ELECTRON BEAM PASTEURIZATION USED TO PROCESS FRESH FRUIT FOR THE NEUTROPENIC DIET: E-BEAM REDUCES BIOBURDEN WHILE PRESERVING QUALITY

A Thesis

by

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ABSTRACT

Fresh produce is often touted for its many health benefits; however, various items have been linked to foodborne disease outbreaks. This is especially a concern for immune suppressed individuals who are classified as severely neutropenic (white blood cell count under 500 neutrophils/μL of blood). At this degree of suppression, many are urged to follow a restrictive diet that reduces the potential of exposure to microbial populations. Currently no processing technique is used to sanitize microorganisms from fresh produce. Electron beam (e-beam) irradiation is a non-thermal process that has been approved by the FDA to treat fresh foods and is able to eliminate bacteria. Another technology used to extend shelf life is modified atmosphere packaging (MAP). The objective of this study was to evaluate the use of e-beam irradiation at current FDA-approved doses (< 1 kGy) to determine whether bioburden on fresh fruits can be reduced while maintaining sensory quality. Aerobic plate count methods were employed to determine the bioburden of treatments over a 21 day storage period under both ambient and MAP conditions. A previously identified bacterial plate count benchmark of < 500 CFU/ gram will be used to determine the applicability of the e-beam treatment. A consumer study using a 9 point hedonic scale as well as instrumentation measuring color, texture, moisture content, total soluble solids and titratable acidity were used to compare the treated fruit to the control. E-beam consistently reduced the bioburden on strawberries, fresh-cut watermelon and significantly ($p<0.05$) for avocado samples. Avocado, grapes and watermelon showed potential to be labeled as clean foods (below

500 CFU/gram). Fruit firmness (as measured by deformation) was not negatively affected by e-beam treatment and was preserved over storage with MAP treatment. Color was not adversely affected by e-beam or MAP, except for avocados that were significantly more grey in the presence of O_2. Most importantly, consumers rated e-beam and MAP treated samples as acceptable (score above 5) in qualities of color, odor, flavor and firmness. E-beam proved to be an effective tool in reducing bioburden at low doses while maintaining fruit quality.

DEDICATION

This is dedicated to my family and friends.

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CHAPTER I

INTRODUCTION

Immunocompromised patients are at a higher risk for developing opportunistic infections, especially foodborne illnesses, when compared to a normal healthy population (1, 2). This is mainly due to the lack of defense their immune systems have in protecting their body against microbial translocation, especially if there is an imbalance in the bacterial ecology (3, 4). Many of these patients are classified as neutropenic, meaning they have abnormally low numbers of neutrophils, a type of white blood cell (2, 5). When the absolute neutrophil count (ANC) falls below 500 neutrophils/µl, individuals are considered at the greatest risk for developing infections (2, 5-7).

Neutrophils are part of the immune system and are responsible for fighting infections. When inadequate numbers are present in the body, any bacteria, even those naturally found on an individual, can cause infection. Neutrophils are produced in the bone marrow and procedures that damage bone marrow, like chemotherapy, radiation therapy and bone marrow transplants, are some of the main causes of this degree of neutropenia (1). Furthermore, neutropenia can be induced pharmacologically. Organ rejection is an immune response and thus anti-rejection medications are aimed at lowering the immune response (4, 6).

Neutropenia associated infections cause a significant increase in morbidity and deaths as well as an economic burden on the healthcare system and patients (8). In the event that these patients develop fungal infections, there is an average 19.2 day hospital

stay extension and these individuals are 5 times more likely to die. These patients on average end up paying $55,400 more than those who do not develop infection (9).

Because of their fragile state, patients suffering from neutropenia are urged by their health care providers to follow a restrictive neutropenic ("clean food") diet which ultimately aims to lower the chances of these patients encountering microorganisms in food products (10, 11). This diet excludes food items that have been minimally processed because these items normally contain a higher amount of microbes compared to foods that have been processed through cooking, pasteurization, or any other bacteria-killing technique (6). Foods that have a history of causing foodborne illness like raw eggs and dairy, and fresh or minimally processed fruits and vegetables, are included on the list of foods to avoid (2, 6). Allowable alternatives include peeled, cooked or canned fruits and vegetables which may not have desirable qualities like, as does fresh fruit(12) .

This lack of desirable food options may play a role in malnutrition, especially in pediatric oncology patients, as approximately 46% are said to suffer from cancer malnutrition (13). Children with cancer need sufficient levels of nutrition in order to properly grow and develop and recover from treatments (4). Treatments such as chemotherapy may cause a decrease in appetite which can be further exacerbated by denying patients appealing food choices like fresh fruit. This greatly contributes to a lower quality of life. (14).

In response to these dietary constraints, ionizing radiation provides an effective non-thermal processing technology that can be a used for microbial inactivation and quality preservation in fresh fruit. Application of this technology to nutrient rich food

products that are currently considered "risky" for immunocompromised populations could increase dietary choices for severely ill patients. Electron beam is one form of ionizing radiation used in numerous applications from medical device sterilization to cross linking of polymers to food processing (15, 16). Food irradiation studies over the last fifty years have proven that irradiation is an effective approach for decontamination and inactivation of pathogens in food as well as shelf life extension (15, 17). While other methods of sanitization used for produce, such as chlorine rinses, are limited in that only microbes on the outside of the fruit are killed, e-beam irradiation is an ideal process as it kills microbes on the surface as well as those that have been internalized within the fruit (18). Low- dose irradiation of < 1 kGy has been approved by the FDA for phytosanitary processing of fresh produce (16).

Another widely used preservation technology is modified atmosphere packaging (MAP) which has been shown to effectively maintain food quality during storage. When used appropriately, MAP can slow the chemical reactions that cause degradation while also inhibiting microbial growth (17).

These technologies have been combined to treat five fruits, which were chosen for this study based on their nutritional benefits and consumer appeal: tomatoes, red grapes, watermelon, strawberries and avocado.

I hypothesize that e-beam irradiation at doses <1 kGy will reduce the bioburden of fresh fruit while preserving sensory qualities and that used in combination with MAP will produce a superior product in terms of microbial safety and organoleptic properties.

The overall objective was to demonstrate the effectiveness of e-beam to develop foods for hospitals patients.

The specific objectives were:

1) To compare the bioburden levels of e-beam treated and non- treated (control) fruit samples in both ambient and modified atmospheres. I hypothesize that e-beam will reduce the bioburden levels of fresh fruit to clean food standards. (<500 CFU/g is the general clean food guideline)

2) To evaluate e-beam processing effects on the sensory qualities of fresh fruit in both ambient and modified atmospheres using instrumental and consumer analyses. I hypothesize that e-beam will not adversely affect attributes of fruits either physically or chemically. I also hypothesize that consumers will rate e-beam treated fruit as acceptable in specific sensory attributes.

CHAPTER II

LITERATURE REVIEW

Neutropenia

Neutropenia is a condition caused by cytotoxic chemotherapy or other bone marrow damaging procedures (19). This disorder is defined by an individual's white blood cell count, more specifically the absolute neutrophil count (ANC). If the ANC is below 1500 neutrophils/ml of blood, a person is classified as neutropenic. However, the most severe cases occur in individuals with an ANC below 500 cells/ml for a prolonged period of time (2, 5). This degree of suppression can have serious consequences as the risk of infection greatly increases. Neutropenia is such a common occurrence that it may affect as many as 80% of all patients receiving cancer treatments (20). This ultimately results in unanticipated hospital stays and interruption of treatments (8, 19). It is also one of the leading causes of death of cancer patients (8). Some of the main avenues by which an individual may become neutropenic include bone marrow transplants, radiation and chemotherapy. These procedures impair the ability of bone marrow to produce sufficient levels of white blood cells (1, 2).

White blood cells are important to the body's defense system because they help protect against infectious disease and foreign matter. There are five types of white cells: neutrophils (account for about 60% of total cells), monocytes, lymphocytes, eosinophils and basophils. These cells arise from hematopoietic stem cells in the bone marrow and participate in the inflammatory and immune responses to protect the body from infection

(21). When a cell is injured, the inflammatory response is initiated; this is a non-specific reaction that responds to chemical mediators released by the injured cell and is one of the first responses the body has to infection. This is important because it allows the body to confine the inflammogen to one area as well as activate the immune response and promote healing. Neutrophils are the first cells stimulated by the inflammatory response and will rush to ingest invading bacteria by migrating through the vessel wall near the site of infection (22).

The immune response on the other hand has a more specific action and is driven by immunological memory and antigen specificity. Immunogens will either illicit a humoral response, which involves antibodies from B lymphocytes, or cellular response, involving T lymphocytes. These cells also aid in the destruction and eradication of deleterious matter (2). Without the normal activity of white blood cells, the inflammatory response is nonexistent and this results in infectious organisms going undetected in the body (2). The infection progresses and the low numbers of T and B lymphocytes are not sufficient help in the weakened immune response. Since these cells are produced in the bone marrow, diseases or treatments that harm these cells can be devastating to immune processes. This greatly increases the vulnerability of patients to infection from any opportunistic bacteria, even in situations where initially there was resistance (23). This can further complicate the diagnosis of disease since often times neutropenic patients do not show signs of infection until they develop a fever. A fever is one of the first sign of infection and at that point can be an indicator of serious illness (19, 24).

Infections in the neutropenic population greatly increase morbidity and death rates, often by as much as 5 fold (9). Costs associated with these infections are also huge burdens to patients and hospitals (8, 19). It is estimated that patients who develop fungal infections on average spend over $55,000 more than patients who do not (9). This highlights the importance of preventing these infections from external sources but internal sources can be a concern as well.

Often times microorganisms that are normally found on or in the body can be potential pathogens if given the chance (4, 25). Keeping the skin clean and monitoring invasive procedures that involve catheters and IV's are vital steps to ensure that bacteria have not infected the skin around these areas or gained access into the body (23, 26). Oral hygiene is of great importance as well as decaying teeth and infected gums can lead to sepsis (1). Approximately 50% of infections in neutropenic patients are caused by translocation of normal microbial flora of the bowel; thus it is of utmost importance to maintain normal bacterial ecology and mucosal integrity of the bowel (3, 27). This can be difficult as mucosal injury is a major discomforting side effect of high dose chemotherapy (and radiation) and can affect any part of the gastrointestinal tract (28). Limiting the number of microbes ingested through diet is one step that can aid in maintaining this microbial balance as well as serving as a precautionary measure from introducing the patient to outside bacteria (8).

Neutropenic Diet

The neutropenic diet is one strategy to reduce the exposure of immunosuppressed individuals to potentially infectious organism (2, 4). These diets are restrictive because

they only allow foods that have been cooked thoroughly or pasteurized. As a general rule of thumb, clean or low microbe foods are considered to be those with bioburden levels less than 500 CFU/gram (29). As a result, the clean food diet often rejects raw and minimally processed foods such as fresh fruits and vegetables, raw eggs and seafood (4, 11, 30, 31). These foods are excluded from the diet as they may contain higher levels of bacteria that could cause infection since they are served without a processing step that specifically kills bacteria (like cooking) (11). Patients are specifically advised to avoid foods that have been linked to foodborne illness outbreaks like leafy green vegetables, cantaloupe and tomatoes (32). Alternatives to fresh fruits and vegetables may include those that can be peeled or cooked; however, these may not be aesthetically or nutritionally equivalent to minimally processed produce thus reducing the appeal to patients (4, 12). Furthermore, thermal processing and freezing is known to degrade compounds like vitamin C and thiamin that are sensitive and have higher benefits to an individual when the food is consumed in its unprocessed state (33).

Despite the intention of the clean food diet, there remains a lack of scientific evidence as to whether the neutropenic diet actually reduces the risk of infection in neutropenic patients (4, 34). As a result, there is a desire by critics to do away with the neutropenic diet and implement standard food safety guidelines for patients to adhere to (11, 31). This involves washing raw foods, like produce, adequately and steering clear from unpasteurized dairy products and cold cut deli meats (4, 35). This would allow the consumption of fresh fruits and vegetables as long as thorough washing and care was taken to ensure that the food is not a potential threat (36, 37). Many studies like that of

Moody et al 2002 have demonstrated that restrictive diets may not reduce the number of infections and is difficult for patients to adhere to; however, this diet is still heavily implemented in some health care environments (37, 38).

