± 0.13^{by}	1.80 ± 0.03^{bx}	2.05 ± 0.05^{abxy}

Note: Data is listed as (Average ± Standard Deviation). The means are an average of 3 replications.
Control, blueberries without vacuum impregnation treatment.
T1 to T6 refers to vacuum impregnation treatment #1 to #6 (experimental design).
a-b Subscript letters within a column which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row which are not the same shows a significant difference between days ($P<0.05$) and vice versa.

Table 7 Effect of vacuum impregnation on Color differences (ΔE *) of blueberries. Samples were stored at stored up to 14 days at 4.5-5ºC, 50-55%RH.

Treatment	Day 0	Day 7	Day 14
Control*	16.58 ± 0.20^{ax}	16.37 ± 0.23^{ax}	16.97 ± 0.07^{ax}
T1	13.46 ± 0.32^{cx}	12.45 ± 0.22^{cx}	13.64 ± 0.20^{bx}
T2	16.01 ± 0.78^{ax}	13.83 ± 0.07^{cy}	16.15 ± 0.27^{ax}
T3	15.34 ± 0.12^{bx}	12.91 ± 0.11^{cy}	14.11 ± 0.67^{bx}
T4	13.30 ± 0.12^{cx}	13.36 ± 0.27^{cx}	13.10 ± 0.40^{bx}
T5	15.75 ± 0.06^{bx}	14.72 ± 0.07^{bx}	13.59 ± 0.12^{bx}
T6	15.01 ± 0.47^{bx}	15.28 ± 0.28^{bx}	16.30 ± 0.41^{ax}

Note: Data is listed as (Average ± Standard Deviation). The means are an average of 3 replications.
Control*, blueberries without vacuum impregnation treatment.
T1 to T6 refers to vacuum impregnation treatment #1 to #6 (experimental design).
a-b Subscript letters within a column which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row which are not the same shows a significant difference between days ($P<0.05$) and vice versa.

Table 8 Effect of vacuum impregnation on Hue* (º) of blueberries. Samples were stored up to 14 days at 4.5-5ºC, 50-55%RH.

Treatment	Day 0	Day 7	Day 14
Control*	278.61 ± 5.04^{ax}	276.21 ± 1.91^{ax}	267.92 ± 0.31^{ax}
T1	272.57 ± 1.49^{ax}	273.40 ± 2.35^{ax}	267.73 ± 1.32^{ax}
T2	274.24 ± 0.51^{ax}	266.40 ± 0.89^{ax}	269.23 ± 0.91^{ax}
T3	265.15 ± 1.53^{ax}	266.70 ± 1.08^{ax}	272.02 ± 0.46^{ax}
T4	269.39 ± 0.23^{ax}	267.74 ± 1.05^{ax}	276.01 ± 2.68^{ax}
T5	267.78 ± 1.85^{ax}	268.17 ± 1.99^{ax}	273.40 ± 2.40^{ax}
T6	267.96 ± 5.30^{ax}	266.26 ± 1.92^{ax}	267.90 ± 0.31^{ax}

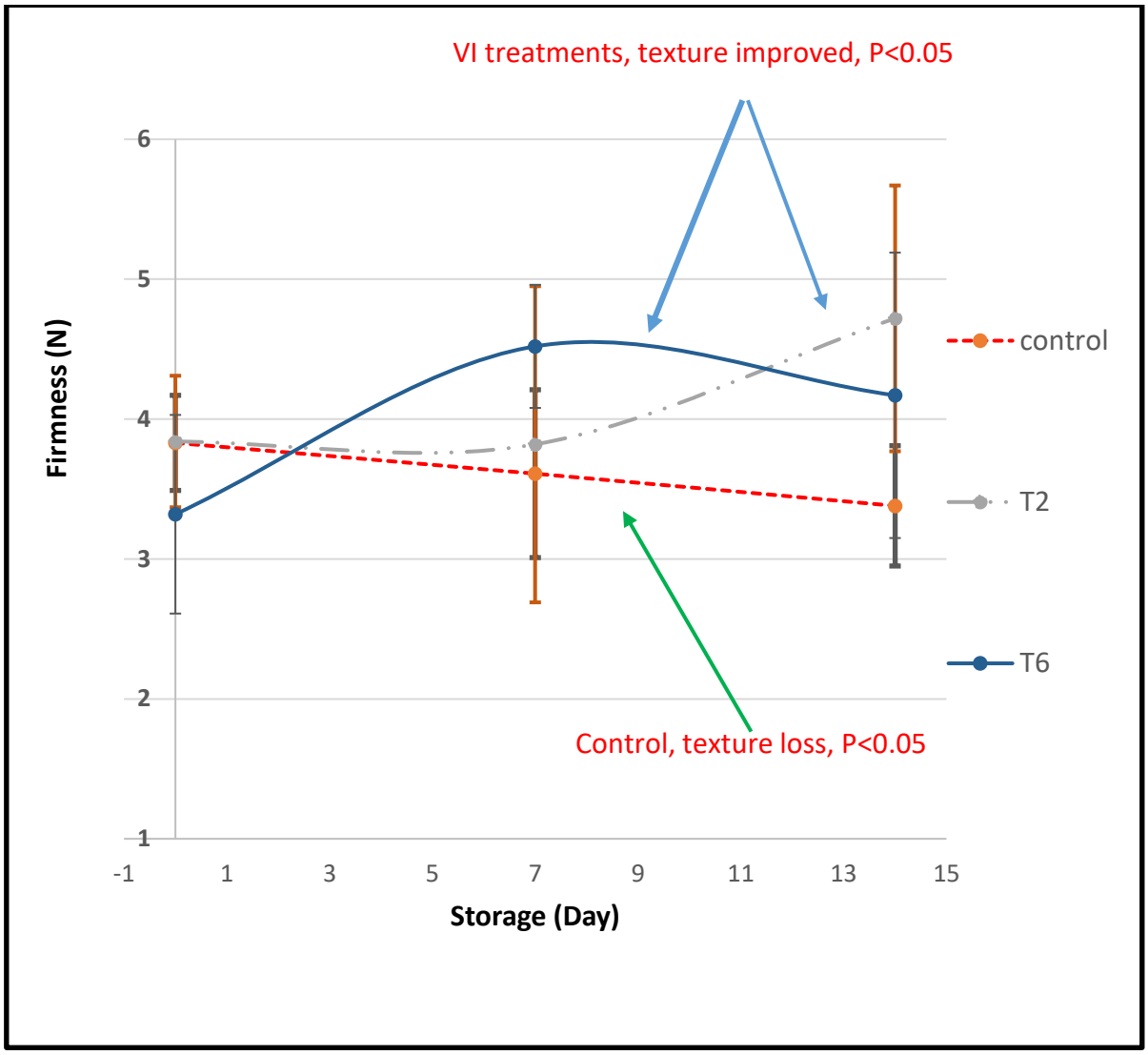
Note: Data is listed as (Average ± Standard Deviation). The means are an average of 6 replications.
Control*, blueberries without vacuum impregnation treatment.
T1 to T6 refers to vacuum impregnation treatment #1 to #6 (experimental design).
a-b Subscript letters within a column which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row which are not the same shows a significant difference between days ($P<0.05$) and vice versa.

3.1.3 Firmness

During storage, the control (Non-VI) blueberries had significant firmness loss ($P<0.05$) over time and Figure 7 shows that the control line was decreasing over 14 days. However, vacuum impregnated blueberries maintained ($P>0.05$) their firmness during the storage. Even blueberries treated by treatment #2 and #6, the firmness got improved ($P<0.05$) throughout the storage. Hence, vacuum impregnation treatment did maintain the fruits' textural characteristic over time.

There were no differences ($P>0.05$) among different treatments on Day 0 and Day 7. However, by the end of the storage, samples from treatment #1, #2, #6 were firmer than the rest (Table 9). This inferred that these three treatments were better than the rest in terms of the firmness improvement. Considering the fact that the firmness of treatments #2 and #6 blueberries got improved over time. It can be concluded that vacuum impregnation treatments #2 and #6 were more effective over other treatments for pre-treating blueberries. Treatment #2 was selected for further study as it was cost effective due to using 4% calcium lactate solution instead of 6% calcium solution of treatment #6.

Figure 7 Effect of vacuum impregnation on firmness (Fmax) of blueberries at storage of 14 days at 4.5-5ºC, 50-55%RH.



Note: Control, blueberries without vacuum impregnation treatment.
T2 and T6 refer to vacuum impregnation treatment #2 and #6 (experimental design).

Table 9 Effect of vacuum impregnation on firmness of blueberries. Samples were stored up to 14 days at 4.5-5ºC, 50-55% RH.

Treatment	Day 0	Day 7	Day 14
Control*	3.83 ± 0.34^{ax}	3.61 ± 0.6^{axy}	3.08 ± 0.15^{by}
T1	3.72 ± 0.33^{ax}	3.91 ± 0.4^{ax}	4.05 ± 0.42^{ax}
T2	3.84 ± 0.47^{ay}	3.82 ± 0.43^{ay}	4.72 ± 0.25^{ax}
T3	3.59 ± 0.41^{ax}	3.10 ± 0.42^{axy}	2.99 ± 0.86^{bx}
T4	3.18 ± 0.56^{ax}	3.29 ± 0.54^{ax}	3.08 ± 0.42^{bx}
T5	3.06 ± 0.69^{ax}	3.10 ± 0.64^{ax}	3.11 ± 0.29^{bx}
T6	3.32 ± 0.71^{ay}	4.52 ± 0.44^{ax}	4.17 ± 1.02^{ax}

Note: Data is listed as (Average ± Standard Deviation). The means are an average of 9 replications.
Control*, blueberries without vacuum impregnation treatment.
T1 to T6 refers to vacuum impregnation treatment #1 to #6 (experimental design).
a-b Subscript letters within a column which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row which are not the same shows a significant difference between days ($P<0.05$) and vice versa.

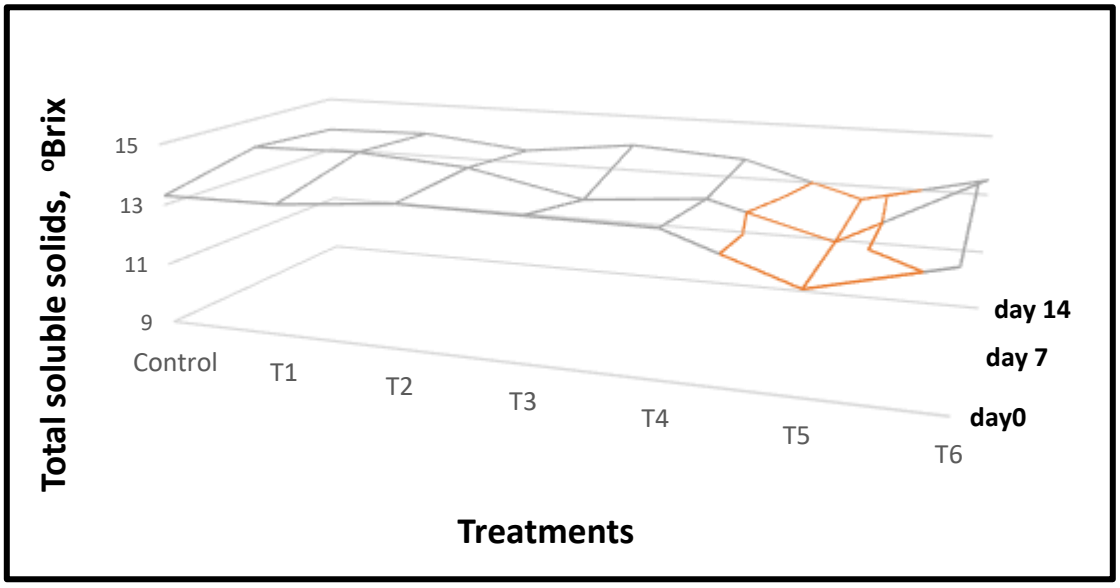
3.1.4 Total soluble solids (TSS)

Total soluble solids (TSS) of blueberries ranged from 9.93 to 14.07 ^{o}Brix in this study (Appendix L). According to Matos and others (2014), the TSS of fresh blueberries were different depending on the variety of cultivars and could be varied from 9-15 ^{o}Brix.

Usually during storage, starch in fruits breaks down and induces an increasing tendency of TSS. In this project, blueberries have low starch content. The slight increase of TSS may be a result of cell wall degradation (Cordenunsi 2003). TSS of all vacuum impregnated blueberries did not change ($P>0.05$) over time. However, the control (Non-VI) samples had significant higher ($P<0.05$) TSS since Day 7. This suggested that vacuum impregnation treatment kept blueberries texture as calcium lactate can prevent the cell wall degradation and thus to maintain the TSS.

In comparisons among vacuum impregnated samples, Figure 8 shows that blueberries from treatment #5 had significant lower ($P<0.05$) TSS than others due to sample variances in the ripening process. ^{o}Brix changed from 12-14 was not affecting the fruit quality (Yang and others 2008; Tosun and others 2008). Blueberries from treatment #5 had a 1 ^{o}Brix difference (12.2-13.2) from others and this difference would not induce any quality change. In conclusion, vacuum impregnation treatment did not affect the total soluble solids of the blueberries.

Figure 8 Effect of vacuum impregnation on pH of blueberries, stored up to 14 days at 4.5-5ºC, 50-55% RH.



Note: Non-vacuum impregnated blueberries served as control.
T1 to T6 refers to vacuum impregnation treatment #1 to #6 (experimental design).

3.1.5 Total Titratable acidity and pH

Fresh blueberries' total titratable acidity usually ranged from 0.3 to 1.3 g citric acid per 100g of fresh weight depending on the maturation stage, storage condition as well as the variety of cultivars (USDA database, 2011). In this study, TTA varied from 0.62 to 1.22 g citric acid (Table 10). Throughout the storage, the TTA of all samples did not change ($P>0.05$) over time. Additionally, TTA of the control and vacuum impregnated blueberries did not show differences ($P>0.05$) on each same date. Moreover, among VI treatments, there was no difference ($p>0.05$) between each other. Therefore, vacuum impregnation treatment did not affect the TTA of blueberries.

The pH of control samples increased ($P<0.05$) from 2.54 to 2.83 at the end of the storage (Appendix M) due to blueberries' maturation process. Ripe samples which had a low acid content had a correspondingly high pH. This agreed Leiva-Valenzuela and others (2013) found mature fruits had slightly higher pH and lower acidity than the "green stage" fruits. Organic acids usually declined during ripening as they were respired or converted to sugars. This data also agreed with what Tosun and others (2008) found on blackberries, which the pH increased over time during storage. All vacuum impregnated blueberries remained unchanged ($P>0.05$) in pH over time.

Comparing different vacuum impregnated samples, there were some differences ($P<0.05$) of pH among each other. The pH of blueberries ranged from 2.11 to 2.88 in this study (Figure 9). Thus, the concentration of the H+ changed from 0.0013 mol/L to 0.00776 mol/L in this study according to the definition of the pH:

where pH = Log 10 (1/ concentration of H+)

Hence,

Log 10 (1/x) = 2.11, x= 0.00776; Log 10 (1/y) = 2.88, y= 0.0013.

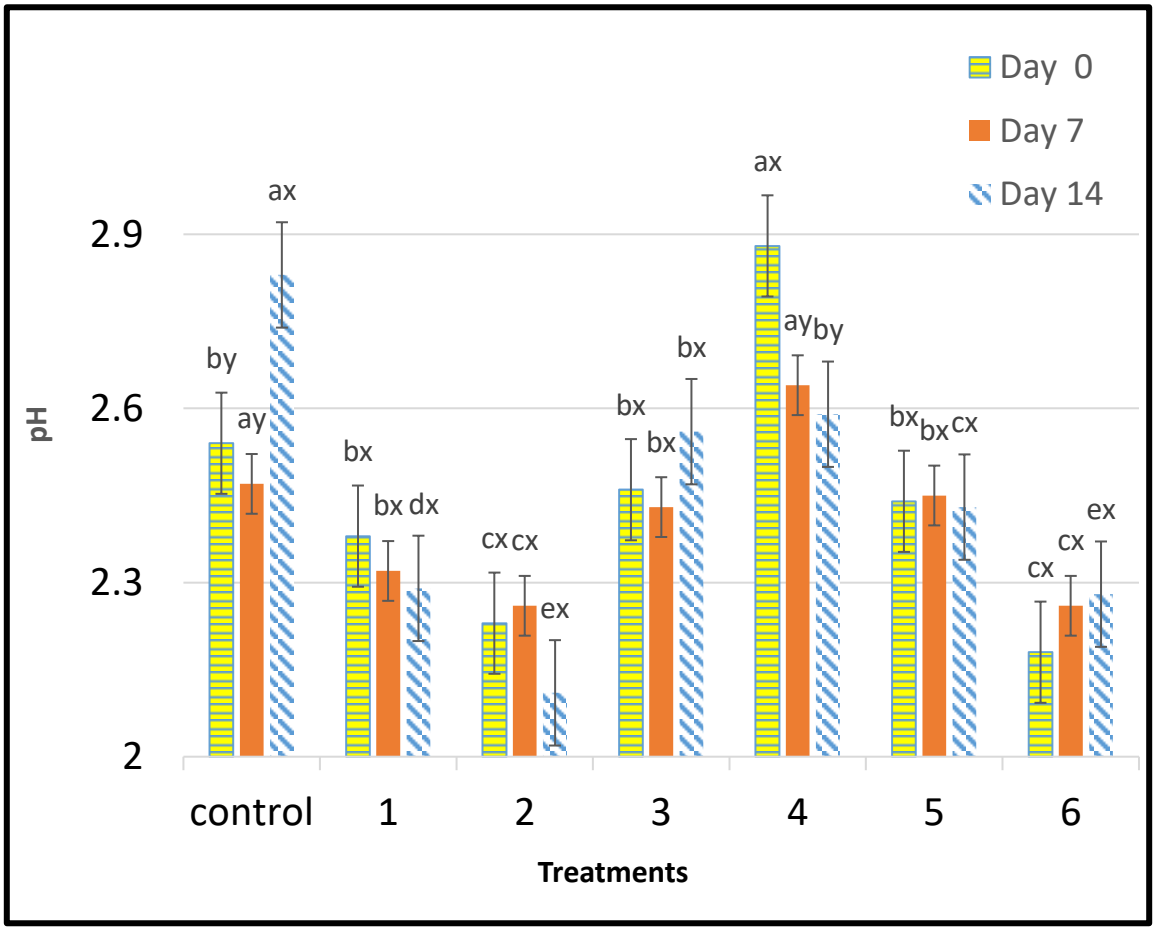
From the equations, which n= m/M and n= CV, the mass concentration (m/v) of H+ is obtained: m/v= CM; where m is the mass (g), v is the volume (L), M is the molar mass (g/mol) and n is the amount of the solute in moles (mol) and C is the molar concentration (mol/L). Thus, the concentration of H+ changed from 0.0013 g/ml to 0.0076 g/ml in this study. This small change in H^+ concentration would not affect any quality changes as when pH changed from 2 to 3, where H^+ concentration changed from 0.001 to 0.01 g/ml was still in the fresh blueberries range. Matiacevich and others (2013) found that fresh blueberries' pH varied from 2 to 3.6 among different cultivars and the soil condition of growing. Additionaly, Basiouny & Chen (1988) found harvest date and storage intervals affected the pH of blueberries. pH changed from 3.1 to 3.8 in his study because of the differnt harvest date. Blueberries' pH ranged from 2.96 to 3.28 in Moreno's study (Moreno and others, 2007). All of these findings pointed out that pH of the blueberries could vary in a relative big range. Thus, 1 pH scale difference in this study was not significant. In summary, combining TTA with pH results, the vacuum impregnation treatment did not affect ($P>0.05$) the acidity of blueberries.

Table 10 Effects of vacuum impregnation on total titratable acidity of blueberries. Samples were stored up to 14 days at 4.5-5ºC, 50-55% RH.

Treatment	Day 0	Day 7	Day 14
Control*	0.87 ± 0.27 ^{ax}	0.80 ± 0.40 ^{ax}	0.89 ± 0.16 ^{ax}
T1	0.98 ± 0.44 ^{ax}	1.08 ± 0.04 ^{ax}	0.97 ± 0.48 ^{ax}
T2	0.72 ± 0.34 ^{abx}	0.79 ± 0.24 ^{abx}	0.82 ± 0.44 ^{ax}
T3	1.04 ± 0.16 ^{ax}	0.96 ± 0.37 ^{ax}	1.15 ± 0.10 ^{ax}
T4	1.08 ± 0.06 ^{ax}	1.16 ± 0.09 ^{ax}	0.84 ± 0.41 ^{ax}
T5	1.11 ± 0.23 ^{ax}	0.75 ± 0.24 ^{abx}	0.87 ± 0.49 ^{ax}
T6	0.88 ± 0.32 ^{ax}	1.03 ± 0.06 ^{ax}	1.22 ± 0.50 ^{ax}

Note: Data is listed as (Average ± Standard Deviation). The means are an average of 3 replications.
Control*, blueberries without vacuum impregnation treatment.
T1 to T6 refers to vacuum impregnation treatment #1 to #6 (experimental design).
a-b Subscript letters within a column which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row which are not the same shows a significant difference between days ($P<0.05$) and vice versa.

Figure 9 Effect of vacuum impregnation on pH of blueberries. Samples stored up to 14 days at 4.5-5ºC, 50-55% RH.



Note: Control: Non-VI samples served as control.
1 to 6 refers to vacuum impregnation treatment #1 to #6 (experimental design).
a-b Subscript letters within a column which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row which are not the same shows a significant difference between days ($P<0.05$) and vice versa.

3.1.6 Total phenol content (TPH)

In this project, the fresh blueberries had a TPH range from 159 to 226 mg/100g GAE (Table 14). This agreed with that TPH could vary from 261.95 to 929.62 mg/100 GAE depending on different cultivars and maturity levels among samples (Sellappan and others, 2002). The TPH of all samples did not change ($P>0.05$) over time and there was no difference ($P>0.05$) among samples from different treatments. Hence, vacuum impregnation treatment did not affect the total phenol content of the blueberries. Blanda and others (2008) found that vacuum impregnation treatment with calcium chloride did decrease ($P<0.05$) the TPH of Granny Smith and Stark Delicious frozen apple slices. They found this reduction was mainly due to the flavan-3-ol class, which was the predominant group in apples. Other phenols such as anthocyanins, flavonoids and carotenes were not significantly changed ($P>0.05$) by vacuum impregnation process. Blueberries in this study had relatively small amount of the flavan-3-ol class (6mg/100g) but large amount of flavonoids (250mg/100g). Thus, TPH of blueberries after VI process did not change was reasonable.

Table 11 Effect of vacuum impregnation. Samples were stored up to 14 days at 4.5-5ºC, 50-55% RH.

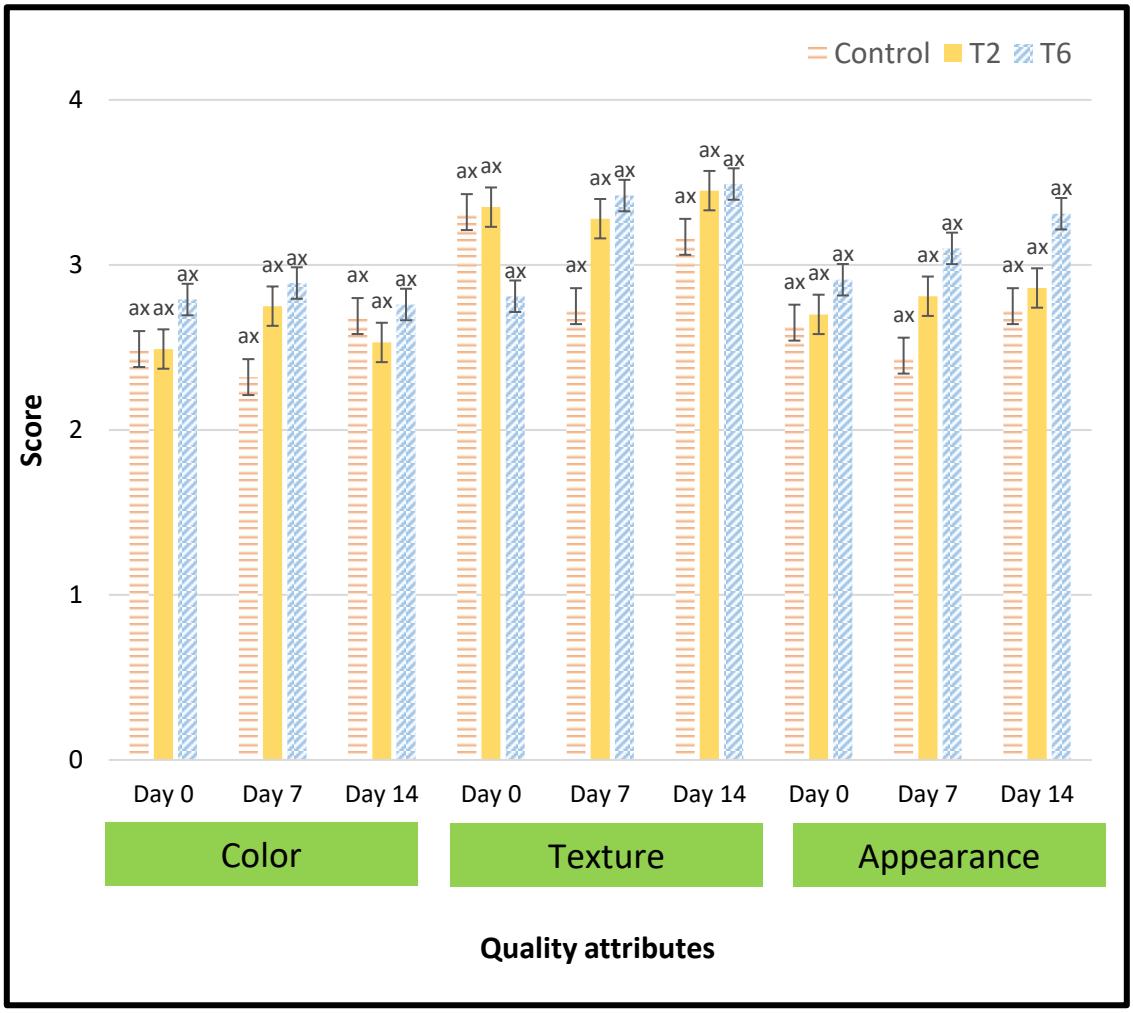
Treatment	Day 0	Day 7	Day 14
Control*	178.31 ± 5.27^{ax}	177.62 ± 11.26^{bx}	193.63 ± 13.68^{ax}
T1	224.71 ± 3.57^{ax}	236.94 ± 16.07^{ax}	203.39 ± 7.36^{ax}
T2	193.83 ± 13.40^{ax}	195.35 ± 16.17^{ax}	191.15 ± 20.77^{ax}
T3	209.78 ± 20.97^{ax}	182.43 ± 8.12^{abx}	181.89 ± 7.58^{ax}
T4	185.94 ± 2.27^{ax}	192.14 ± 8.50^{ax}	191.11 ± 10.37^{ax}
T5	189.77 ± 9.45^{ax}	199.10 ± 6.33^{ax}	192.22 ± 10.24^{ax}
T6	189.96 ± 5.25^{ax}	195.25 ± 2.47^{ax}	199.54 ± 6.24^{ax}