Although health care facilities cater to a vulnerable population, it has been shown that processed foods and meals prepared in hospital kitchens contain high numbers of contaminants resulting in unnecessary outbreaks of foodborne illness (29-31). Foods such as cereals, coffee, peanuts, tea, soy sauce and powdered milk have been reported to be contaminated with *Aspergillus spp. (39)*. Furthermore, *Aspergillus spp.* is commonly found in hospital locations around the world. In French health care facilities, pepper, tea, fruits and freeze dried soup tested positive for *Aspergillus spp.* in a study evaluating non-heat sterlizable foods in hematology units (40).

Another concern in hospital and healthcare settings is the ability of viruses and other diseases to spread quickly from patient to patient. A study examined a *Listera monocytogenes* outbreak in hospitals in north east England (41). It was discovered that the outbreak was caused by sandwiches provided by a catering company at the hospital shops (41). *Listeria monocytogenes* is a huge threat to immunocompromised individuals and is known to cause septicemia and meingoencephalitis. Listeriosis reportedly has between a 20%-40% death rate for those infected by the disease (42). An additional concern in hospital settings is norovirus (43). This pathogen is the number one cause of foodborne illness outbreaks in the US. According to the CDC, it contributes to 21 million illnesses each year, resulting in 70,000 hospitalizations and 800 deaths. This is a very contagious virus that is easily spread through contact with infected persons,

contaminated food and water as well as contaminated surfaces (43, 44). Almost two-thirds of the total reported norovirus outbreaks take place in long-term care facilities. This is also a concern overseas as in 2012, the Health Protection Agency reported 1,513 wards to be closed as a result of 1,818 norovirus outbreaks in England (45).

Another diet concern facing the neutropenic population is malnutrition (46). In some instances, 50% of patients were reported as being malnourished (46-48). Malnutrition in hospital patients is not usually caused by the primary disease, but rather a result of insufficient food intake. Undesirable food options and loss of taste perception in cancer patients make this condition a very common occurrence (49, 50). Malnutrition is one major limiting treatment factor in hospital patients worldwide (48, 51). When immunocompromised patients do not get sufficient nourishment, complications related to wound healing and ulcers are increased (47). This can lead to prolongation of hospital stays by 50% and three times higher mortality rates (52). The costs associated with treating malnourished patients can be over 300% more expensive than those associated with patients maintaining adequate nutritional status (53). Countries outside of the U.S. are equally affected by patient malnutrition. A study in Iran from December 2008 – June 2010 reported patients complained that low food quality contributed to their disappointment about being hospitalized. Reason for this included the food's lack of freshness, poor ingredient quality, poor preparation, a lack of spices and vegetables and problems with desserts. Moody et al 2006 found that oncology patients rated "decreased pleasure from food" as the second greatest negative experience of being hospitalized (14). Food dissatisfaction is common in many hospitals world- wide (48, 49, 52).

For patients undergoing immunosuppressive therapies, restrictive diets are but one of many forms of intervention that are intended to protect the severely immunocompromised. Other interventions that can increase patient dissatisfaction may include: isolation, air filtration, protective clothing, elimination of flowers, toys and animals and use of disinfectants (54). Thus therapeutic restrictions and limitations that patients face can have psychological effects on them as well (55, 56). A high frequency of mental disturbances have been found in bone marrow transplant (BMT) patients who are isolated and not able to participate in normal activities. This emotional distress is believed to often lead to physical complications such as infections (57).

Ionizing Radiation and Electron Beam Technology

Ionizing radiation is a form of electromagnetic energy comprised of particles carrying sufficient energy to knock out electrons from the orbit of atoms or molecules; this results in electrically charged particles, or ions (58). Irradiation is often referred to as cold pasteurization since a 1 kGy absorbed dose corresponds to 0.24 ˚C increase in temperature (15). There are three main kinds of ionizing radiation that have been approved by the FDA for processing food: gamma rays, electron beams and x-rays.

Gamma rays are high energy photons emitted by radioactive isotopes. These particles are considered to be indirectly ionizing because they are electrically neutral and do not interact with atomic electrons through Coulombic forces (68). Coulombic forces involve the attraction or repulsion of charged particles (like charges repel each other; opposite charges attract each other). One example of a gamma producing source is Cobalt-60 (Co-60). This is a man-made radionuclide produced when Co-59 absorbs a

neutron given off from the fission of U-235 (58). Operationally, Co-59 slugs are activated to Co-60 in a nuclear power reactor. Co-60 is often used in facilities for medical device sterilization and food irradiation. The gamma rays given off by Co-60 have energies of 1.17 and 1.33 Million electron Volts (MeV) that are produced by two different rays. Co-60 has a relatively deep penetrating ability; however, one limitation is the fact that radioactive emissions from Co-60 degrade 12.35% each year (half-life of 5.26 years) (58). As a result, the source must either must be replenished or replaced when it decays (58). Cesium-137 is another FDA approved gamma source with 0.662 MeV energies (16) and a half-life of 30 years. However, Co-60 facilities are more prevalent than Cesium-137 since the source is more readily available (58).

Electron beams (e-beams) are generated using a particle accelerators, such as a linear accelerator (16). These particles carry a charge and are considered directly ionizing because they interact directly with atomic electrons that participate in Coulombic interactions (68). Unlike gamma irradiation, no radioactive material is needed to create electron beams and the beam-generating instrument is able to be switched on or off when needed (16). Although e-beams have a more shallow penetration depth, this can be compensated for by dual exposure (i.e. top and bottom of product) or adjusting various parameters such beam energy (MeV) of the instrument to a higher level. Higher beam energies allow electrons to penetrate the product to a greater depth. Energies up to 10 MeV are allowed by the FDA for accelerated electrons. This 10 MeV limit ensures that food will not be processed at energies that could affect the energy balance in the nuclei in a way that would induce radioactivity in the food (15).

Low energy beams of 1 or 2 MeV are also available and are mainly used to treat thin packaged items such as grains and fluids. When processing packaged foods, it is often advised to use machines with at least 5 MeV as density of a product can limit e-beam effectiveness. Areal density can be used to assess the possibility of using e-beam for a product. The areal density is used to verify that the thickness of the product is within the single beam penetration limit of 3.8 g/cm^2 or dual beam processing limits of <8.5g/cm^2 (15). It is calculated using product height and density. If the product is outside of these limits, reconfiguring the packaging to provide a less dense product is one way to overcome the limitation.

As with gamma rays, x-rays are also photons that have a high penetrating ability. X-rays are produced when accelerated electrons strike a high atomic metal like tantalum or tungsten. This collision process converts electrons to x-rays and is known as bremsstrahlung (15). X-ray photons participate in Compton scattering in which an inelastic collision of a photon and electron occurs and part of the photon's energy is transferred into the scattering electron (15). This collision result in further scattering of electrons and photons called Compton events (16)The efficacy of the conversion of electrons to x-rays is low. Energies of 5 or 7.5 MeV are used in x-ray processing facilities.

Food Irradiation

Ionizing radiation and its effect on foods have been studied extensively for over half a century. Some of the main benefits provided by this technology include, insect control on fresh produce, sprout inhibition and microbial decontamination of produce,

meat, shellfish and eggs (15, 59). Food irradiation technology is approved by the FDA for treating specific foods at approved doses (Table 2.1) and is endorsed by various other agencies including the EPA, WHO, FAO, CDC and USDA in their implementation programs for food safety.

The existence of food irradiation technology ultimately began upon the discoveries of x-rays and radioactive sources in the mid-1890's (15). Research was then carried out investigating the effects of radiation from these sources on biological organisms. The success of this work then led to the approach of using these technologies for food applications (17). Initially, five varying kinds of ionizing radiation were examined and compared for their effectiveness in preserving food. These sources included ultraviolet light, x-rays, electrons, neutrons and alpha particles (60). Through comparative studies carried out in the 1940's and 50's it was found that electrons were the most suitable form of irradiation for food processing. This conclusion was based on the shallow penetration of ultraviolet rays and alpha particles and while x-rays had good penetration ability, they have a very low conversion efficiency of the electron beams into x-rays. Neutrons emitted from nuclear fusion or nuclear reactions (radioactive decay or particle accelerators) are good at penetrating materials and are effective at eliminating bacteria; however, since they have the ability to create radionuclides in food, they are not seen as safe for the purpose of food irradiation or sterilization of materials that humans may come in contact with (15). These considerations lead to the conclusion that electrons were the most efficient, safe and practical form of ionizing radiation for food preservation (15).

Table 2.1 Foods permitted to be irradiated under FDA's regulations (21 CFR 179.26)

Food	**Purpose**	**Dose**
Fresh, non-heated processed pork	Control of *Trichinella spiralis*	0.3 kGy min. to 1 kGy max.
Fresh foods	Growth and maturation inhibition	1 kGy max.
Foods	Arthropod disinfection	1 kGy max.
Dry or dehydrated Enzyme preparations	Microbial disinfection	10 kGy max.
Dry or dehydrated spices/seasonings	Microbial disinfection	30 kGy max.
Fresh or frozen, uncooked poultry products	Pathogen control	3 kGy max.
Frozen packaged meats (solely NASA)	Sterilization	44 kGy min.
Refrigerated, uncooked meat products	Pathogen control	4.5 kGy max.
Frozen uncooked meat products	Pathogen control	7 kGy max.
Fresh shell eggs	Control of *Salmonella*	3.0 kGy max.
Seeds for sprouting	Control of microbial pathogens	8.0 kGy max.
Fresh or frozen molluscan shellfish[1]	Control of *Vibrio* species and other foodborne pathogens	5.5 kGy max.
[1] (FDA 2005)		

Gamma irradiation is heavily used for food irradiation; yet, recently the cobalt source has become more difficult and expensive to obtain given the recent public unease concerning the nuclear industry (15). It is therefore predicted that linear accelerator generated electron beams will become the predominate source for ionizing radiation applications (15).

Today, there exists over 1,000 e-beam accelerators which are primarily used to treat polymers and medical devices (58). E-beam pasteurization of food is a small market and a relatively new process but the effects that ionizing radiation has on foods is similar between source types. Irradiation induces changes in biological organisms and chemical compounds present in foods as a result of the way in which the energies are absorbed and redistributed (15, 61). These chemical changes are caused by direct (primary) ionizing events or in-direct (secondary) free radical attack and are completely random events. Primary effects of the beam occur when the beam directly breaks chemical bonds. Electrons can also indirectly achieve this action through secondary effects when there are adequate levels of water present in the food product. Radiolysis of water results in ions, free radicals and other reactive species (such as hydroxyl radicals, hydrated electrons and hydrogen atoms). These ions can then interact with bacterial DNA causing inactivation (60). While bacteria are usually more susceptible to ionizing radiation when present in high water content foods, they can be expected to be more resistant in frozen or dried foods where water is bound or nonexistent (15). Electron beam decontamination of low water activity foods can be estimated to be reduced at a

given dose as there is both a lower production of free radicals and free radical mobility to interact with bacteria.

Typically, bacteria present on foods are classified into three groups: beneficial (useful) or commensal bacteria, spoilage bacteria and pathogenic bacteria. Useful bacteria are those that provide a useful purpose such as sugar fermentation while commensal bacteria provide neither an advantage nor disadvantage to the food product (62). Spoilage bacteria are comprised of microbes that cause undesirable changes in the quality of foods but are not necessarily harmful if consumed. On the other hand, pathogens are the microorganisms known to cause disease when ingested and are the pathogenic mediators of foodborne disease. Common food borne pathogens include *Salmonella spp., E. coli* O157:H7, *Clostridium botulinum, Listeria monocytogenes*. These pathogenic bacteria cause disease by means of invasion or intoxication (63). Yeasts and molds also present a food safety concern as some have the ability to produce poisonous toxins that cause foodborne illness. An example would be the production of aflatoxins by *Aspergillus flavus* ; this is an extremely dangerous toxin that can cause liver damage (15). Other infectious particles, such as viruses, cause concern as they are linked to foodborne outbreaks, especially norovirus, hepatitis A and poliovirus. Although viruses do not grow on food, they care capable of contaminating food and invading bacteria that may be present on foods (62,63). In addition, other organisms including parasites and insects present a food safety hazard as they can cause illness and high levels of spoilage in fresh produce. Ionizing radiation is used on foods primarily to

target disease causing bacteria but since this is a non-specific process, any bacteria that is present is susceptible to radiation induced destruction (64).