Note: Data is listed as (Average ± Standard Deviation). The means are an average of 3 replications.
Control*, blueberries without vacuum impregnation treatment.
T1 to T6 refers to vacuum impregnation treatment #1 to #6 (experimental design).
a-b Subscript letters within a column which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row which are not the same shows a significant difference between days ($P<0.05$) and vice versa.

3.1.7 Sensory tests

The moisture content, water activity, total soluble solids, phenolic content, pH and titratable acidity of blueberries were not affected ($P>0.05$) by vacuum impregnation treatment. Only the firmness was affected by the vacuum impregnation process. Hence, samples from treatment #2 and #6 were selected for sensory evaluation due to firmness improvement performance. The control (Non-VI), VI treatment #2 and #6 blueberries were tested below for appearance, color (vision evaluation) and texture (hand touch feeling) preference test (Appendix G). Samples were evaluated by 50 consumers. In general, consumers liked vacuum impregnated blueberries and the control (non-VI) blueberries equally. Figure 10 shows that there was no difference ($P>0.05$) among two vacuum impregnated products and the control throughout the storage of 14 days.

Figure 10 Sensory test on samples on different date, sample stored up to 14 days at 4.5-5ºC, 50-55% RH.



Note: Non-vacuum impregnated samples served as control.
T2 and T6 refer to vacuum impregnation treatment #1 to #6 (experimental design).
a-b Subscript letters within a column which are not the same shows a significant difference between treatments (P<0.05) and vice versa.
x-y Subscript letters within a row which are not the same shows a significant difference between days (P<0.05) and vice versa.

- **Appearance**

Consumers' scores did not show significant differences (P>0.05) in preference over time for all samples. In addition, the differences of preference among VI groups and the control were not observed (both comparisons' P>0.05). Hence, vacuum impregnated fruits and fresh blueberries were equally liked by consumers in terms of their appearance.

- **Color**

Consumers did not express (P>0.05) preference over time within all samples. No differences (P>0.05) in sensory test were found among VI treated groups and the control. Similarly, consumers equally liked the color of control and VI blueberries.

- **Texture**

During storage period, no textural difference (P>0.05) of preference was observed over time. Besides, comparing control group to the treated groups, consumers like all samples equally. In conclusion, vacuum impregnated blueberries and fresh (Non-VI) samples were equally liked by consumers.

3.2 Effect of vacuum impregnation as a pretreatment on the quality of E-beam irradiated blueberries

The best vacuum impregnation (VI) treatment for the irradiation study was selected based on objective measurements of quality and sensory scores by a consumer panel. Finally, VI treatment #2 was selected as a pre-treatment for E-beam irradiation test,

which was vacuum impregnating blueberries with 4% (w/w) calcium lactate solution under a two-step VI process. VI treatment was conducted under 160 mm mercury bar pressure with a 8% liquid/solids (solution/blueberries) ratio.

Both control (Non-VI) and vacuum impregnated blueberries were irradiated on both sides with a 1.35 MeV Van de Graaff accelerator. Samples were irradiated using low, medium and high dose levels of 0.5 kGy; 1.0 kGy; 1.5 kGy and 2.0 kGy. After irradiation, fruits were kept at 4.5-5°C, 50-55% RH for 14 days and tested for quality attributes on days 0, 7 and 14.

3.2.1 Absorbed Dose Calculations with Radiochromic Film

Average counts/ kGy in the accelerator was 419923, that is, 419923 electrons were released from the accelerator at per kGy dosage. An irradiation dose sheet was generated shown as Table 12. There was a slight but not significant difference ($P>0.05$) between absorbed dose and target dose.

Table 12 Irradiation dose with electrons counts for the 1.35 MeV Van der Graaff accelerator used in this study.

Target Dose, kGy	Target Counts	Actual Counts	Actual Dose , kGy
0.0	0	0	0
0.5	209961	209900	0.49
1.0	419923	420001	1.05
1.5	629885	629782	1.52
2.0	839846	841010	2.03

Note: The actual counts was the average counts of six replications.
The actual dose was calculated as: Actual dose = actual counts / 419923 *1 kGy.

3.2.2 Texture of irradiated blueberries

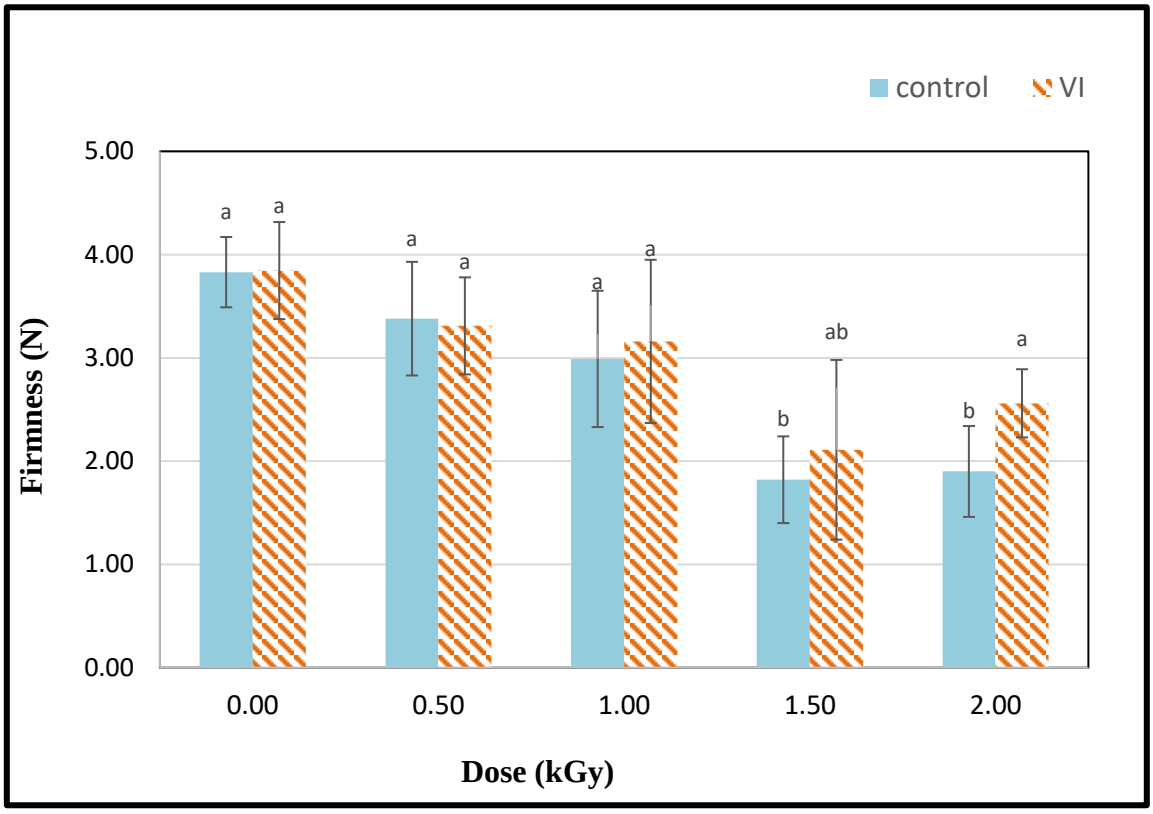
Firmness of the blueberries deteriorated due to exposure to ionizing radiation. In general, the higher the applied dose, the softer the samples by the end of storage. On the day of irradiation (Day 0), the firmness of control (non-VI) irradiated blueberries decreased ($P<0.05$) from 3.83 N to 1.98 N as the dose increased. The same effect was observed of the VI treated irradiated blueberries where the firmness decreased ($P<0.05$)

from 3.84 N to 2.11 N. (Figure 11). In addition, the control (Non-VI) fruits were significantly softer ($P<0.05$) than the VI samples when exposed to dose of 2.0 kGy.

On the Day 7, Figure 12 shows that control (non-VI) blueberries significantly lost ($P<0.05$) the firmness when exposed to doses greater than 1.0 kGy. However, the VI blueberries maintained ($P>0.05$) the firmness even when irradiated up to 2 kGy. At the end of the storage (Day 14), vacuum impregnated blueberries were significantly firmer ($P<0.05$) than the control (Figure 13).

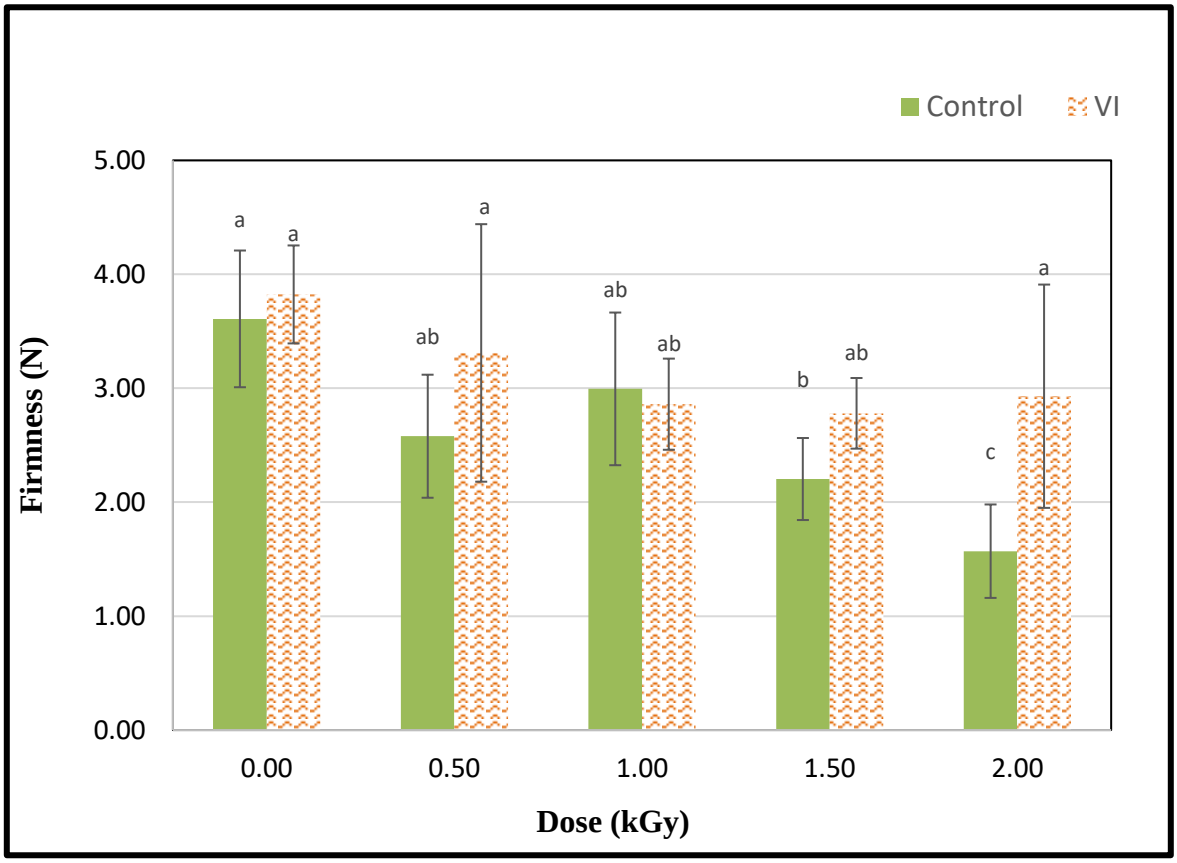
Firmness of control (Non-VI) irradiated blueberries decreased ($P<0.05$) over storage time (Table 13) while firmness of the VI pre-treated irradiated blueberries remained constant ($P>0.05$) with irradiation up to 1.0 kGy. Hence, the VI process did help reduce the negative effects of ionizing radiation on the cellular structure of the fruits and this was due to the calcium's function. Kovacs and others (1988) used SEM for ultrastructural investigations and found that calcium did improve the texture of irradiated pears and apples slices. The authors found that calcium could not prevent the breakdown the middle lamella of irradiated tissues, but had positive effects on the cell membranes. In addition, Magee and others (2003) found that 1-2% (w/w) calcium chloride did enhance ($P<0.05$) the firmness of diced tomatoes under Gamma irradiation with dose up to 1.25 kGy. The authors found that using calcium resulted in decreasing the water-soluble pectin, which yielded a firmer cell structure. In this study, VI pretreatment with 4% calcium lactate solution was effective in improving the firmness of irradiated blueberries up to 1.0 kGy.

Figure 11 Effect of irradiation and VI pre-treatment on firmness of blueberries on Day 0 of storage at 4.5-5ºC, 50-55% RH.



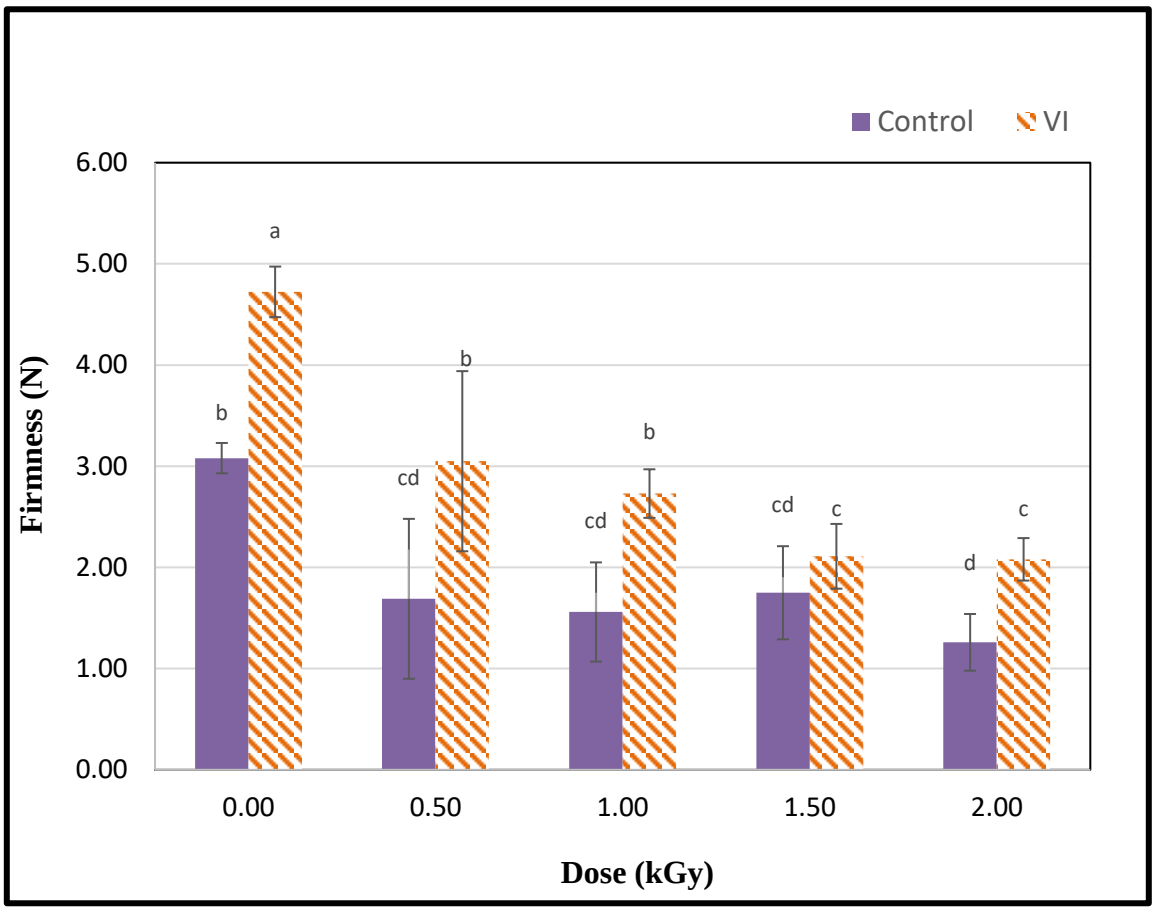
Note: Day 0: The day of irradiation test
Control: Non-VI pretreated blueberries
Samples were irradiated at room temperature.

Figure 12 Effect of irradiation and VI pre-treatment on firmness of blueberries on Day 7 of storage at 4.5-5ºC, 50-55% RH.



Note: Control: Non-VI pretreated blueberries.
Samples were irradiated at room temperature.

Figure 13 Effect of irradiation and VI pre-treatment on firmness of blueberries on Day 14 of storage at 4.5-5ºC, 50-55% RH.



Note: Control: Non-VI pretreated blueberries.
Samples were irradiated at room temperature.

Table 13 Effect of irradiation and VI pre-treatment on firmness of blueberries of storage at 4.5-5ºC, 50-55% RH up to 14 days.

Treatment Dose	Day 0	Day 7	Day 14
0.0 kGy control	3.83 ± 0.34^{ax}	3.61 ± 0.60^{ax}	3.08 ± 0.15^{by}
0.0 kGy VI	3.84 ± 0.47^{ay}	3.82 ± 0.43^{ay}	4.72 ± 0.25^{ax}
0.5 kGy control	3.38 ± 0.55^{ax}	2.58 ± 0.54^{aby}	1.69 ± 0.79^{cdz}
0.5 kGy VI	3.31 ± 0.47^{ax}	3.31 ± 1.13^{ax}	3.05 ± 0.89^{bx}
1.0 kGy control	2.99 ± 0.66^{ax}	2.29 ± 0.67^{abxy}	1.56 ± 0.49^{cdy}
1.0 kGy VI	3.16 ± 0.79^{ax}	2.86 ± 0.4^{abx}	2.73 ± 0.24^{bx}
1.5 kGy control	1.82 ± 0.42^{bx}	2.20 ± 0.36^{bx}	1.75 ± 0.26^{cdy}
1.5 kGy VI	2.11 ± 0.87^{abx}	2.78 ± 0.31^{abx}	2.11 ± 0.32^{cy}
2.0 kGy control	1.90 ± 0.44^{bx}	1.57 ± 0.41^{cy}	1.26 ± 0.28^{dz}
2.0 kGy VI	2.56 ± 0.23^{ax}	2.93 ± 0.98^{ax}	2.08 ± 0.21^{cy}

Note: Data is listed as (Average ± Standard Deviation). The means are an average of 9 replications.
Control, blueberries without vacuum impregnation treatment.
a-b Subscript letters within a column which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row which are not the same shows a significant difference between days ($P<0.05$) and vice versa.
Samples were irradiated at room temperature.

The loss of textural quality (loss of firmness) of VI-treated and control (Non-VI) irradiated blueberries as a function of irradiation dose can be described by an exponential relationship,

$$F = A\, e^{-kD} \qquad \text{(Equation 7)}$$

Where *F* is the firmness (N) and *D* is the dose (kGy), *k* is the kinetic parameter (firmness loss constant, kGy $^{-1}$) and A is a constant determined by sample properties (Appendix O). Table 14 below shows the firmness constant *k* of the irradiated blueberries.

Table 14 Firmness loss constant (*k*) and R^2 values of irradiated blueberries. Samples were stored up to 14 days at 4.5-5ºC, 50-55% RH.

Date	**Control, *k* (kGy $^{-1}$)**	**R^2**	**VI pretreated, *k* (kGy^{-1})**	**R^2**
0	0.202	0.88750	0.126	0.7246
7	0.364	0.8286	0.141	0.7171
14	0.175	0.6976	0.201	0.8971

Note: Control: Non-VI treated samples.
Samples were irradiated at room temperature.

Control (Non-VI) irradiated blueberries had larger *k* values than the VI pretreated samples on Day 0 and Day 7 of the storage, while only at the end of the storage, the control and VI pretreated blueberries had similar *k* values. This finding indicates that throughout a 14-day storage, control (Non-VI) blueberries lost firmness at a faster rate over the dosage than the VI treated samples. When fruits were exposed to irradiation, cell wall constituents such as polysaccharides, cellulose and hemicellulose decomposed and fruits became softer. Calcium has a function as a firming agent and thus, VI pretreated samples lost less firmness than the non-VI samples.

3.2.3 Total phenolic content (TPH)

Previous results in this study show that vacuum impregnation treatment did not ($P>0.05$) affect TPH of blueberries. In this study, for both control (non-VI) and vacuum impregnated irradiated blueberries, the TPH of all samples remained constant ($P>0.05$) over the storage time (Table 15). Figure 14 and 15 show that samples from each dosage level were stable in TPH over time.

In addition, irradiation did not affect the TPH of the blueberries on Day 0 and Day 7. However, when the storage came to an end, the non-irradiated samples had significantly more ($P<0.05$) TPH than the irradiated samples. This could be a result of irradiated blueberries lost the firmness ($P<0.05$) on Day 14. The cell wall structure of the fruits was broken down and the enzymes which can decompose the TPH could easily come out and interact with the TPH and hence, TPH decreased. Moreover, TPH ranged from 90 to 200

mg/100g GAE in this study. The difference of 110 mg/100g could not yield any quality differences as TPH of blueberries could vary approximately 600mg/100g due to sample variances (Sellappan and others 2002). Miller (1994) found that the color of E-beam irradiated blueberries did not change with dose up to 1.25kGy, and gamma irradiation up to 3 kGy did not yield blueberries' color change either, which indicated that TPH did not change as it was related to fruits color closely. Moreno and others (2007) found that electron beam irradiation up to 3.1 kGy did not ($P>0.05$) affect the TPH on blueberries within 10 days at 5 ^{o}C storage.

However, several studies showed that irradiation treatment decreased the TPH of the fruits. Tomas and Espin (2001) observed that 1.75–2.50 kGy irradiation decreased the TPH of potato tubers, banana, mango and peach. In addition, Breitfellner and others (2002) found that γ-irradiation reduced ($P<0.05$) TPH of strawberries at the dose level of 6kGy, particularly degrading the flavonoids. Similarly, Schindler and others (2005) found that γ-irradiation up to 6 kGy reduced ($P<0.05$) the TPH significantly in tomatoes. In a more recent study, Santillo and others (2014) found that grapes had darker color when irradiated at 4.5 kGy and it was due to increase in anthocyanins. Hence, irradiation did not affect the TPH of blueberries can be a result of relative low dose irradiation applied in this study, which was only up to 2 kGy.

Figure 14 Effect of irradiation dose on TPH of control (Non-VI) samples, samples were stored up to 14 days at 4.5-5ºC, 50-55% RH.

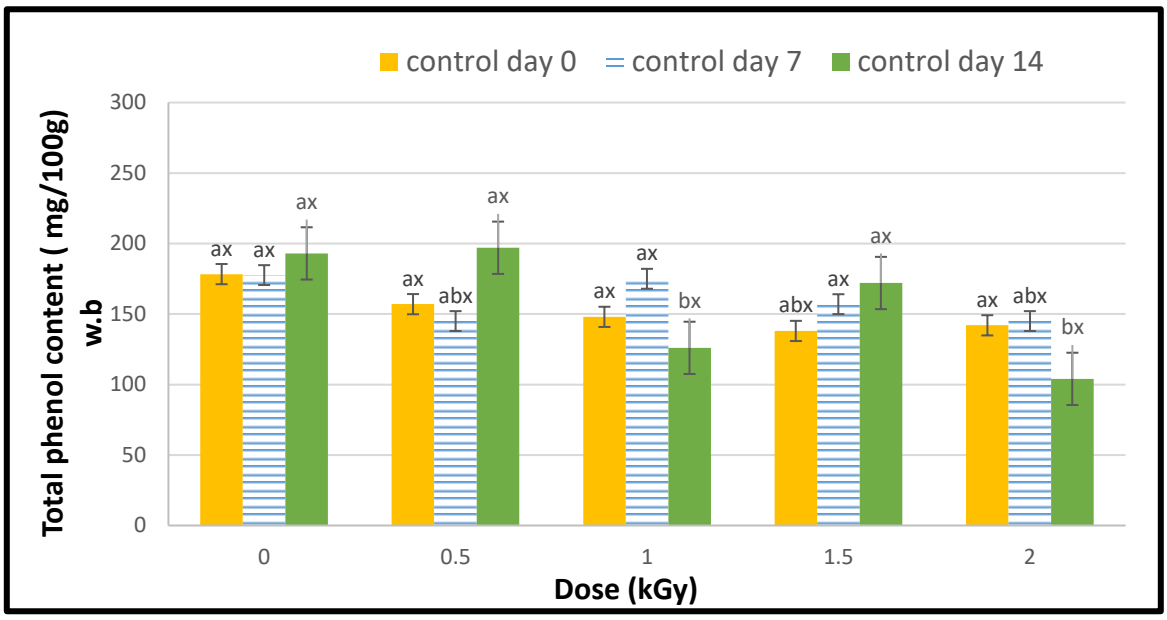


Figure 15 Effect of irradiation dose on TPH of VI samples, samples were stored up to 14 days at 4.5-5ºC, 50-55% RH.