It is a generally accepted concept that ionizing radiation is able to inactivate bacteria through the disruption of chemical bonds in the DNA (64). The sugar phosphate backbone and base pairs are vulnerable to free radical attack which can leave scissions (breaks) in the DNA strands. DNA is essential to metabolic functions including cell division and transcription of gene products involved in synthesis of proteins and enzymes that regulate the cell metabolism. Therefore, disruption to this macromolecule often results in cell death (15). DNA is an easy target for ionizing radiation because of its relatively large size compared to other cellular molecules (11,63). These single and double stranded breaks in DNA may or may not prove to be immediately lethal; however, there is a possibility that mutations can surface in the DNA after replication (65). Since larger molecules are more susceptible to the effects of radiation, smaller molecules and particles such as virus and spores are more resistant to the technology (15). Minimum doses required to inactivate these organisms are usually higher than other biological organisms.

Measuring the effects of various doses of ionizing radiation on the survival of certain bacterial strains is important in determining the doses to use in food processing (11,16). The units used to measure the absorbed irradiation dose are grays (Gy) and is equivalent to 1 joule of energy deposited in 1 kilogram of mass. Formally, the rad (radiation absorbed dose) was used to measure absorbed dose before being replaced with the gray (1 gray = 100rads) (63). Food irradiation target doses vary depending on the

food product and need. Fresh foods are typically treated with low doses (< 1 kGy) for insect control and sprout inhibition. Medium doses range from 1-10 kGy for foods that have pathogen food safety risks. High doses are anything above that for food or products that need to be sterilized (15, 16).

Doses required to reduce an initial population of bacteria by 1 log is known as the D-10 value. This is often used to assess the radiation sensitivity of certain microorganisms in a given food environment (15, 16). These D-10 values are important in food safety applications and can be used as a general guideline for setting irradiation limits (17). While setting minimum doses are important to ensure that the effects and goals of irradiation are reached, maximum limits are also essential as high doses can result in a loss of sensory quality of foods. These maximum limits are mainly established by government agencies after thorough investigation and assurance that the irradiation dose will not negatively affect the aspects of the food in toxicological safety and nutritional values. Doses up to 10 kGy on any food item have been approved by a joint organization (comprised of the FAO, IAEA and WHO) as acceptable limits to process food. However, this decision was based on research assessing the wholesomeness (toxicologically, microbiologically and nutritionally safe) status of foods irradiated at 10 kGy or less (66). Data was lacking on the effects of ionizing radiation on foods beyond this dose. Furthermore, the technology was intended for food items that required a dose less than 10 kGy, such as meats and fresh produce. Low doses are used with these products as high doses would drastically decrease the quality of these foods. This ideology served as another reason to enforce this limit (66). Foods that may require

higher doses had not at that time been assessed for toxicological effects and overall nutrition therefore needing further studies before approval (63).

Upper irradiation limits are mainly set to ensure food quality as high doses can have undesirable changes in attributes like color, texture, smell, and taste. Ionizing radiation can have an effect on various components of food from macro to micronutrients much like other processing techniques like cooking. This is mainly due to the fact that excess energy is absorbed by the molecule in areas where bonds are the weakest or where electron density is the greatest (15).

Carbohydrates for example undergo a chemical change when subjected to ionizing radiation. Ether linkages are disrupted and C-H bonds are broken when exposed to low doses of irradiation (13). More complex carbohydrates like starches (cellulose or glycogen) will break down into smaller sugar units when glucosidic linkages are broken (13,17). Simple carbohydrates such as monosaccharides are broken into sugar acids and ketones when subjected to irradiation, much like hydrolysis reactions. Only when these sugars are treated with high irradiation doses will texture be drastically affected. Minimal effects are seen when carbohydrates are treated with low or medium levels or irradiation (67).

Proteins (or polypeptides) are another major compound found in foods. Proteins consist of chains of amino acids linked through peptide bonds. Functions of proteins depend on their tertiary structure, a feature that varies with each protein in accordance with its primary amino acid sequence (19,64). Collagen is an example where amino acid chains are parallel while globular proteins including enzymes have structures that are

twisted. Although individual amino acids are sensitive to ionizing radiation, the location of individual amino acids within the tertiary structure of the protein can create shielding effects. For example, amino acids located deep within the protein's tertiary structure are sensitive to free radical attack (61). Hence low dose irradiation only results in slight break down of proteins into smaller fragments. Higher doses on the other hand can completely denature the protein structure and alter food quality (61).

Another macronutrient in food is lipids. These are mainly triglycerides (glycerol backbone with three long fatty acid side chains). Low irradiation doses have not been found to adversely affect the nutritional quality of these compounds (15, 61). Some medium to high irradiation doses (3-10 kGy) can induce lipid oxidation and the formation of lipid hydroperoxides leading to rancidity in the product; however, these effects caused by irradiation on lipids can be minimized with the exclusion of oxygen from the system before processing (67).

These low dose irradiation effects on macronutrients are negligible and in most cases, result in less degradation of compounds when compared to heating, drying and cooking (15). Vitamins are smaller molecules that are vital to human health. They include water soluble varieties like the B vitamins as well as vitamin C while vitamin A, D E and K are fat soluble vitamins. Irradiation typically does not have a significant effect on these compounds since they are smaller molecules. Although, antioxidant vitamins can react with free radicals formed during irradiation and lose some of their effectiveness. Because doses are important to monitoring qualities of food, effectively

measuring the absorbed dose of a product is always vital and is achieved through dosimetry.

Dosimetry

A dosimetry system is an integral part of an irradiation process. It involves dosimeters, instruments to measure doses recorded by dosimeters and software to outline the procedures and use of the system (16). Dosimeters are objects that are able to exhibit a quantifiable change when exposed to irradiation (68) . This physical or chemical change can then be measured on an instrument that reads the absorbed dose. Food irradiation processes include an upper and lower dose. Upper doses are in place to ensure good manufacturing practices are being followed (53,66). It may also be set from a food quality standpoint to make sure food is not over processed to the point of being unacceptable to consumers (68). However, it is even more essential that lower doses are met so that the food sanitation goal of the irradiation process is ensured. Dosimetry is vital in order to effectively monitor the dose range and to guarantee that the process is in compliance with various regulations (68). Before a product can be commercially irradiated or processed for research purposes, the product must be dose mapped. This is a tedious process that allows the user to measure the dose distribution through a certain product and to find if it is uniformly distributed. In this process, dosimeters are placed throughout the product in areas where the doses are thought to be differently absorbed. The closer the dose maximum and dose minimum ratio is to 1, the more evenly distributed the dose is (68).

Fruit and Vegetable Shelf Life

Shelf life can be defined as "the length of time that corresponds to a tolerable loss in quality of a processed food and other perishable items" (69). However, shelf life can be described differently depending on the current needs. For instance, a market shelf life may not be the same as a sensory or microbial shelf life. In the market, the "sell by" or "use by" dates set for items depend on the food safety regulations the retailer is required to follow. Although an average shelf life of fresh cut produce is about 10-14 days, markets may enforce a 1-2 day maximum shelf life storage before discarding the item (69). Sensory shelf life varies by food product. Carrots provide a good example of foods whose shelf life can be defined by different criteria. Cut carrots develop a white surface color due to moisture loss well before they are considered to have unsafe levels of microbes. This would be an example where sensory shelf life preceding microbial contamination; the food is still safe to eat, but since the appeal is lost, consumers often find this product as low quality and unacceptable (70).

Furthermore, the shelf life of fresh produce varies widely depending upon the commodity in question. In fresh fruit and vegetables, the storage life is directly related to its rate of respiration, so produce items with high respiration rates will therefore have a shorter storage life than produce with low respiration rates (17, 18). Storage temperature is another important factor to shelf life prediction of fruits and vegetables as every 10°C increase in temperature typically increases respiration 2-3 fold thus decreasing shelf life. (16-18) Cooler temperatures can slow ripening and ethylene production allowing fruit senescence to occur at slower rates (71). The rate of water loss (produce contain between

65-95% water) and exhaustion of food reserves (starches) also has a great influence on the shelf life. Once these vital components are diminished, produce begin to perish since their source of energy has been depleted. This illustrates the point that external conditions have the potential to speed up or slow plant death, so it is important that as many factors be controlled to aid in preserving the fruit as long as possible (72).

Maintaining produce quality begins in the field. Contaminated irrigation water or manure, cross contamination by harvesters, food preparers, or workers with poor hygiene are primary avenues for introducing pathogens and spoilage microbes onto produce. Good manufacturing practices are not only essential in pre-harvest stages, but are just as vital during post-harvest to both the grower and consumer.

It is estimated by the FAO that about 25% of produce are lost after harvesting due to factors such as mishandling, spoilage and pest infestation. Some commodities are more perishable than others and fresh produce such as tomatoes, bananas and citrus fruit may see losses upwards of 50% in some areas. These losses result in great economic distress for growers and a lower amount of food available for consumers to purchase (63,64). Food products are often more successfully preserved if hurdle technologies are applied. This strategy involves the use of multiple techniques such as controlling temperature, water activity, using modified atmospheres, irradiation and competitive microorganisms all together. These techniques can be applied minimally which as a result has negligible effects on food quality but greatly suppresses microbial growth.

One of the main hurdle technologies involves proper cooling of harvested fruits. This is needed to preserve the quality of the produce as well as prevent the growth of

harmful microorganisms. Since refrigeration is one of the most important control points, it is vital that storage rooms be sufficiently insulated and cooled to prevent temperature variation (73). Washing produce is another major step in preventing the spread and transfer of pathogens and spoilage bacteria. Disinfectants such as chlorine may be used to kill microorganisms. Various factors such as temperature and organic debris can affect the concentration of chlorine and its ability to sanitize. Other disinfectants such as ozone and hydrogen peroxide are also used (74). In some areas, methyl bromide, a harmful chemical, is used for disinfestation of produce (13). Packaging is an especially important process because these materials may cause contamination. Proper storage of the packaging material is vital to ensure that the produce will not be contaminated in this way. For example, keeping the materials off of the floor and carefully packaged together before use can be one way in which the materials can stay clean until they are used (75). Various needs should be met based on the sensitivity of the food when developing and implementing an effective HACCP (Hazard Analysis and Critical Control Point) plan. Development of such procedures not only ensures that the food product is safe from pathogens, but also reduces the prevalence of spoilage microorganisms that tend to infest foods, causing them to rapidly deteriorate.

Microbial Spoilage of Fruit

Fruit surfaces are home to a large population of bacteria, most of which are native bacteria. A majority of the microbes that are introduced onto a fruit or vegetable are soil inhabitants (69). These microbes are spread through air and irrigation water and live with the produce as a commensal entity (76). Some native microorganism even

provide a protective system to the fruit or vegetable by forming a barrier that prevents harmful bacteria to invade and damage the commodity (77). Although spoilage microorganisms are a small number of bacteria that cause the deterioration of fruits and vegetables, they can easily be spread during harvesting, storage and distribution. These organisms are also are found on equipment used in harvesting produce, worker's tools, packing houses and on food contact surfaces. These relationships highlight the need to stress the importance of good agricultural practices to reduce the occurrence of spoilage microorganisms (69). Losses associated with spoilage in fruits and vegetables have been estimated to be at least 20% of all fruits and vegetables produced each year resulting in huge economic burdens.