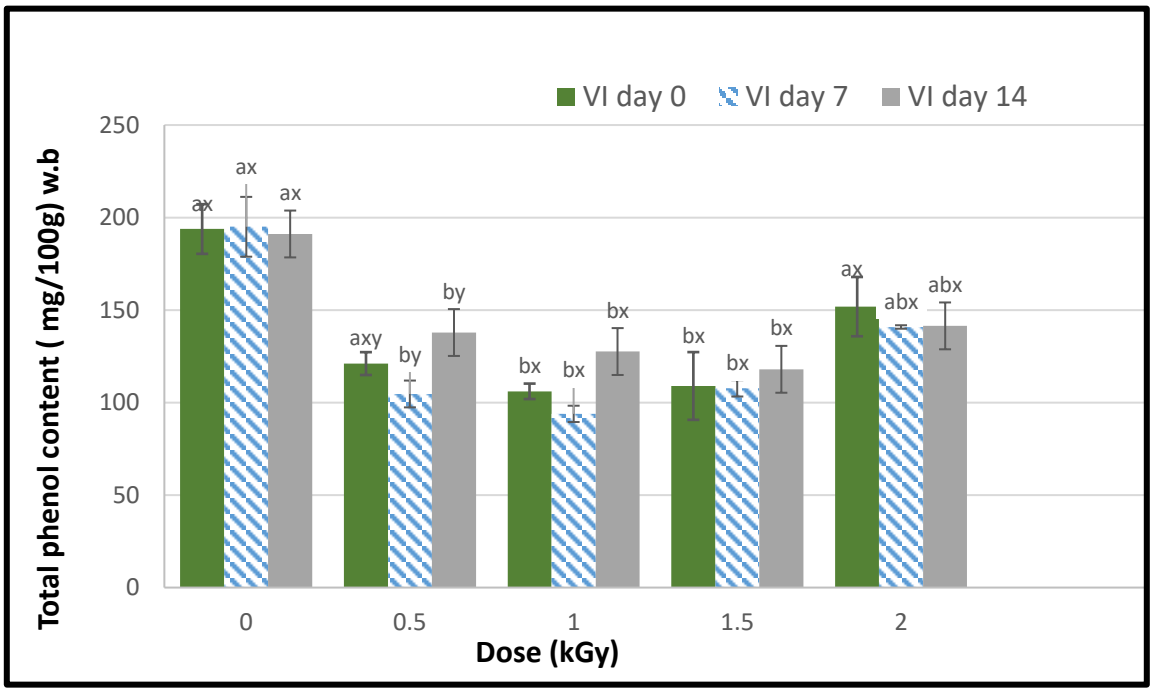


Table 15 Effect of E-beam irradiation and VI pre-treatment on the Total Phenolic Content of blueberries at storage up to 14 days at 4.5-5ºC, 50-55%RH.

Treatment	**Day 0**	**Day 7**	**Day 14**
0.0 kGy control	178.31 ± 5.27^{ax}	177.62 ± 11.27^{ax}	193.63 ± 3.68^{ax}
0.0 kGy VI	183.79 ± 13.41^{ax}	195.28 ± 16.16^{ax}	191.11 ± 20.76^{ax}
0.5 kGy control	157.44 ± 46.42^{axy}	145.00 ± 11.14^{abx}	196.60 ± 7.04^{ay}
0.5 kGy VI	121.06 ± 6.17^{axy}	104.64 ± 7.25^{by}	137.85 ± 10.80^{bx}
1.0 kGy control	148.49 ± 17.93^{ax}	175.15 ± 14.15^{ax}	125.80 ± 20.10^{bx}
1.0 kGy VI	106.07 ± 4.17^{bx}	93.88 ± 4.41^{bx}	127.57 ± 13.90^{bx}
1.5 kGy control	138.05 ± 3.88^{ax}	157.98 ± 8.41^{abx}	172.95 ± 22.33^{abx}
1.5 kGy VI	108.96 ± 18.30^{bx}	107.68 ± 4.41^{bx}	117.96 ± 10.37^{bcx}
2.0 kGy control	142.89 ± 43.38^{ax}	145.00 ± 11.14^{abx}	103.01 ± 3.73^{cx}
2.0 kGy VI	151.84 ± 16.02^{ax}	140.84 ± 0.94^{abx}	141.44 ± 4.43^{bx}

Note: Data is listed as (Average ± Standard Deviation). Means are average of 3 replications.
Control, blueberries without vacuum impregnation treatment.
a-b Subscript letters within a column which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row which are not the same shows a significant difference between days ($P<0.05$) and vice versa.
Samples were irradiated at room temperature.
*Total phenolic content is expressed with Gallic acid equivalent (GAE) mg/100g fresh blueberry weight.

3.2.4 pH

Previous results in this study showed that vacuum impregnation process did not affect the pH of blueberries. The pH was stable over 14 days without irradiation for both control (non-VI) and vacuum impregnated blueberries. When fruits were exposed to the irradiation where dosage was over 1.0 kGy, the pH of irradiated blueberries significantly increased ($P<0.05$) at the middle of the storage and then decreased ($P<0.05$) by the end. However, there was no difference ($P>0.05$) on pH between Day 0 and Day 14. For blueberries irradiated with 0.5 kGy dosage, the control (non-VI) samples had significant higher ($P<0.05$) pH while the vacuum impregnated blueberries had lower ($P<0.05$) pH by the end of the storage. Among different dosage levels, the pH was slightly fluctuating over the doses (Table 16). Nevertheless, as the pH changed from 2.74 to 3.26 in this study and this pH difference was a result of the maturation variety, the irradiation did not affect the pH of fruits. Similar facts were also found by others which irradiation did not affect the fruits' pH (Miller and others 1994; Golding and others 2014; Tong and others 2015).

Table 16 Effect of irradiation and VI pre-treatment on pH of blueberries of storage at 4.5-5ºC, 50-55% RH up to 14 days.

Treatment Dose	Day 0	Day 7	Day 14
0.0 kGy control	2.96 ± 0.00^{cx}	2.97 ± 0.01^{cdy}	2.99 ± 0.01^{bx}
0.0 kGy VI	2.99 ± 0.01^{cx}	2.92 ± 0.00^{dx}	3.02 ± 0.01^{ax}
0.5 kGy control	2.83 ± 0.00^{dy}	3.03 ± 0.00^{cx}	3.05 ± 0.02^{ax}
0.5 kGy VI	3.09 ± 0.00^{bx}	3.12 ± 0.00^{bx}	2.88 ± 0.01^{dy}
1.0 kGy control	2.97 ± 0.00^{ay}	3.13 ± 0.01^{bx}	2.94 ± 0.00^{cy}
1.0 kGy VI	2.87 ± 0.00^{dy}	3.26 ± 0.00^{abx}	2.92 ± 0.00^{cy}
1.5 kGy control	2.90 ± 0.00^{dy}	3.31 ± 0.00^{ax}	2.91 ± 0.01^{cdy}
1.5 kGy VI	2.74 ± 0.00^{ey}	3.10 ± 0.01^{bx}	3.07 ± 0.02^{abxy}
2.0 kGy control	3.15 ± 0.00^{ax}	2.94 ± 0.00^{cy}	2.99 ± 0.02^{by}
2.0 kGy VI	2.87 ± 0.01^{dy}	3.11 ± 0.00^{bx}	2.97 ± 0.01^{by}

Note: Data is listed as (Average ± Standard Deviation). Means are average of 3 replications.
Control, blueberries without vacuum impregnation treatment.
a-b Subscript letters within a column which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row which are not the same shows a significant difference between days ($P<0.05$) and vice versa.
Samples were irradiated at room temperature.

CHAPTER IV

CONCLUSIONS

This study focused on the effects of vacuum impregnation as a pre-treatment on the quality of blueberries exposed to electron beam irradiation up to a dose of 2.0 kGy. The following conclusions can be drawn:

1. Moisture content, water activity, pH, total titratable acidity, sugar content, total phenolic content of blueberries were not affected ($P>0.05$) by the treatment with vacuum impregnation.
2. Texture of the blueberries was enhanced after vacuum impregnation treatment ($P<0.05$). Firmness of the vacuum impregnated blueberries remained constant ($P>0.05$) during the 14-day storage study at 4.5-5ºC, 50-55% RH while the non-VI treated blueberries were soft and mushy ($P<0.05$).
3. The vacuum impregnation treatment that yielded blueberries with enhanced firmness was 160 mm Hg bar vacuum pressure and 8% solid/liquid ratio (blueberries/impregnation solution) with 4% (w/w) calcium lactate solution.
4. A consumer test (50 panelists) found no significant differences of preference ($P>0.05$) among the vacuum impregnated fruits and the untreated controls throughout the storage period. Some comments from the panelists pointed out the sample size did affect their judgements and the smaller fruits were more disliked by the consumers.

5. Vacuum impregnation of blueberries with 4% (w/w) calcium lactate solution was effective in maintaining the firmness ($P>0.05$) of blueberries irradiated up to 1.0 kGy.
6. Vacuum impregnated blueberries were consistently firmer ($P<0.05$) than the non-VI treated fruits at each irradiation dose level. Vacuum impregnation helped maintain the texture of the blueberries ($P>0.05$) throughout the refrigerated storage while the non-VI fruits showed considerable softness.
7. Electron beam irradiation did not affect the pH and total phenolic content of the blueberries during the 14-day storage at 4.5-5ºC, 50-55% RH.

CHAPTER V

RECOMMENDATIONS FOR FUTURE STUDY

Recommendations for future research include:

- Evaluate the effects of a wider range of concentrations of calcium lactate on quality of the product.
- Evaluate the feasibility of applying vacuum impregnation to other fruits such as strawberries, raspberries and blackberries.
- Access the effectiveness of other vacuum impregnation solutions to achieve the project objectives, for example sugar solution.
- Explore the effectiveness of other combinations of non-thermal technologies to maintain firmness of fresh produce.

REFERENCES

Abbott JA. 1999. Quality measurement of fruits and vegetables. Postharvest Biol.Technol. 15(3):207-25.

Alothman M, Bhat R, Karim A. 2009. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chem. 115(3):785-8.

Anino SV, Salvatori DM, Alzamora SM. 2006. Changes in calcium level and mechanical properties of apple tissue due to impregnation with calcium salts. Food Res.Int. 39(2):154-64.

Arena V. 1971. Ionizing radiation and life: An introduction to radiation biology and biological radiotracer methods. Mosby: St. Louis.

Balasundram N, Sundram K, Samman S. 2006. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem. 99(1):191-203.

Ballinger W, Kushman L. 1970. Relationship of stage of ripeness to composition and keeping quality of highbush blueberries. Journal of the American Society of Horticultural Science 95239-42.

Basiouny FM, Chen Y. 1988. Effects of harvest date, maturity and storage intervals on postharvest quality of rabbiteye blueberry (Vaccinium ashei Reade). 101281-4.

Blanda G, Cerretani L, Cardinali A, Barbieri S, Bendini A, Lercker G. 2009. Osmotic dehydrofreezing of strawberries: Polyphenolic content, volatile profile and consumer acceptance. LWT-Food Science and Technology 42(1):30-6.

Blanda G, Cerretani L, Bendini A, Cardinali A, Scarpellini A, Lercker G. 2008. Effect of vacuum impregnation on the phenolic content of Granny Smith and Stark Delicious frozen apple cvv. European Food Research and Technology 226(5):1229-37.

Bourne M. 2002. Food texture and viscosity: concept and measurement. Academic press.

Bowtell J, Geer A, Adlam A, Bakkar ZA, Fulford J. 2015. Blueberry consumption enhances brain function in healthy elderly participants. Proceedings of the Physiological Society.

Bravo L. 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr.Rev. 56(11):317-33.

Breitfellner F, Solar S, Sontag G. 2002. Effect of γ-Irradiation on Phenolic Acids in Strawberries. J.Food Sci. 67(2):517-21.

Castrejón ADR, Eichholz I, Rohn S, Kroh LW, Huyskens-Keil S. 2008. Phenolic profile and antioxidant activity of highbush blueberry (Vaccinium corymbosum L.) during fruit maturation and ripening. Food Chem. 109(3):564-72.

Chiabrando V, Giacalone G, Rolle L. 2009. Mechanical behaviour and quality traits of highbush blueberry during postharvest storage. J.Sci.Food Agric. 89(6):989-92.