Various factors that influence the levels and types of microbes on produce include: location grown, farming methods, processing and storage of produce (78). Although these factors dictate the vast microbial community, there are still some commonalities among produce (78). According to Leff et al, Enterobacteriacea and Bacillacea (30% and 4.6% respectively) were the families most heavily represented in the produce varieties they studied. With this being said, not all microorganisms present on fruit contribute to spoilage. Spoilage depends on the ability of the bacteria or fungi to produce metabolites that are known to result in off odors and tastes. For example, in vegetables, spoilage microorganisms like *Erwinia carotovora* and *Pseudomonas fluorescens* are examples of microbes that can degrade pectin content in the cell wall, thus resulting in unpleasant by products that would be deemed as spoilage (79).

Spoilage microorganisms thrive on fresh fruits and vegetables because of their nutrient rich tissues that easily support microbe life. More specifically with fresh produce, these foods contain starch that can be used to fuel bacterial growth. Various spoilage microorganisms have extracellular lytic enzymes that can be used to break down these starch polymers and subsequently use those by-products to facilitate growth. Fungi are especially efficient at spoilage as many contain various pectinases and hemicellulases that easily break down plant components (80). Internalization of spoilage bacteria is possible by invading fruit during its development or entering through other openings like the calyx. Examples of pathogens that infest and spoil fresh produce are *Penicillium expansum, Botrytis cinerea, Erwinia carotovora* and *Pseudomonas spp*. These microbes can cause devastating loss if fruits and vegetables are not cleaned properly or if infected produce are not removed before cross contaminating other fruits (80).

Modified Atmosphere Packaging

Fresh fruits and vegetables are especially prone to spoilage because of the increased respiration rate once harvested. Preservation technologies, such as canning, dehydration, freeze drying, low temperatures and modified atmosphere packaging (MAP) may be used to prolong shelf life of product. MAP technologies have become of increasing value to fresh produce industries as it can be applied to fresh and minimally processed (fresh cut) produce to prolong the foods initial fresh state. In modified atmosphere packaging, the normal atmospheric contents in the package around the food are altered or modified (71). This modified gas mixture is different from the normal

composition of air which is 20% oxygen, O_2, 1% carbon dioxide, CO_2 and 79% nitrogen, N_2, and consequently is able to slow natural deterioration by lowering the respiration rate and so reduce moisture loss. MAP can be used for an array of food products but the mixture of gas is dependent upon the kind of food, packaging material and temperature in which the product is stored. Fruits and vegetables have specific needs because they continue to respire after harvest. They are therefore, better maintained in packages with permeable films that allow the transmission of gases at a rate optimal for the commodity to balance the CO_2 and O_2 levels produced by the food (71).

Another characteristic of produce to consider when choosing MAP conditions is the fact that various commodities have different internal air space levels. Low air space amounts like potatoes (1-2%) are more resistant to diffusion of gases like O_2, CO_2 and ethylene, C_2H_4. Ethylene is a natural plant hormone that is responsible for initiating fruit ripening. This hormone can be controlled with lower O_2 levels as its production is cut in half when O_2 is reduced to 2.5% thus slowing the rate of ripening (71).

Ripening and respiration are especially affected by temperature because these metabolic processes increase with a rise in temperature. Just as important to the MAP system, elevated temperatures can alter film permeability. Fluctuations in temperature can also cause condensation inside the package. Temperature should be properly maintained to prevent undesirable changes in the food product.

Three predominate gasses are used in modified atmospheres beingCO_2, O_2 and N_2. These gasses can be used singly or combined to produce the optimal mixture for the food product.

For fresh produce, the O_2 content is often lowered from the normal atmospheric of 20% to 5% or below (71). This low O_2 atmosphere alone hinders many reactions that are known to reduce food quality. This includes slowing down respiration rates and enzymatic browning as well as C_2H_4 production by limiting the substrate O_2 that is needed for these reactions (59, 60). Other metabolic processes that require oxygen, such as lipid oxidation and browning reactions, are similarly delayed without the availability of its needed substrate. Low amounts of O_2 also prohibit the growth of spoilage bacteria and fungi. Typically, O_2 concentrations are kept as low as possible without allowing anaerobic fermentation of the product (71).

Carbon dioxide is another widely used gas for packaging. Elevated CO_2 in modified atmospheres has an antimicrobial effect on microorganisms as it lowers the pH to a level below that suitable for the growth of many bacteria (81-83). When CO_2e dissolves with water, it creates carbonic acid. This product elevates the acidity by lowering the pH of the fruit. Because of the high solubility of CO_2, appropriate balance is needed to avoid package collapse when CO_2 goes into solution (71).

Nitrogen is advantageous to the MAP system because it is un-reactive gas with low solubility in water. These traits make it ideal for balancing the gas composition in a modified atmosphere package. It is able to help stabilize the package to guard against collapse that may be caused by CO_2 (83). Other gases minimally used for MAP include carbon monoxide, sulphur dioxide and noble gases such as helium, argon and neon.

Various studies have demonstrated the increased value added to fresh or minimally processed fruits when MAP is effectively applied. Avocadoes (Hass) were

stored long term for up to 9 weeks under MA conditions (84). Chinese cabbage was packaged in various polypropylene films at low O_2 and high CO_2 atmospheres. The gas composition and packaging material aided in maintaining the overall appearance, odor and some bioactive compounds. More importantly, it remained marketable for up to 10 days when stored at 10˚C (85). La Storia (2012) used a combination of MAP and antimicrobials to extend shelf life of beefsteaks (86). They found that antimicrobial sheets in combination with MAP storage at 4˚ C were effective for the storage of beefsteaks by retarding the growth of spoilage bacteria, producing a lower concentration of volatile organic compounds and keeping acceptable levels of color and other sensory parameters for more than 10 days (86).

When irradiation is combined with MAP, significant increases in shelf life of produce have been seen. In a study by Lafortune et al 2005, peeled carrots were irradiated at doses of 1 kGy and the aerobic bacteria was reduced by 4 logs when packaged with ambient air. However, there was a 4.5 log reduction when the carrots were packed in MAP (87). Other studies demonstrating the usefulness of MAP is seen by Gunes et al 2011. MAP ($3\%O_2$, 50% CO_2, $47\%N_2$) was proven to reduce the undesirable effects of irradiation on the quality of meatballs and extend the shelf life for up to 21 days when refrigerated (88).

Packaging Material

Packaging films are a vital parameter to the success of a MAP system. The polymeric films used for these systems all have different permeability and durability properties. Films used alone may not encompass all of the physical properties desired for

a product and are often laminated or co-extruded together to produce films that have a more desirable trait for the food product (71). Film types include polystyrene, poly amide (nylon), cellulose, polypropylene and polyethylene. There are several factors that can influence the properties of packaging films and gas mixtures within the package. Increased temperatures cause an increase in respiration rate of the produce and also increase the film permeability. The produce as a result consumes more O_2 and gives off higher amounts of CO_2. This leads to a loss of quality of the food product because the film cannot effectively balance the gas mixtures inside the package. (89, 90).

Models have been developed to determine the levels of O_2 and CO_2 to incorporate into a package. The most widely used mathematical model is the Michaelis-Menten respiratory model. This respiratory model is typically used alongside the Arrhenius equation which considers the temperature sensitivity of the film permeability to estimate O_2 partial pressure as a function of other parameters (temperature, product mass, surface area and film thickness) (90).

It is often easier to customize packaging for lower respiring foods. High respiring commodities such as broccoli need a film with a high gas transmission rate so that gasses are entering or leaving the package at sufficient rates to support the aerobic respiration needs (66). This kind of film that improves gas transmission rates can be achieved through layers or 2 or 3 of different films. Each layer may provide a specific benefit such as strength, durability or a better gas transmission ability. These materials can be blended or laminated together to produce the desired materials (71).

Also, airtight packages are not advised because the O_2 levels will be depleted and anaerobic respiration that results in off flavors and smells could result. It is also just as important that the packaging material not be too porous or the modified atmosphere would escape and not be able to exert its benefits on the product (71,72).

It is also important to keep in mind that only certain packaging materials (Table 2.2) have been approved by the FDA for use in irradiation. This includes polyethylene, nylon and polyolefin among others; so it is vital that the materials have been approved before the produce are packaged and irradiated.

Most gas barrier films are composed of multiple layers. Packaging used in this study contained two films: polypropylene on the inside and nylon on the outside. Polypropylene is a vinyl polymer that is very much like polyethylene (the most commonly used plastic). Polypropylene however is very useful as it does not melt below 160°C unlike polyethylene that will anneal at temperatures around 100°C (58). Polypropylene can be produced from propylene (a monomer) through processes called Ziegler-Natta polymerization and metallocene catalysis polymerization.

The outside layer of nylon (polyamide) is used to protect the bag from tears, making it more durable and able to hold in the gas mixtures. Nylon can be made from diacid chlorides and diamines (58, 65).

Oxygen transmission rate (OTR) is the rate at which oxygen can permeate through a film and is expressed within a 24 hour time span. The lower the value, the more slowly oxygen is able to migrate into the package and cause undesirable reactions (65). The poly nylon bags used had an oxygen transmission rate of 63 cc/m^2 (per 24

hours). Polypropylene film alone has an OTR of 3,500 cc/m^2, thus demonstrating how multiple layers can have an effect on gas transmission properties. The moisture vapor transmission rate (MVTR) was 4.8 g/m^2/day meaning that the lower the rate, the longer the package is able to shelter the contents from moisture and maintain the moisture content of the product throughout storage (61,66).

Table 2.2 Packaging materials listed in 21 CFR 179.45 for use during irradiation of prepackaged foods.

21 CFR Citation	Packaging Materials	Max Dose [kGy]
Section 179.45(b)	Nitrocellulose-coated cellophane	10
	Glassine paper	10
	Wax-coated paperboard	10
	Polyolefin film	10
	Kraft paper	0.5
	Polyethylene terephthalate film (basic polymer)	10
	Polystyrene film	10
	Rubber hydrochloride film	10
	Vinylidene chloride-vinyl chloride copolymer film	10
	Nylon 11 [polyamide-11]	10
Section 179.45(c)	Ethylene-vinyl acetate copolymer	30
Section 179.45(d)	Vegetable parchment	60
	Polyethylene film (basic polymer)	60
	Polyethylene terephthalate film	60
	Nylon 6 [polyamide-6]	60
	Vinyl chloride-vinyl acetate copolymer film	60

Consumer Acceptance of Irradiated Fruits and Vegetables

Food irradiation has been studied for over 5 decades and is known to be the "most extensively studied food processing technology in the history of mankind" (91). Despite this, consumers are still fully unaware of the advantages this technology can provide (91). However, studies have shown that with increased consumer education, willingness to purchase or eat irradiated foods increases (91-93).

Consumer trends on reluctance to accept "new" technology can be seen with the introduction of pasteurization. It took almost 70 years for this technology to be accepted as a beneficial and necessary process (76). Some of the arguments brought on by critics included concerns about changing the properties of food, toxic chemicals being formed from this process, increases in the cost of purchasing milk, and that it was an unnecessary step in the processing of food, similar to the arguments against food irradiation (91). Several studies have been performed to evaluate the consumer acceptance of irradiated foods.

In 1994, the Nations Pride label was created by a Florida based company to help food companies bring irradiated products to market. These products included meat, poultry and produce (94). In February of that year, irradiated chicken wings and strawberries were offered to individuals at the Florida state fair. Over 17 days, 23,000 chicken wings and 5,000 strawberries were given out and over 95% of the fairgoers that were offered this food ate it. The individuals were told that the food had been irradiated to reduce *Salmonella* and that this was legal and that both the FDA and American Medical Associate had approved this process. One interesting finding from this study

was that the consumers seemed to be more accepting of the word “zapped” instead of “irradiation” (both words were displayed). When zapped was advertised, it was appreciated by consumers because it was not a technical word, but rather a word that they already used in their vocabulary. Also, it was somewhat comical as it made consumers smile when hearing the word. This “zapped” description has been used in media and food industry; thus most consumers knew that it referred to irradiation and many thought it was much easier to say than irradiated (94).

Various studies have shown that consumers are willing to buy irradiated foods; however, the problem is that irradiated food is not as readily available as non-irradiated foods in the grocery stores. Although some grocers are educated on the benefits of offering irradiated foods, many are concerned of the negative media attention they might get from activists (94). In a study carried out at Purdue, (Pohlman et. l, 1994) consumers were shown a video on the irradiation process and educated on the regulations and food products allowed to be irradiated in the US. This information increased the consumer willingness to buy from about 50% to 90%. When these subjects were allowed to taste irradiated fruit, such as strawberries, as well as watch the video, willingness to buy increased to 99% (95).

Other countries have their concerns with irradiated foods as well. In Turkey, a study was performed and it was found that 80% of consumers were uncertain about irradiated foods and their safety while only 11% stated that irradiated foods were safe (93). Some consumers were concerned that irradiated foods could become radioactive.

However, when consumers were told of the benefits of irradiation, positive feelings towards the technology increased to 62% (93).

Objective Sensory Analysis of Irradiated Fresh Fruits and Vegetables

Consumers have expressed skepticism about eating irradiated foods because they are often concerned that the process alters physical properties of color or texture of the fruit resulting in a loss of quality. In a study by Yu et al. 1996, strawberries were irradiated at 0, 1 and 2 kGy and stored at 2˚C. The firmness and pectin content was compared over an 8 day storage period. Flesh firmness increased on the first two days and from day 3 on, firmness decreased with storage time (96). Fruit softening caused by irradiation was possibly due to an alteration of pectic substances as proposed by various other studies (97-100).

Hammad et al. 2005 used low dose irradiation on fresh cut celery, green beans and lettuce and demonstrated that shelf life (with refrigeration) could be extended and no sensory qualities were affected by the process (89).

Mushrooms were e-beam treated with 0.5-5 kGy doses. The firmness was not affected by irradiation except for the doses above 5 kGy. Microbial induced browning was prevented when doses above 0.5 kGy were used. Color was not negatively affected by e-beam treatment (101).

Another e-beam study looked at the effects of irradiation on romaine lettuce at doses of 1, 1.5 and 3.2 kGy. Irradiated samples did not have a significant difference in color when compared to control samples. Firmness in the leaves decreased only as the

doses absorbed increased. Higher doses (3.2 kGy) also had lower sensory scores for overall quality, color, sogginess and off-flavor (102).

Cherry tomatoes were irradiated at doses of 0.5-3.0 kGy and the doses above 0.5 kGy were found to have significantly higher a-values of redness compared to control samples stored at room temperature. Overall, it was concluded that parameters of color, soluble solids, cell membrane permeability and sensory quality of tomatoes and carrots were not significantly affected at doses under 2.0 kGy stored at both room temperature and refrigerated. (103).

Another concern that consumers have with ionizing radiation is that the process will deteriorate beneficial compounds, such as vitamins and bioactive compounds found in fresh produce. This is however not necessarily true. In a study involving irradiated grapes, it was found that irradiation increased anthocyanin yield in the extraction process. Anthocyanins are natural compounds found in foods red and blue colored fruits such as grapes and strawberries. Anthocyanins have been reported as having chemoprotective properties and can also be used as natural food color agents (104). Abiotic stresses including excess heat, water and O_2 deficiencies or air pollution are unfavorable conditions for plants. However, most plants have evolved to adapt to these situation by initiating pathways that produce compounds that help to protect them from harm. This could explain some of the increases in bioactive compounds after various stresses on the commodity.

In another study by Reyes et al. 2007, e-beam irradiation was used as a controlled stress in mangos to evaluate the effects on mango compounds both

immediately after processing and during storage. Doses of 1.0-3.1 kGy were employed and HPLC was used to determine any changes in the phenolic compound profile between treatments. After 18 days of storage, irradiated samples had an increase in flavonol compounds. There were no differences in the total phenol content (as measured by the Folin-Ciocalteu method) or antioxidant capacity. Ascorbic acid content was decreased by 50-54% when the samples underwent doses equal to or greater than 1.5 kGy. Carotenoid content was not different between treatments (105).

In a similar study, mangoes and blueberries were treated with 1, 1.5 and 3.1 kGy doses and stored for 21 and 14 days, respectively. There was an 8% and 14% reduction in total sugars seen in mangoes when treated with 1 and 1.5 kGy. All treated mangoes had significantly lower ascorbic acid content; however, the phenolic compounds increased by 27.4% and 18.3 % at doses of 1.5 and 3.1 kGy respectively. Irradiation of blueberry fruits (1.1kGy) had no significant effects on the physiochemical traits except for a 17% decrease in ascorbic acid after the samples had been stored for 14 days. Again, similar to mangoes, total sugars decreased in the irradiated samples while the total phenols and tannins experienced a 10-20 % increase (106).

Minimally processed carrots, cucumbers and pineapples were irradiated at 2kGy and bioactive compounds were compared between treatments. Vitamin C and total carotenoids were extracted in the irradiated and control samples. There were no significant differences ($p<0.05$) in the total carotenoids and Vitamin C content when samples were irradiated at 2 kGy(107).

Degradation of some bioactive compounds when fresh produce is irradiated are often far less than the losses associated with food that has been cooked, frozen or freeze dried (108).

CHAPTER III

BIOBURDEN REDUCTION AFTER E-BEAM PROCESSING OF FRESH FRUIT IN AMBIENT AND MODIFIED ATMOSPHERES

Introduction

Fresh produce contain an abundantly diverse amount of bacteria on their surfaces, many of which are introduced from the soil where the commodity is grown. The various kinds of bacteria affect the produce in different ways. The most commonly studied microorganisms are pathogens which may not necessarily cause harm to the fruit themselves, but are hazardous to individuals who consume them. These microbes include *Salmonella, E.Coli* O157:H7 and *Listeria* and they may only make up a small amount of all bacteria present but are notorious for causing foodborne illness outbreaks (109-111). Produce surfaces also contain spoilage bacteria. These microbes result in a loss of quality of the food and unlike pathogens do not necessarily cause foodborne disease if consumed (78). The majority of bacteria found on fresh fruit and vegetable surfaces are commensal and typically do not harm the fruit or the individual who consumes it. Some bacteria even protect the fruit from pathogens by inhibiting the growth of pathogenic bacteria on the surface (109, 112-115). Although generally these bacteria may not adversely affect the healthy population, certain medical conditions prevent the inclusion of these raw foods in their diets because of the presence of any bacterial populations. Neutropenic individuals that are severely immunocompromised (neutrophil counts below 500 cells/ μl of blood) are advised against eating fresh produce

since it is a raw and unprocessed food likely to convey bacteria to the immunocompromised individuals. There is a concern that the microbial inhabitants of produce surfaces may act as opportunist pathogens and cause infection (3, 55). These infections can lead to increased morbidity and mortality in these patients (8).

Fruits and vegetables are often touted for their healthful compounds that are believed to have chemotherapeutic power. Since these foods are most often banned from neutropenic diets, neutropenics are not able to take advantage of their purported health benefits from these foods. The standard neutropenic diet restricts all food microbe levels to 500 CFU/g of food, for which fresh produce may not meet the requirements (1-3). Not to mention the concern of foods like tomato and leafy greens that has a history of *E. coli* O157:H7 and *Salmonella* spp. contamination (14, 15). There is no technology currently used to cleanse these fruits to acceptable levels. Chemical sanitizers such as chlorine are routinely used in industry to destroy bacteria on the fruit surface, but since microbes have the potential to invade tissue cells and form biofilms, fruits may still pose a threat the immunocompromised individuals.

Ionizing radiation is a non-thermal processing technology that has been approved by the FDA for certain foods. Doses < 1 kGy are allowed for processing fresh foods (5). This technology is effective at eliminating pathogens of concern and spoilage microorganisms. Electron beam is a form of ionizing radiation that is derived from a linear accelerator machine (15). This machine is able to be turned on or off when needed unlike radioactive sources used in food irradiation. The shallow penetrating depth of electron beam is one performance challenge, but dual beams (top and bottom exposure)

can be used to overcome this shortfall as long as the density of the food package is less than 8.0 cm/g^2 (16).

Modified atmosphere packaging (MAP) is another technology shown to be effective at prolonging fresh food shelf life by slowing the respiration rate of the commodity (44-46, 78). Coupling the e-beam and MAP processes has the potential to provide the optimal product quality in terms of microbial safety and organoleptic properties for use in hospitals, as well as anywhere else fresh produce may be distributed.

I hypothesize that low dose e-beam and MAP treatments will be effective in reducing the bioburden on our target fresh fruits of strawberry, grape, watermelon, tomato and avocado. The hypothesis will be tested by comparing the bioburden levels of e-beam treated and MAP fruits to control (non-irradiated) samples.

Materials and Methods

Fruit samples

The following fruit samples: strawberry (*Fragaria ananassa*), grapes (*Vitis vinifera*), watermelon (*Citrullus lanatus*), cherry tomatoes (*Solanum lycopersicum var. cerasiforme*) and avocados (*Persea americana*) were purchased from a local farmers market. This farmers market was chosen because the fruit sold here (versus a supermarket) are claimed to be produced environmentally friendly without the use of excess pesticides. These fruit were assumed to therefore provide a better representation of the native microbial populations of fresh produce.

Fruit sample preparation for ambient atmosphere conditions

Gloves and knives were rendered sterile prior to use by disinfection with 10% bleach solution followed by distilled water rinses. Utensils were used to portion the fruit into 50 ± 1 gram samples. Samples were weighed in sterile weigh boats. Portioned samples were placed in plastic clamshells (Sambrailo, Santa Maria, CA), with triplicate clamshells prepared for each treatment to be tested.

Fruit sample preparation for modified atmosphere conditions

The fruit samples were portioned into clamshells in accordance with previous fruit preparation protocol. Briefly, the clamshells were then placed into poly nylon vacuum bags (S-15151, ULINE, Ontario, CA). The bags were flushed and filled with the appropriate gas mixture (Praxair, Danbury, CT) and then sealed using an Accu-Seal 635HP-S Validatable Vacuum Sealer (San Marcos, CA). Gas mixtures (Table 3.1) are shown.

Table 3.1 Atmospheric conditions of modified atmosphere packaged fruit

Fruit	O_2 %	CO_2 %	N_2 %
Strawberry	5	15	80
Watermelon	5	10	85
Grape	5	3	92
Tomato	5	0	95
Avocado	5	10	85

Fruit sample irradiation

Samples were irradiated at the National Center for Electron Beam Research in College Station, TX. A 15kW, 10 MeV linear accelerator instrument (L3 Pulse Sciences) was used for irradiation. The conveyor belt speed was set to 58.5 feet per minute. Ten wooden boards were used to attenuate the beam in order to achieve the target dose of < 1kGy. The samples were irradiated from both sides.

Dosimetery

L-α-alanine pellets (Gamma-Service Produkbestrahlung GmbH, Germany) traceable to ASTM standards and the E-scan electron paramagnetic resonance spectroscopy (Bruker,BioSpin., Billerica, Mass.) were used to measure the absorbed dose. Two alanine dosimeters were used for each speed check. One pellet was placed on top of the fruit while the second was attached to the bottom of the fruit. An X-acto knife was used to cut a hole into the fruit for dosimeter placement so that the pellets were an integral part of the fruit. Two speed checks were used for each irradiation run. Upon completion of irradiation, all samples were stored at 4 ± 1˚C until microbial analysis.

Microbial analysis

Microbial analysis was carried out periodically on storage days 0, 1, 3, 7, 14 and 21. All analyses were performed in triplicate for each sampling day.

Homogenizing samples

Microbial enumeration was performed as instructed by the FDA Bacterial Analytical Manual (BAM) on Food Sampling and Preparation of Sample Homogenate.

Each 50 g sample was aseptically transferred into a sterile stomacher bag with filter (VWR International) and 450 ml of Butterfield's Phosphate Buffer (Sigma Chemical CO, St. Louis, MO) was added; this resulted in a 10^{-1} dilution. Samples were homogenized for 2 minutes on normal speed settings using a stomacher machine (Seward 350, London, UK). Tenfold dilutions were made with Butterfield's Phosphate Buffer by transferring 100 µl into 900µl dilution blanks.