Cleland MR. 1983. Radiation processing: basic concepts and practical aspects. J.Ind.Radiat.Technol 1191-218.

Constenla D, Lozano J, Crapiste G. 1989. Thermophysical properties of clarified apple juice as a function of concentration and temperature. J.Food Sci. 54(3):663-8.

Cordenunsi BR, Nascimento JRO, Lajolo FM. 2003. Physico-chemical changes related to quality of five strawberry fruit cultivars during cool-storage. Food Chem. 83(2):167-73.

Damodaran S, Parkin KL, Fennema OR. 2007. Fennema's food chemistry. CRC press.

Diehl JF. 2002. Food irradiation—past, present and future. Radiat.Phys.Chem. 63(3):211-5.

Duan J, Wu R, Strik BC, Zhao Y. 2011. Effect of edible coatings on the quality of fresh blueberries (Duke and Elliott) under commercial storage conditions. Postharvest Biol.Technol. 59(1):71-9.

Eaton G, Meehan C, Turner N. 1970. Some physical effects of postharvest gamma radiation on the fruit of sweet cherry, blueberry, and cranberry. Canadian Institute of Food Technology Journal 3(4):152-6.

Ehlermann DA. 2016. Particular applications of food irradiation: Meat, fish and others. Radiat.Phys.Chem. 12953-7.

Feng Y, Bruhn C, Marx D. 2016. Evaluation of the Effectiveness of Food Irradiation Messages. Food Protection Trends 36(4):272-83.

Fito P, Chiralt A, Betoret N, Gras M, Cháfer M, Martínez-Monzó J, Andrés A, Vidal D. 2001. Vacuum impregnation and osmotic dehydration in matrix engineering: Application in functional fresh food development. J.Food Eng. 49(2–3):175-83.

Giovanelli G, Buratti S. 2009. Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties. Food Chem. 112(4):903-8.

Golding JB, Blades BL, Satyan S, Jessup AJ, Spohr LJ, Harris AM, Banos C, Davies JB. 2014. Low dose gamma irradiation does not affect the quality, proximate or nutritional profile of 'Brigitta'blueberry and 'Maravilla'raspberry fruit. Postharvest Biol.Technol. 9649-52.

Gong Z, Gao L, An J, Zhang M, Mujumdar A, Sun J. 2009. Effects of predrying and vacuum impregnation with nano-calcium carbonate solution on strawberries, carrots, corn, and blueberries. Drying Technol 28(1):36-41.

Gould GW. 2012. New methods of food preservation. Springer Science & Business Media.

Gras M, Vidal D, Betoret N, Chiralt A, Fito P. 2003. Calcium fortification of vegetables by vacuum impregnation: interactions with cellular matrix. J.Food Eng. 56(2):279-84.

Grecz N, Rowley D, Matsuyama A. 1983. The effect of irradiation on bacteria and viruses. Preservation of food by ionizing radiation.CRC Press, Boca Raton 167

Guimarães IC, Menezes EGT, Abreu PSd, Rodrigues AC, Borges PRS, Batista LR, Cirilo MA, Lima, Luiz Carlos de Oliveira. 2013. Physicochemical and microbiological quality of raspberries (Rubus idaeus) treated with different doses of gamma irradiation. Food Science and Technology (Campinas) 33(2):316-22.

Hallman GJ, Thomas DB. 1999. Gamma irradiation quarantine treatment against blueberry maggot and apple maggot (Diptera: Tephritidae). J.Econ.Entomol. 92(6):1373-6.

Hancock J, Callow P, Serçe S, Hanson E, Beaudry R. 2008. Effect of cultivar, controlled atmosphere storage, and fruit ripeness on the long-term storage of highbush blueberries. HortTechnology 18(2):199-205.

Hernández-Muñoz P, Almenar E, Ocio MJ, Gavara R. 2006. Effect of calcium dips and chitosan coatings on postharvest life of strawberries (Fragaria x ananassa). Postharvest Biol.Technol. 39(3):247-53.

Horwitz W. 2002. Instructions for inserting: Official methods of analysis of AOAC International. AOAC international.

Irfan P, Vanjakshi V, Prakash MK, Ravi R, Kudachikar V. 2013. Calcium chloride extends the keeping quality of fig fruit (Ficus carica L.) during storage and shelf-life. Postharvest Biol.Technol. 8270-5.

Ju ZY, Howard LR. 2003. Effects of solvent and temperature on pressurized liquid extraction of anthocyanins and total phenolics from dried red grape skin. J.Agric.Food Chem. 51(18):5207-13.

Kalt W, McDonald J, Donner H. 2000. Anthocyanins, phenolics, and antioxidant capacity of processed lowbush blueberry products. J.Food Sci. 65(3):390-3.

Kirkin C, Mitrevski B, Gunes G, Marriott PJ. 2014. Combined effects of gamma-irradiation and modified atmosphere packaging on quality of some spices. Food Chem. 154255-61.

Kita A, Bąkowska-Barczak A, Lisińska G, Hamouz K, Kułakowska K. 2015. Antioxidant activity and quality of red and purple flesh potato chips. LWT - Food Science and Technology 62(1, Part 2):525-31.

Koca I, Karadeniz B. 2009. Antioxidant properties of blackberry and blueberry fruits grown in the Black Sea Region of Turkey. Scientia Horticulturae 121(4):447-50.

Kong Q, Wu A, Qi W, Qi R, Carter JM, Rasooly R, He X. 2014. Effects of electron-beam irradiation on blueberries inoculated with Escherichia coli and their nutritional quality and shelf life. Postharvest Biol.Technol. 9528-35.

Kovacs E, Keresztes A, Kovacs J. 1988. The effects of gamma irradiation and calcium treatment on the ultrastructure of apples and pears. Food Structure 7(1):2.

Leiva-Valenzuela GA, Lu R, Aguilera JM. 2013. Prediction of firmness and soluble solids content of blueberries using hyperspectral reflectance imaging. J.Food Eng. 115(1):91-8.

Leunda Ma, Guerrero Sn, Alzamore S. 2000. Color and chlorophyll content changes of minimally processed kiwifruit. J.Food Process.Preserv. 24(1):17-38.

Lohachoompol V, Srzednicki G, Craske J. 2004. The Change of Total Anthocyanins in Blueberries and Their Antioxidant Effect After Drying and Freezing. J.Biomed.Biotechnol. 2004(5):248-52.

Luna-Guzmán I, Barrett DM. 2000. Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupes. Postharvest Biol.Technol. 19(1):61-72.

Magee RL, Caporaso F, Prakash A. 2003. Effects of exogenous calcium salt treatments on inhibiting irradiation-induced softening in diced Roma tomatoes. J.Food Sci. 68(8):2430-5.

Maraei RW, Elsawy KM. 2017. Chemical quality and nutrient composition of strawberry fruits treated by γ-irradiation. Journal of Radiation Research and Applied Sciences

Martin-Diana AB, Rico D, Frias JM, Barat J, Henehan G, Barry-Ryan C. 2007. Calcium for extending the shelf life of fresh whole and minimally processed fruits and vegetables: a review. Trends Food Sci.Technol. 18(4):210-8.

Matiacevic S, Silv P. 2012. Evaluation of Blueberry Color during Storage by Image Analysis. Color in Food: Technological and Psychophysical Aspects 211.

Matos S, Guiné R, Gonçalves F, Costa D. 2014. Evaluation of the physical-chemical properties of blueberries from diferente geographic origin.

Mclellan M, Lind L, Kime R. 1995. Hue angle determinations and statistical analysis for multiquadrant Hunter L, a, b data. J.Food Qual. 18(3):235-40.

Meilgaard MC, Carr BT, Civille GV. 2006. Sensory evaluation techniques. CRC press.

Miller W, McDonald R, Smittle B. 1995. Quality ofSharpblue'Blueberries after Electron Beam Irradiation. HortScience 30(2):306-8.

Miller W, McDonald R, McCollum T, Smittle B. 1994. Quality of ‘Climax’blueberries after low dosage electron beam irradiation. J.Food Qual. 17(1):71-9.

Molins RA, Motarjemi Y, Käferstein FK. 2001. Irradiation: a critical control point in ensuring the microbiological safety of raw foods. Food Control 12(6):347-56.

Monk JD, Beuchat LR, Doyle MP. 1995. Irradiation inactivation of food-borne microorganisms. Journal of Food Protection® 58(2):197-208.

Moraga MJ, Moraga G, Fito PJ, Martínez-Navarrete N. 2009. Effect of vacuum impregnation with calcium lactate on the osmotic dehydration kinetics and quality of osmodehydrated grapefruit. J.Food Eng. 90(3):372-9.

Moreno M, Castell-Perez ME, Gomes C, Da Silva PF, Moreira RG. 2006. Effects of electron beam irradiation on physical, textural, and microstructural properties of “Tommy Atkins” mangoes (Mangifera indica L.). J.Food Sci. 71(2):E80-6.

Moreno MA, Elena Castell-Perez M, Gomes C, Da Silva PF, Moreira RG. 2007. Quality of electron beam irradiation of blueberries (Vaccinium corymbosum L.) at medium dose levels (1.0–3.2 kGy). LWT - Food Science and Technology 40(7):1123-32.

Neto CC. 2007. Cranberry and blueberry: evidence for protective effects against cancer and vascular diseases. Molecular nutrition & food research 51(6):652-64.

Niemira BA, Fan X. 2006. Low-dose irradiation of fresh and fresh-cut produce: safety, sensory and shelf life. Food irradiation research and technology 32(5):45-52.

Nunes MCN, Emond J, Brecht JK. 2004. Quality curves for highbush blueberries as a function of the storage temperature. Small Fruits Rev. 3(3-4):423-40.

Olivas G, Barbosa-Cánovas G. 2005. Edible coatings for fresh-cut fruits. Crit.Rev.Food Sci.Nutr. 45(7-8):657-70.

Paniagua A, East A, Hindmarsh J, Heyes J. 2013. Moisture loss is the major cause of firmness change during postharvest storage of blueberry. Postharvest Biol.Technol. 7913-9.

Perkins-Veazie P, Collins JK, Howard L. 2008. Blueberry fruit response to postharvest application of ultraviolet radiation. Postharvest Biol.Technol. 47(3):280-5.

Poovaiah B, Glenn G, Reddy A. 1988. Calcium and fruit softening: physiology and biochemistry. Horticultural Reviews, Volume 10 107-52.

Redgwell RJ, Fischer M. 2002. Fruit texture, cell wall metabolism and consumer perceptions. Fruit quality and its biological basis.Sheffield: Sheffield Academic Press 46-88.

Ribera A, Reyes-Diaz M, Alberdi M, Zuñiga G, Mora M. 2010. Antioxidant compounds in skin and pulp of fruits change among genotypes and maturity stages in highbush blueberry (Vaccinium corymbosum L.) grown in southern Chile. Journal of soil science and plant nutrition 10(4):509-36.

Rico D, Martin-Diana AB, Frias JM, Barat J, Henehan G, Barry-Ryan C. 2007. Improvement in texture using calcium lactate and heat-shock treatments for stored ready-to-eat carrots. J.Food Eng. 79(4):1196-206.

Rimando AM, Nagmani R, Feller DR, Yokoyama W. 2005. Pterostilbene, a new agonist for the peroxisome proliferator-activated receptor α-isoform, lowers plasma lipoproteins and cholesterol in hypercholesterolemic hamsters. J.Agric.Food Chem. 53(9):3403-7.

Rockland LB, Stewart GF. 2013. Water activity: influences on food quality: a treatise on the influence of bound and free water on the quality and stability of foods and other natural products. Academic Press.

Rohrbach R, Franke J, Willits D. 1982. A firmness sorting criterion for blueberries. Trans.ASAE 25(2):261-5.

Salwin H. 1959. Defining minimum moisture contents for dehydrated foods. Food Technol. 13(10):594-5.

Sams CE. 1999. Preharvest factors affecting postharvest texture. Postharvest Biol.Technol. 15(3):249-54.

Santillo AG, Rogovschi VD, Araujo MM, Silva PV, Silveira AP, Villavicencio AL. 2014. Influence of gamma radiation on grapes color during storage period.

Sapers GM, Burgher AM, Phillips JG, Jones S. 1984. Color and composition of highbush blueberry cultivars. J.Am.Soc.Hort.Sci. 109(1):105-11.

Sathe SK, Teuber SS, Roux KH. 2005. Effects of food processing on the stability of food allergens. Biotechnol.Adv. 23(6):423-9.

Schindler M, Solar S, Sontag G. 2005. Phenolic compounds in tomatoes. Natural variations and effect of gamma-irradiation. European Food Research and Technology 221(3-4):439-45.

Scott W. 1957. Water relations of food spoilage microorganisms. Adv.Food Res. 783-127.

Sellappan S, Akoh CC, Krewer G. 2002. Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. J.Agric.Food Chem. 50(8):2432-8.

Slinkard K, Singleton VL. 1977. Total phenol analysis: automation and comparison with manual methods. Am.J.Enol.Vitic. 28(1):49-55.