Plating samples

Samples (100µl) were spread plated onto Plate Count Agar (VWR International) for enumeration of aerobic bacteria and onto Sabouraud's Dextrose agar (VWR International) for enumeration of yeasts and molds. Plates were incubated at 28 ˚C for 5 days and counted in accordance with the FDA bacterial analytical manual.

Statistical analysis

Results were expressed in colony forming units per gram (CFU/g). Descriptive statistics were used as the data was portrayed as boxplot graphs. The maximum, minimum, interquartile ranges and medians were shown. This was done using GraphPad Prism 5.0 (GraphPad Software Inc., California). Also, for each day, the treated and control samples were compared using the Mann-Whitney test to determine statistical difference between samples.

Possible Shortfalls

This experiment was designed to investigate the effect of e-beam on the native bacteria found on fresh fruit. For this reason, the samples were not washed to remove bacteria and they were not inoculated with any exogenous microorganism as is usually

done when studying inactivation kinetics of a very specific strain of bacteria. My goal was to target the bacteria already present on the fruit at the time of purchase to investigate the magnitude of reduction brought about by e-beam treatment alone and in combination with MAP. The specific strain that was being treated was not known, but a very broad target of the general aerobic group of bacteria was selected. Previous studies found that this target population of bacteria may include commensal microbes, spoilage microbes or even pathogens. Fresh fruit inherently have a wide variety and number of microorganisms that are introduced when they are grown, harvested and distributed. Therefore, the particular species of bacteria and strains present will always vary from fruit to fruit. Furthermore, various uncontrollable factors contribute to the bioburden levels on fruit. Exposure to microbes throughout processing will dictate the levels of bacteria on fruit. Because of these occurrences, the initial population of bacteria of these fruit samples was not known (unlike in a spiked study where the starting bacterial load is known). Consequently, when comparing how e-beam treatment reduced the bacterial load, it was not compared to the actual starting amount of bacteria on that fruit but to a completely different control sample. While this provides a very robust test of what a consumer may encounter with any given piece of fruit, but provides a poor estimate of log-reductions of bacteria that e-beam causes due to the variable starting bioburden. For example, if the treatment is used to process fruit with a large amount of bacteria and then compared to a control that unknowingly had a relatively low bacteria population the true reduction is not shown. The reverse also holds true.

The term reduction was still used to express lower levels of bacteria in treated samples compared to non-treated samples. These are the possible shortfalls of the study but the results are still useful in showing how e-beam is an effective treatment.

Results

E-beam absorbed doses

The average absorbed doses for each fruit ranged from 0.61 kGy to 0.98 kGy.

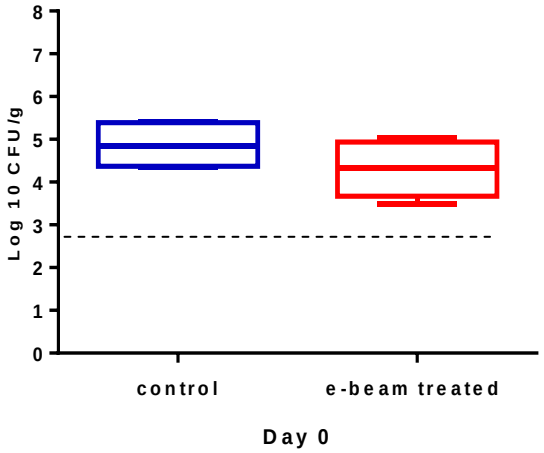


Figure 3.1Effect of e-beam on the bioburden of strawberries stored for 0 days at refrigerated temperatures. — control — e-beam treated - - clean food diet upper limit = 500 CFU/g

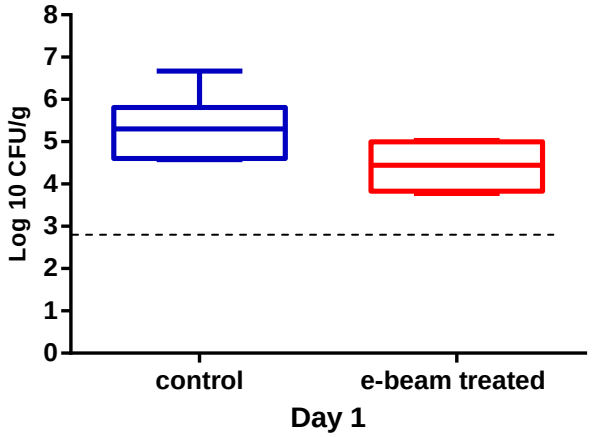


Figure 3.2 Effect of e-beam on the bioburden of strawberries stored for 1 day at refrigerated temperatures. — control — e-beam treated - - clean food diet upper limit = 500 CFU/g

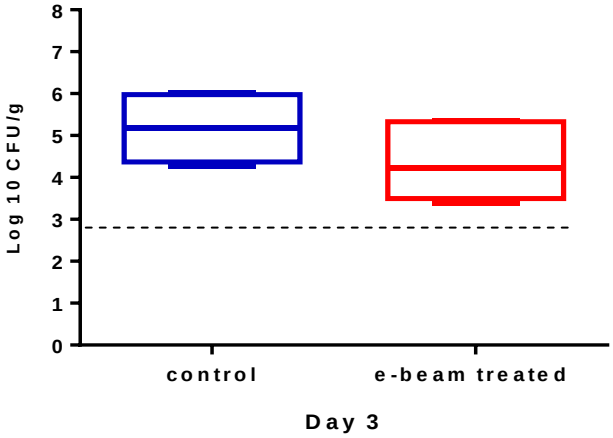


Figure 3.3 Effect of e-beam on the bioburden of strawberries stored for 3 days at refrigerated temperatures. — control — e-beam treated - - clean food diet upper limit = 500 CFU/g

Bioburden analysis of strawberry in ambient atmosphere

E-beam processing reduced the bioburden levels found on strawberries. They had a lower CFU/g count when compared to control samples at each storage time point. Figure 3.1-3.6 shows the range of the microbial load throughout 21 days of storage. Although e-beam did reduce the bioburden, it was not below the 500 CFU/g suggested for clean food diets.

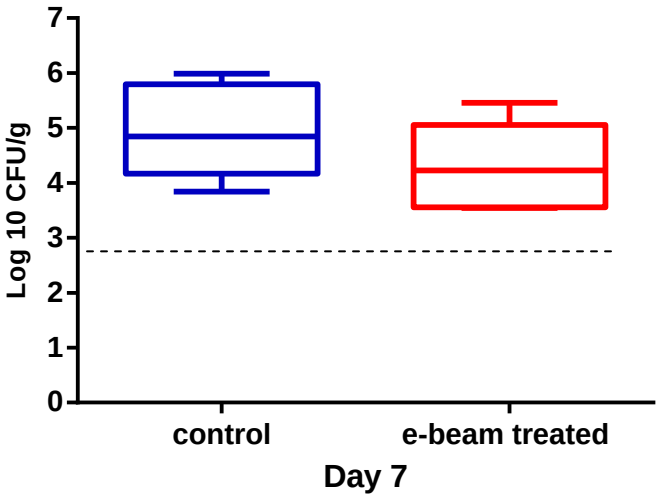


Figure 3.4 Effect of e-beam on the bioburden of strawberries stored for 7 days at refrigerated temperatures. control e-beam treated clean food diet upper limit = 500 CFU/g

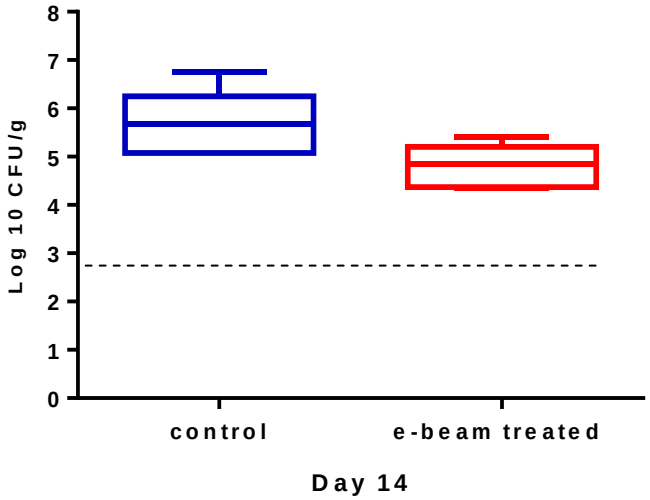


Figure 3.5 Effect of e-beam on the bioburden of strawberries stored for 14 days at refrigerated temperatures. — control — e-beam treated - - clean food diet upper limit = 500 CFU/g

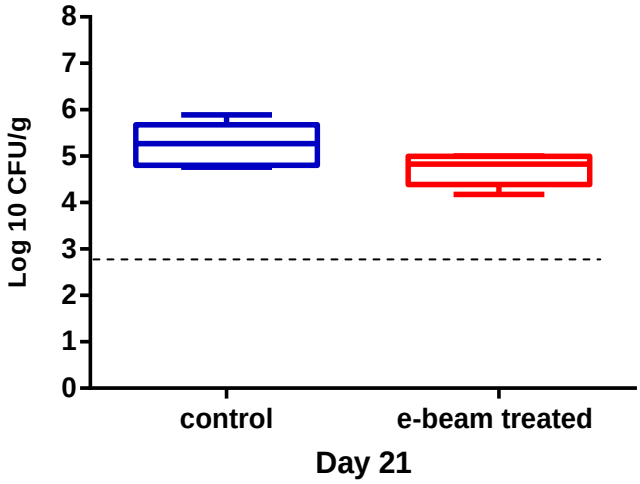


Figure 3.6 Effect of e-beam on the bioburden of strawberries stored for 21 days at refrigerated temperatures. — control — e-beam treated - - clean food diet upper limit = 500 CFU/g

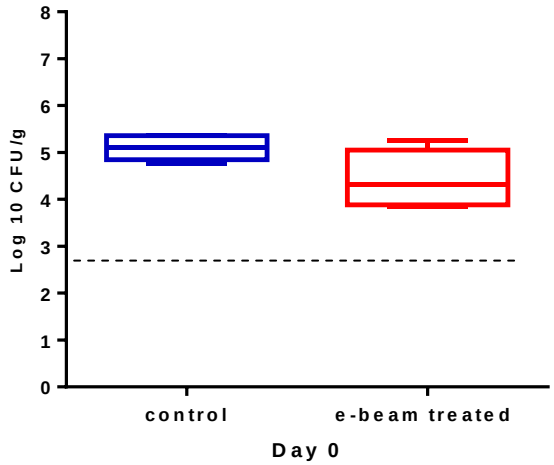


Figure 3.7 Effect of e-beam and MAP on the bioburden of strawberries stored for 0 days at refrigerated temperatures. — control — e-beam treated - - clean food diet upper limit = 500 CFU/g

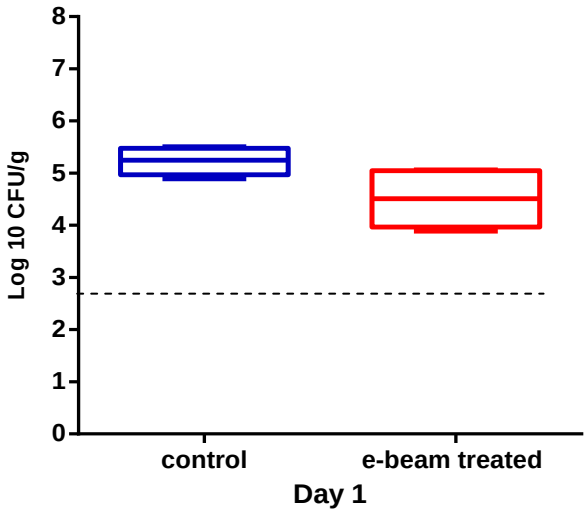


Figure 3.8 Effect of e-beam and MAP on the bioburden of strawberries stored for 1 day at refrigerated temperatures. — control — e-beam treated - - clean food diet upper limit = 500 CFU/g

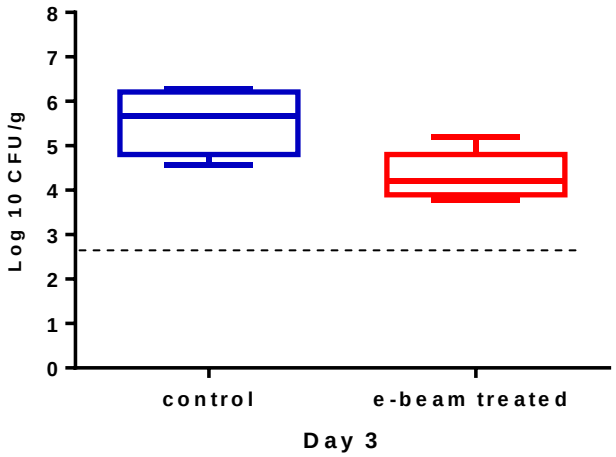


Figure 3.9 Effect of e-beam and MAP on the bioburden of strawberries stored for 3 days at refrigerated temperatures. — control — e-beam treated - - clean food diet upper limit = 500 CFU/g

Bioburden analysis of strawberry in modified atmosphere (5% O_2: 15%CO_2: 80%N_2)

Modified atmosphere-treated strawberries that underwent e-beam processing had lower bacterial counts through 21 days of storage compared to control samples (Fig 3.7-3.12). Again, this reduction was not below 500 CFU/g, but e-beam still proved to be consistently effective at reducing the amount of bacteria on the strawberries.

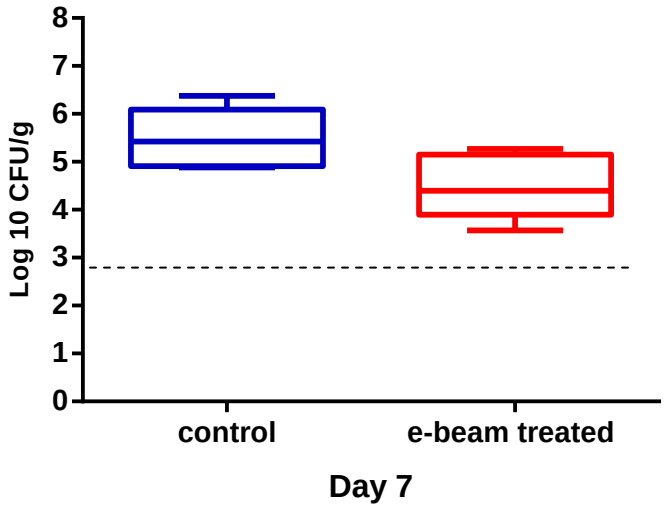


Figure 3.10 Effect of e-beam and MAP on the bioburden of strawberries stored for 7 days at refrigerated temperatures. control e-beam treated clean food diet upper limit = 500 CFU/g

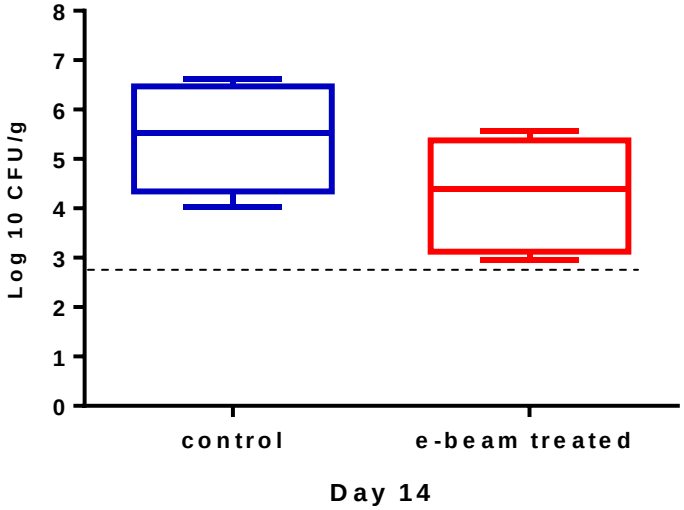


Figure 3.11 Effect of e-beam and MAP on the bioburden of strawberries stored for 14 days at refrigerated temperatures. control e-beam treated clean food diet upper limit = 500 CFU/g

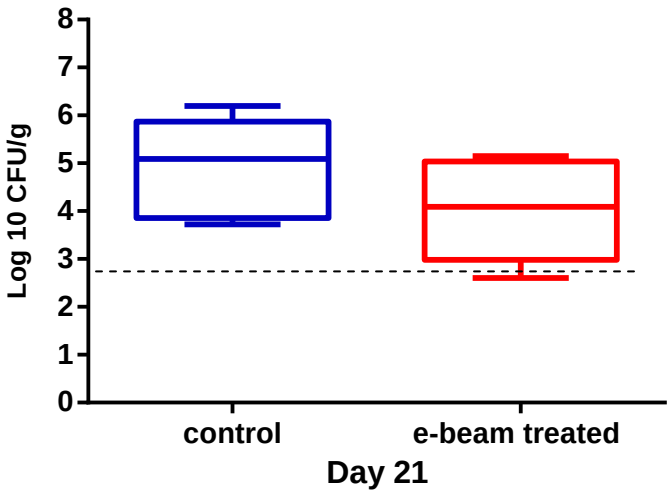


Figure 3.12 Effect of e-beam and MAP on the bioburden of strawberries stored for 21 days at refrigerated temperatures. control e-beam treated clean food diet upper limit = 500 CFU/g

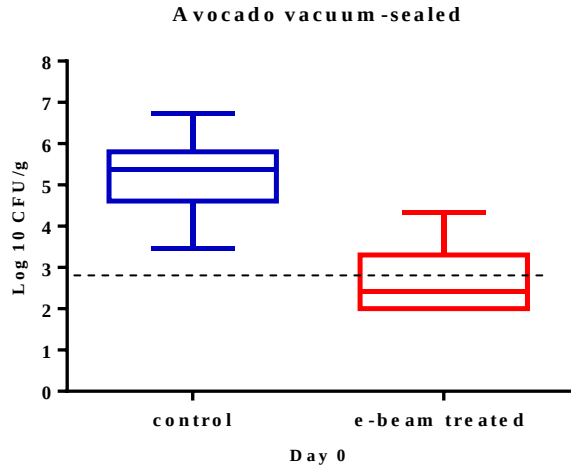


Figure 3.13 Effect of e-beam on the bioburden of avocado vacuum sealed and stored for 0 days at refrigerated temperatures. — control — e-beam treated - - clean food diet upper limit = 500 CFU/g

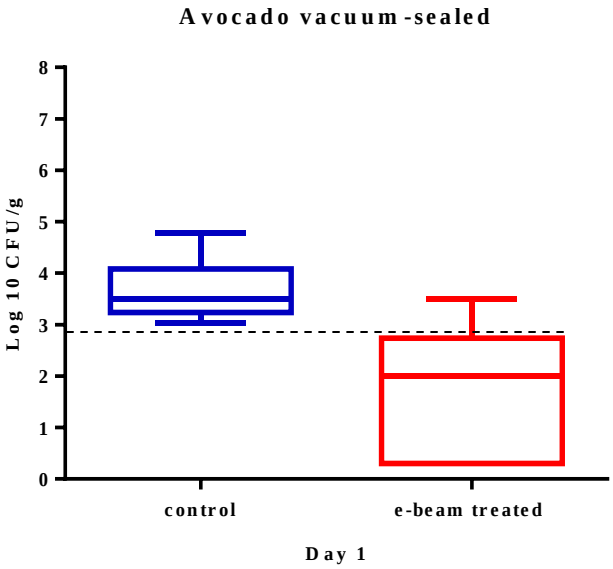


Figure 3.14 Effect of e-beam on the bioburden of avocado vacuum sealed and stored for 1 day at refrigerated temperatures. — control — e-beam treated - - clean food diet upper limit = 500 CFU/g

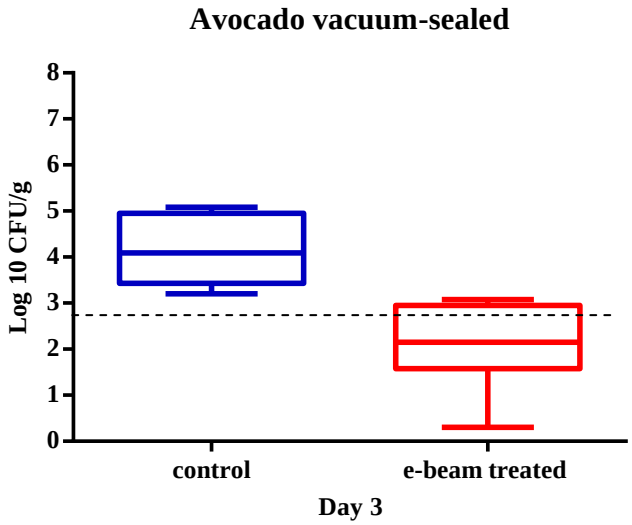


Figure 3.15 Effect of e-beam on the bioburden of avocado vacuum sealed and stored for 3 days at refrigerated temperatures. — control — e-beam treated - - clean food diet upper limit = 500 CFU/g

Bioburden analysis of avocado in vacuum-sealed package

E-beam significantly ($p<0.05$) reduced the bacteria present on avocado compared to control samples on all days of storage. The reductions were near or below the clean food diet guideline. None of the control avocados were below 500 CFU/g (Fig 3.13-3.18).

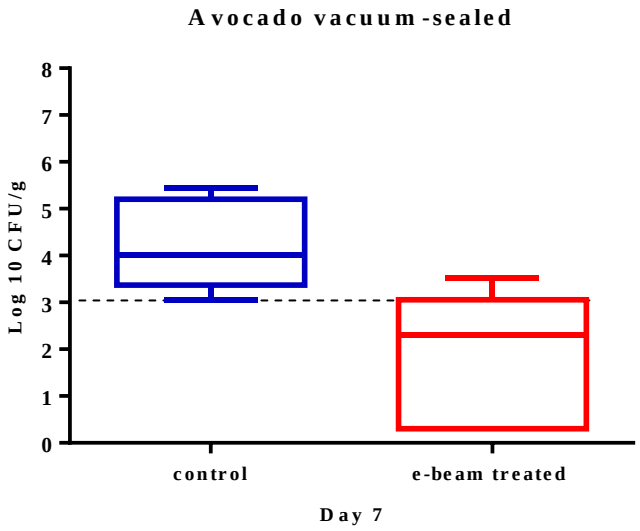


Figure 3.16 Effect of e-beam on the bioburden of avocado vacuum sealed and stored for 7 days at refrigerated temperatures. — control — e-beam treated - - clean food diet upper limit = 500 CFU/g

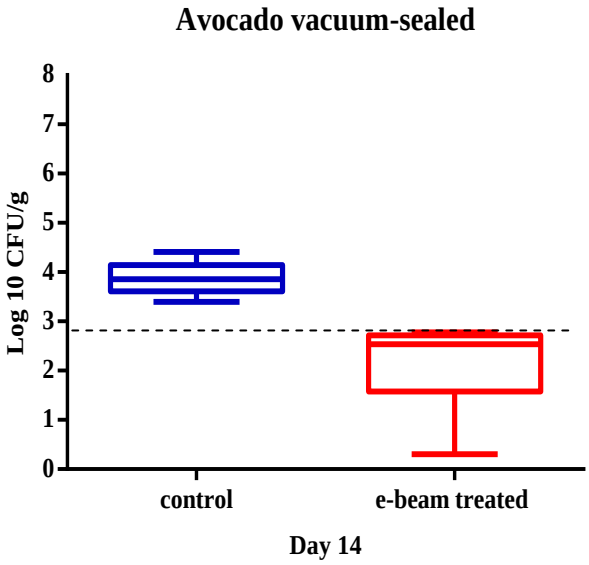


Figure 3.17 Effect of e-beam on the bioburden of avocado vacuum sealed and stored for 14 days at refrigerated temperatures. — control — e-beam treated - - clean food diet upper limit = 500 CFU/g