Smith M, Marley K, Seigler D, Singletary K, Meline B. 2000. Bioactive properties of wild blueberry fruits. J.Food Sci. 65(2):352-6.

Steinmetz KA, Potter JD. 1996. Vegetables, fruit, and cancer prevention: a review. J.Am.Diet.Assoc. 96(10):1027-39.

Steyn WJ. 2012. The physiology and functions of fruit pigments: An ecological and horticultural perspective.

Sturm K, Koron D, Stampar F. 2003. The composition of fruit of different strawberry varieties depending on maturity stage. Food Chem. 83(3):417-22.

Toivonen PM, Brummell DA. 2008. Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. Postharvest Biol.Technol. 48(1):1-14.

Tomás-Barberán FA, Espin JC. 2001. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. J.Sci.Food Agric. 81(9):853-76.

Tong J, Rakovski C, Prakash A. 2015. Phytosanitary Irradiation Preserves the Quality of Fresh Blueberries and Grapes during Storage. HortScience 50(11):1666-70.

Tosun I, Ustun NS, Tekguler B. 2008. Physical and chemical changes during ripening of blackberry fruits. Scientia agricola 65(1):87-90.

Valero D, Serrano M. 2010. Postharvest biology and technology for preserving fruit quality. CRC press.

Wang SY, Lin H. 2000. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. J.Agric.Food Chem. 48(2):140-6.

Xie J, Zhao Y. 2003. Nutritional enrichment of fresh apple (Royal Gala) by vacuum impregnation. Int.J.Food Sci.Nutr. 54(5):387-98.

Yang W, Harpole J, Finn C, Strik B. 2008. Evaluating berry firmness and total soluble solids of newly released highbush blueberry cultivars. 863-8.

Yu L, Reitmeier C, Gleason M, Nonnecke G, Olson D, Gladon R. 1995. Quality of electron beam irradiated strawberries. J.Food Sci. 60(5):1084-7.

Yurttas ZS, Moreira RG, Castell-Perez E. 2014. Combined Vacuum Impregnation and Electron-Beam Irradiation Treatment to Extend the Storage Life of Sliced White Button Mushrooms (Agaricus bisporus). J.Food Sci. 79(1):E39-46.

Zhao Y, Xie J. 2004. Practical applications of vacuum impregnation in fruit and vegetable processing. Trends Food Sci.Technol. 15(9):434-51.

Zheng Y, Yang Z, Chen X. 2008. Effect of high oxygen atmospheres on fruit decay and quality in Chinese bayberries, strawberries and blueberries. Food Control 19(5):470-4.

APPENDIX A

Appendix A.1 Nutrition profile of blueberries (Adapted from USDA food composition database, 2016).

Source: USDA National Nutrient Database for Standard Reference		
Full Report (All Nutrients)		
Report Run at: March 21	2016 11:12 EDT	
Nutrient data for: 09050, Blueberries, raw		
Food Group: Fruits and Fruit Juices		
Common Name:		
Carbohydrate Factor: 3.6 Fat Factor: 8.37 Protein Factor: 3.36 Nitrogen to Protein Conversion Factor: 6.25		
Refuse: 5% Refuse Description: Stems and green or spoiled berries		
Nutrient	Unit	1Value per 100 g
Proximates		
Water	g	84.21
Energy	kcal	57
Energy	kJ	240
Protein	g	0.74
Total lipid (fat)	g	0.33

Ash	g	0.24
Carbohydrate, by difference	g	14.49
Fiber, total dietary	g	2.4
Sugars, total	g	9.96
Sucrose	g	0.11
Glucose (dextrose)	g	4.88
Fructose	g	4.97
Lactose	g	0
Maltose	g	0
Galactose	g	0
Starch	g	0.03
Minerals		
Calcium, Ca	mg	6
Iron, Fe	mg	0.28
Magnesium, Mg	mg	6
Phosphorus, P	mg	12
Potassium, K	mg	77
Sodium, Na	mg	1
Zinc, Zn	mg	0.16
Copper, Cu	mg	0.057
Manganese, Mn	mg	0.336

Selenium, Se	µg	0.1
Vitamins		
Vitamin C, total ascorbic acid	mg	9.7
Thiamin	mg	0.037
Riboflavin	mg	0.041
Niacin	mg	0.418
Pantothenic acid	mg	0.124
Vitamin B-6	mg	0.052
Folate, total	µg	6
Folic acid	µg	0
Folate, food	µg	6
Folate, DFE	µg	6
Choline, total	mg	6
Betaine	mg	0.2
Vitamin B-12	µg	0
Vitamin B-12, added	µg	0
Vitamin A, RAE	µg	3
Retinol	µg	0
Carotene, beta	µg	32
Carotene, alpha	µg	0
Cryptoxanthin, beta	µg	0

Vitamin A, IU	IU	54
Lycopene	µg	0
Lutein + zeaxanthin	µg	80
Vitamin E (alpha-tocopherol)	mg	0.57
Vitamin E, added	mg	0
Tocopherol, beta	mg	0.01
Tocopherol, gamma	mg	0.36
Tocopherol, delta	mg	0.03
Vitamin D (D2 + D3)	µg	0
Vitamin D	IU	0
Vitamin K (phylloquinone)	µg	19.3
Lipids		
Fatty acids, total saturated	g	0.028
4:00	g	0
6:00	g	0
8:00	g	0
10:00	g	0
12:00	g	0
14:00	g	0
16:00	g	0.017
18:00	g	0.005

Fatty acids, total monounsaturated	g	0.047
16:1 undifferentiated	g	0.002
18:1 undifferentiated	g	0.047
20:01	g	0
22:1 undifferentiated	g	0
Fatty acids, total polyunsaturated	g	0.146
18:2 undifferentiated	g	0.088
18:3 undifferentiated	g	0.058
18:04	g	0
20:4 undifferentiated	g	0
20:5 n-3 (EPA)	g	0
22:5 n-3 (DPA)	g	0
22:6 n-3 (DHA)	g	0
Fatty acids, total trans	g	0
Cholesterol	mg	0
Amino Acids		
Tryptophan	g	0.003
Threonine	g	0.02
Isoleucine	g	0.023
Leucine	g	0.044
Lysine	g	0.013

Methionine	g	0.012
Cystine	g	0.008
Phenylalanine	g	0.026
Tyrosine	g	0.009
Valine	g	0.031
Arginine	g	0.037
Histidine	g	0.011
Alanine	g	0.031
Aspartic acid	g	0.057
Glutamic acid	g	0.091
Glycine	g	0.031
Proline	g	0.028
Serine	g	0.022
Other		
Alcohol, ethyl	g	0
Caffeine	mg	0
Theobromine	mg	0
Flavonoids		
Anthocyanidins		
Cyanidin	mg	8.46
Petunidin	mg	31.5

Delphinidin	mg	35.4
Malvidin	mg	67.6
Pelargonidin	mg	0
Peonidin	mg	20.3
Flavan-3-ols		
(+)-Catechin	mg	5.3
(-)-Epigallocatechin	mg	0.7
(-)-Epicatechin	mg	0.6
(-)-Epicatechin 3-gallate	mg	0
(-)-Epigallocatechin 3-gallate	mg	0
(+)-Gallocatechin	mg	0.1
Flavanones		
Hesperetin	mg	0
Naringenin	mg	0
Flavones		
Apigenin	mg	0
Luteolin	mg	0.2
Flavonols		
Kaempferol	mg	1.7
Myricetin	mg	1.3
Quercetin	mg	7.7

Isoflavones		
Daidzein	mg	0
Genistein	mg	0
Glycitein	mg	0
Total isoflavones	mg	0
Formononetin	mg	0
Coumestrol	mg	0
Proanthocyanidin		
Proanthocyanidin dimers	mg	6.4
Proanthocyanidin trimers	mg	4.9
Proanthocyanidin 4-6mers	mg	20.5
Proanthocyanidin 7-10mers	mg	14.3
Proanthocyanidin polymers (>10mers)	mg	136

A.2 Effect of vacuum impregnation on moisture content % (g water/g blueberries) of blueberries. Samples were stored up to 14 days at 4.5-5ºC, 50-55% RH.

Treatment/Date	Day 0	Day 7	Day 14
Control*	86.84 ± 1.75^{ax}	85.85 ± 1.06^{bx}	87.09 ± 2.78^{ax}
T1	85.99 ± 3.14^{ax}	86.38 ± 0.77^{bx}	85.17 ± 1.57^{ax}
T2	85.71 ± 1.33^{ax}	84.78 ± 0.12^{bx}	84.95 ± 1.92^{ax}
T3	89.02 ± 0.68^{ax}	89.76 ± 0.21^{ax}	87.95 ± 1.15^{ax}
T4	87.71 ± 0.50^{ax}	89.53 ± 0.95^{ax}	89.05 ± 2.18^{ax}
T5	85.71 ± 1.20^{ax}	87.28 ± 1.69^{ax}	88.41 ± 2.14^{ax}
T6	86.15 ± 0.04^{ax}	84.25 ± 1.34^{bx}	86.37 ± 1.43^{ax}

Note: Data is listed as (Average ± Standard Deviation). The means are an average of 3 replications.
Control, blueberries without vacuum impregnation treatment.
T1- T6 means treatment #1 to #6 , referred to DOE.
a-b Subscript letters within a column (comparing treatments) which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row (over time) which are not the same shows a significant difference between days ($P<0.05$) and vice versa.
Non-Vacuum impregnated samples served as control.

A.3 Effect of vacuum impregnation on water activity of blueberries. Samples were stored up to 14 days at 4.5-5ºC, 50-55% RH.

Treatments	Day 0	Day 7	Day 14
Control*	0.988 ± 0.12^{ax}	0.978 ± 0.15^{ax}	0.988 ± 0.12^{ax}
T1	0.978 ± 0.21^{ax}	0.979 ± 0.15^{ax}	0.980 ± 0.15^{ax}
T2	0.979 ± 0.25^{ax}	0.976 ± 0.10^{ax}	0.978 ± 0.25^{ax}
T3	0.978 ± 0.07^{ax}	0.980 ± 0.06^{ax}	0.978 ± 0.21^{ax}
T4	0.981 ± 0.06^{ax}	0.975 ± 0.15^{ax}	0.978 ± 0.21^{ax}
T5	0.979 ± 0.25^{ay}	0.985 ± 0.11^{ax}	0.987 ± 0.12^{ax}
T6	0.979 ± 0.26^{ax}	0.972 ± 0.22^{ax}	0.983 ± 0.12^{ax}

Note: Data is listed as (Average ± Standard Deviation). The means are an average of 3 replications.
Control, blueberries without vacuum impregnation treatment.
T1 to T6 refers to vacuum impregnation treatment #1 to #6 (experimental design).
a-b Subscript letters within a column (comparing treatments) which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row (over time) which are not the same shows a significant difference between days ($P<0.05$) and vice versa.
Non-Vacuum impregnated samples served as control.

A.4 Effect of vacuum impregnation treatment on color of blueberries. Samples were stored at stored up to 14 days at 4.5ºC, 55% RH.

(1)L, lightness of blueberries.

Treatment/Day	day 0	day 7	day 14
Control	16.44 ± 0.20^{ax}	16.22 ± 0.22^{ay}	16.80 ± 0.08^{ay}
T1	13.42 ± 0.32^{abx}	12.44 ± 0.22^{bcy}	13.59 ± 1.57^{bx}
T2	15.87 ± 0.18^{ax}	13.77 ± 0.08^{by}	16.05 ± 0.25^{ax}
T3	15.25 ± 0.11^{ax}	12.85 ± 0.12^{bz}	14.02 ± 0.26^{aby}
T4	13.25 ± 0.11^{ax}	13.31 ± 0.40^{bx}	13.03 ± 0.26^{bx}
T5	15.63 ± 0.06^{ax}	14.63 ± 0.06^{by}	13.53 ± 0.12^{by}
T6	14.93 ± 0.46^{by}	16.17 ± 0.28^{ay}	15.17 ± 0.41^{ax}

(2) a*, redness to greenness of blueberries.

Treatment/Day	day 0	day 7	day 14
Control	0.13 ± 0.20^{ax}	0.13 ± 0.07^{ax}	0.14 ± 0.02^{ax}
T1	0.04 ± 0.02^{ax}	0.04 ± 0.02^{ax}	0.05 ± 0.03^{bx}
T2	0.16 ± 0.02^{ax}	-0.09 ± 0.02^{by}	0.17 ± 0.03^{ax}
T3	-0.15 ± 0.04^{by}	-0.07 ± 0.02^{by}	0.06 ± 0.01^{bx}
T4	-0.01 ± 0.01^{by}	0.14 ± 0.02^{ax}	0.15 ± 0.05^{ax}
T5	-0.08 ± 0.01^{by}	-0.05 ± 0.03^{by}	0.08 ± 0.06^{bx}
T6	-0.06 ± 0.02^{bx}	-0.12 ± 0.02^{bx}	-0.08 ± 0.01^{bx}

(3) b*, yellowness to blueness of blueberries.

Treatment/Day	day 0	day 7	day 14
Control*	-2.15 ± 0.06^{ax}	-2.14 ± 0.07^{ax}	-2.41 ± 0.06^{ax}
T1	-0.98 ± 0.08^{cy}	-0.65 ± 0.14^{cy}	-1.18 ± 0.06^{bx}
T2	-2.09 ± 0.03^{ax}	-1.35 ± 0.02^{bz}	-1.79 ± 0.09^{by}
T3	-1.74 ± 0.03^{bx}	-1.26 ± 0.04^{bz}	-1.56 ± 0.02^{by}
T4	-1.26 ± 0.07^{bx}	-1.22 ± 0.05^{bx}	-1.34 ± 0.12^{bx}
T5	-1.93 ± 0.01^{bx}	-1.59 ± 0.07^{by}	-1.29 ± 0.03^{bz}
T6	-1.58 ± 0.13^{bx}	-1.80 ± 0.03^{bx}	-2.05 ± 0.05^{ax}

Note: Data is listed as (Average ± Standard Deviation). The means are an average of 3 replications.
Control, blueberries without vacuum impregnation treatment.
T1- T6 means treatment #1 to #6, inferred to DOE.
a-b Subscript letters within a column (comparing treatments) which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row (over time) which are not the same shows a significant difference between days ($P<0.05$) and vice versa.
Non-Vacuum impregnated samples served as control.

A.5 Effect of vacuum impregnation on TSS of blueberries. Samples were stored up to 14 days at 4.5-5ºC, 50-55% RH.

Treatments/ Day	Day 0	Day 7	day 14
Control*	13.33 ± 0.06^{ay}	14.07 ± 0.23^{ax}	13.83 ± 0.06^{ax}
T1	13.33 ± 0.06^{ax}	14.07 ± 0.23^{ax}	13.87 ± 0.06^{ax}
T2	13.67 ± 0.11^{ax}	13.80 ± 0.20^{ax}	13.47 ± 0.15^{ax}
T3	13.63 ± 0.35^{bx}	13.00 ± 0.00^{ax}	13.93 ± 0.62^{ax}
T4	13.6 ± 0.35^{ax}	13.34 ± 0.20^{bx}	13.67 ± 0.31^{ax}
T5	12.2 ± 0.20^{bx}	12.27 ± 0.11^{bx}	12.53 ± 0.03^{bx}
T6	13.23 ± 0.06^{ax}	13.43 ± 0.06^{ax}	13.50 ± 0.06^{ax}

Note: Data is listed as (Average ± Standard Deviation). The means are an average of 3 replications.
Control, blueberries without vacuum impregnation treatment.
T1 to T6 refers to vacuum impregnation treatment #1 to #6 (experimental design).
a-b Subscript letters within a column which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row which are not the same shows a significant difference between days ($P<0.05$) and vice versa.
Non-Vacuum impregnated samples served as control.

A.6 Effect of vacuum impregnation on pH of blueberries. Samples were stored up to 14 days at 4.5ºC, 55% RH.

Treatment	Day 0	day 7	Day 14
Control*	2.54± 0.02 ^{by}	2.47± 0.02 ^{ay}	2.83± 0.01 ^{ax}
T1	2.38± 0.01 ^{bx}	2.32± 0.01 ^{bx}	2.29± 0.00 ^{dx}
T2	2.23± 0.02 ^{cx}	2.26± 0.01 ^{cx}	2.11± 0.01 ^{ex}
T3	2.46± 0.04 ^{bx}	2.43± 0.01 ^{bx}	2.56± 0.02 ^{bx}
T4	2.88± 0.01 ^{ax}	2.64± 0.01 ^{ay}	2.59± 0.01 ^{by}
T5	2.44± 0.01 ^{bx}	2.45± 0.01 ^{bx}	2.43± 0.01 ^{cx}
T6	2.18± 0.03 ^{cx}	2.26± 0.02 ^{cx}	2.28± 0.00 ^{ex}

Note: Data is listed as (Average ± Standard Deviation). The means are an average of 50 replications.
Control, blueberries without vacuum impregnation treatment.
T1 to T6 refers to vacuum impregnation treatment #1 to #6 (experimental design).
a-b Subscript letters within a column (comparing treatments) which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row (over time) which are not the same shows a significant difference between days ($P<0.05$) and vice versa.
Non-Vacuum impregnated samples served as control.

A.7 Sensory results of samples stored up to 14 days at 4.5-5ºC, 50-55% RH.

(1) Appearance

Treatments	Day 0	Day 7	Day 14
Control*	2.65 ± 1.34^{ax}	2.45 ± 1.02^{ax}	2.75 ± 1.1^{ax}
T2	2.70 ± 1.18^{ax}	2.81 ± 1.28^{ax}	2.86 ± 1.13^{ax}
T6	2.91 ± 1.31^{ax}	3.10 ± 1.29^{ax}	3.31 ± 1.43^{ax}

(2) Color

Treatments	Day 0	Day 7	Day 14
Control*	2.49 ± 1.22^{ax}	2.32 ± 1.03^{ax}	2.69 ± 1.68^{ax}
T2	2.49 ± 1.05^{ax}	2.75 ± 1.19^{ax}	2.53 ± 1.10^{ax}
T6	2.79 ± 1.47^{ax}	2.89 ± 1.12^{ax}	2.76 ± 1.43^{ax}

(3) Texture

Treatments	Day 0	Day 7	Day 14
Control*	3.32 ± 1.51^{ax}	2.75 ± 1.33^{ax}	3.17 ± 1.16^{ax}
T2	3.35 ± 1.59^{ax}	3.28 ± 1.19^{ax}	3.45 ± 1.15^{ax}
T6	2.81 ± 1.57^{ax}	3.42 ± 1.42^{ax}	3.49 ± 1.50^{ax}

Note: Data is listed as (Average ± Standard Deviation). The means are an average of 50 replications.
Control, blueberries without vacuum impregnation treatment.
T1 to T6 refers to vacuum impregnation treatment #1 to #6 (experimental design).
a-b Subscript letters within a column (comparing treatments) which are not the same shows a significant difference between treatments ($P<0.05$) and vice versa.
x-y Subscript letters within a row (over time) which are not the same shows a significant difference between days ($P<0.05$) and vice versa.
Appendix B The degradation of texture (loss of firmness) of VI and control samples over IR dosage where y is the firmness (N) and x is the dose (kGy).

A.8 Firmness lost over dosage equations.

Control (Non-VI) samples:

On day 0: $y = 4.8825e^{-0.202x}$, $R^2 = 0.8857$

On day 7: $y = 3.5899e^{-0.364x}$, $R^2 = 0.8286$

On day 14: $y = 3.0127e^{-0.175x}$, $R^2 = 0.6976$

VI pre-treated samples:

On day 0: $y = 4.2864e^{-0.126x}$, $R^2 = 0.7246$

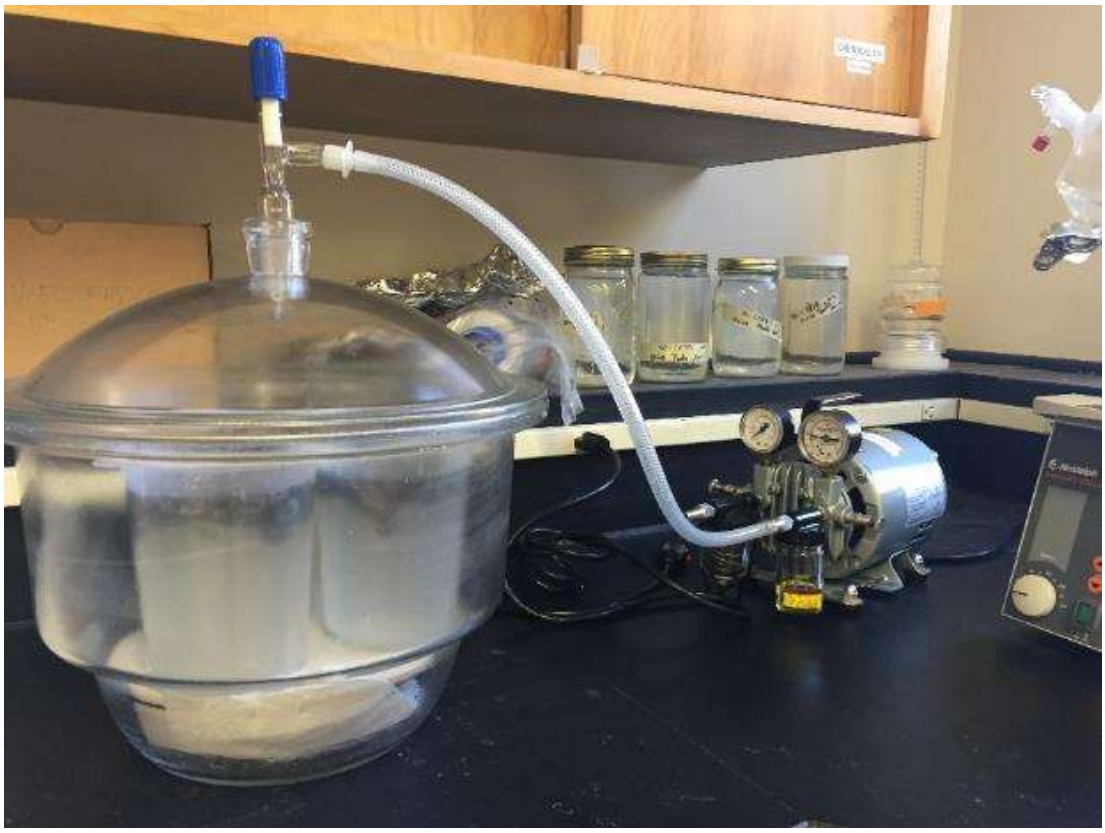
On day 7: $y = 3.5917e^{-0.141x}$, $R^2 = 0.7171$

On day 14: $y = 5.1183e^{-0.201x}$, $R^2 = 0.8971$

APPENDIX B

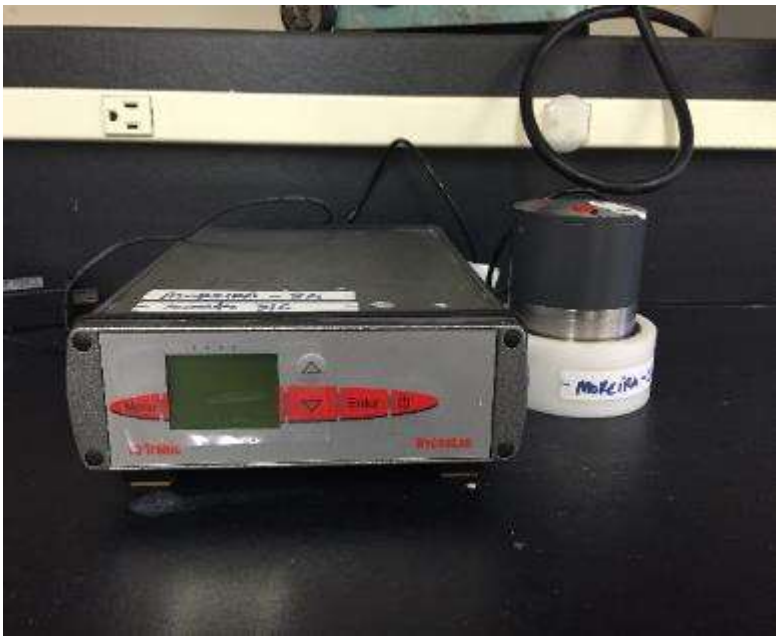
B.1 1.35 MeV Van de Graaff accelerator used in this study , Texas A&M University facility.

B.2 Vacuum impregnation system used in this study, Texas A&M University.



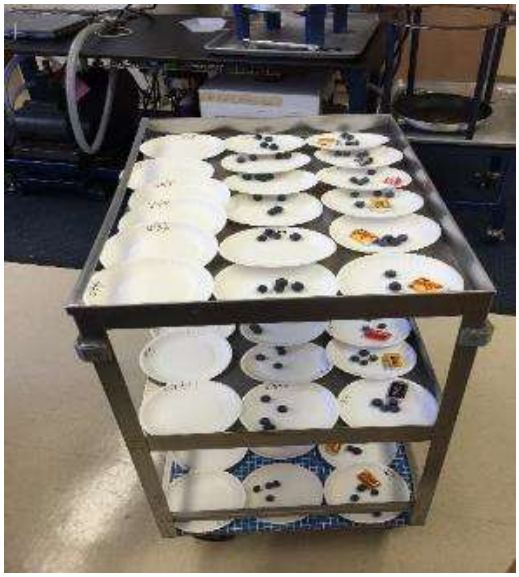
B.3 Left: Blueberries samples before vacuum dry; Right: Samples after vacuum dry at 70^{o}C for 6 hours.

B.4 Hydrometer for water activity measurement



B.5 Blueberries puree for TAA test; Top: before titration; Bottom: after titration by NaOH.

B.6 Sensory test sample preparation and testing.



B.7 Blueberries packed for E-beam Irradiation.