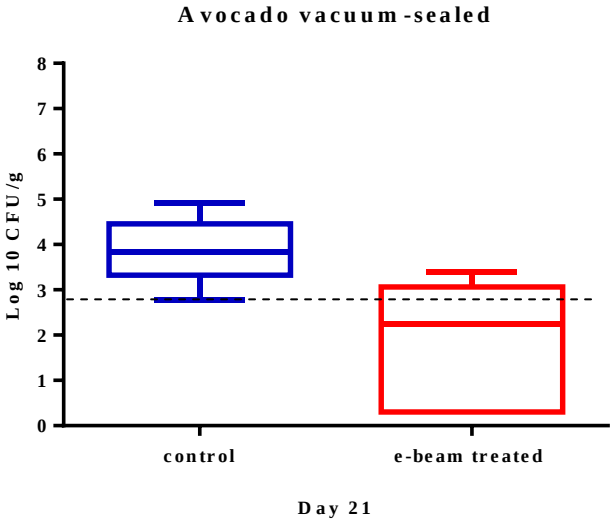


Figure 3.18 Effect of e-beam on the bioburden of avocado vacuum sealed and stored for 21 days at refrigerated temperatures. — control — e-beam treated - - clean food diet upper limit = 500 CFU/g

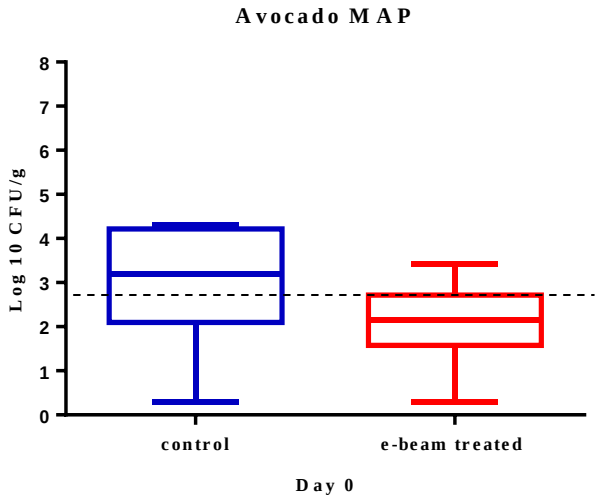


Figure 3.19 Effect of e-beam and MAP on the bioburden of avocado stored for 0 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

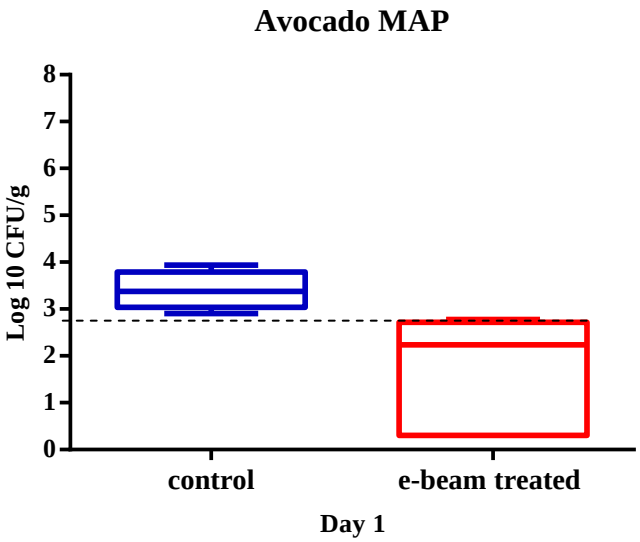


Figure 3.20 Effect of e-beam and MAP on the bioburden of avocado stored for 1 day at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

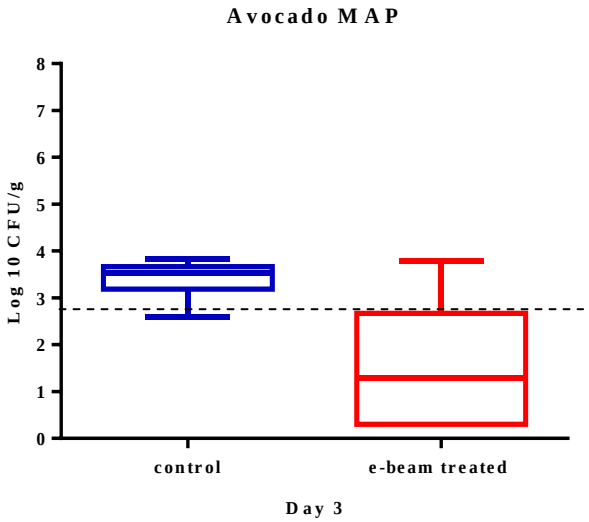


Figure 3.21 Effect of e-beam and MAP on the bioburden of avocado stored for 3 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

Bioburden analysis of avocado in modified atmosphere packaging

(5%:10%CO_2:85%N_2)

Modified atmosphere packaged avocado exposed to e-beam had significantly ($p<0.05$) lower bioburdens than control MAP-treated avocado. Furthermore, e-beam reduced CFU counts to the clean food standard of <500 CFU/g (Fig.3.19- 3.24)

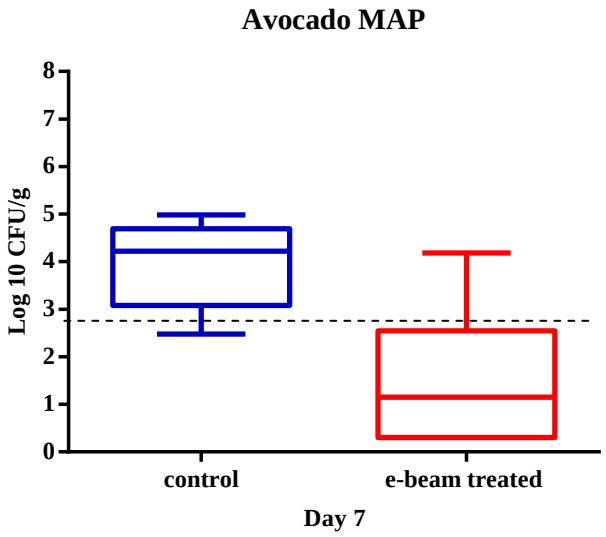


Figure 3.22 Effect of e-beam and MAP on the bioburden of avocado stored for 7 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

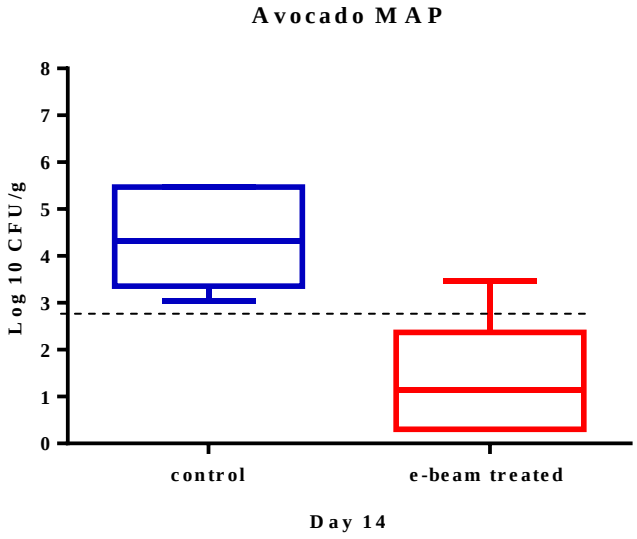


Figure 3.23 Effect of e-beam and MAP on the bioburden of avocado stored for 14 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

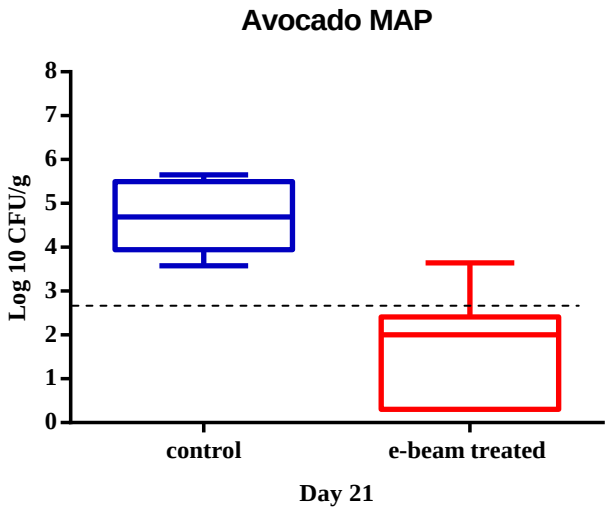


Figure 3.24 Effect of e-beam and MAP on the bioburden of avocado stored for 21 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

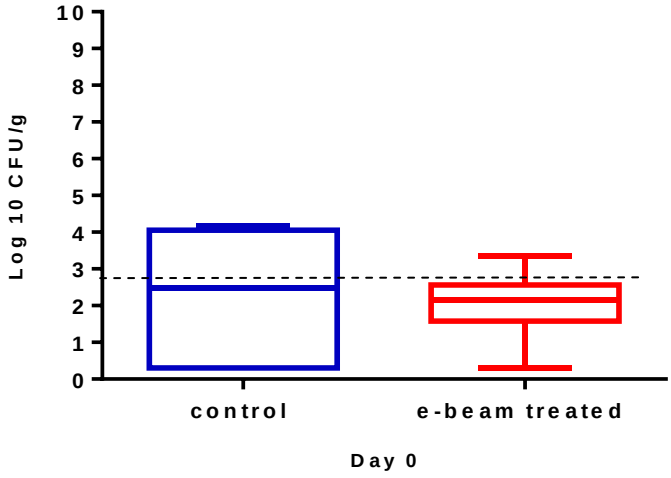


Figure 3.25 Effect of e-beam on the bioburden of watermelon stored for 0 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

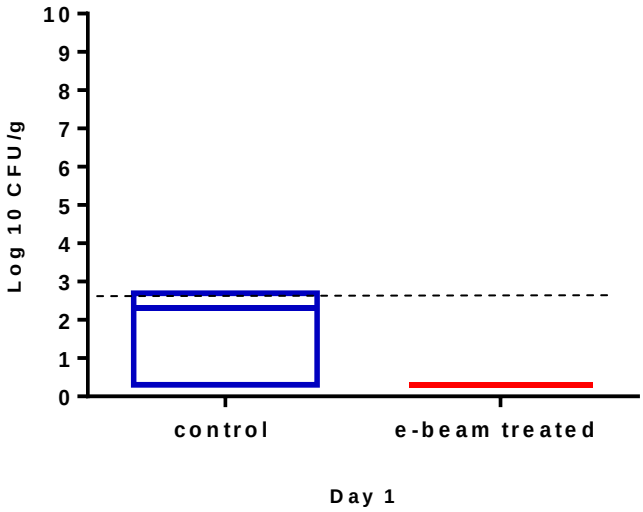


Figure 3.26 Effect of e-beam on the bioburden of watermelon stored for1 day at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

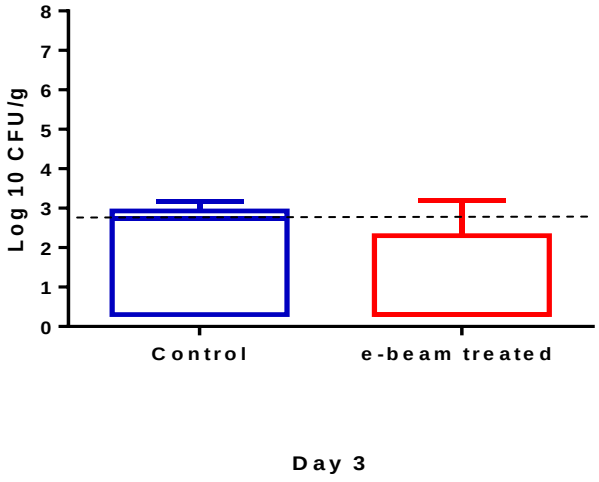
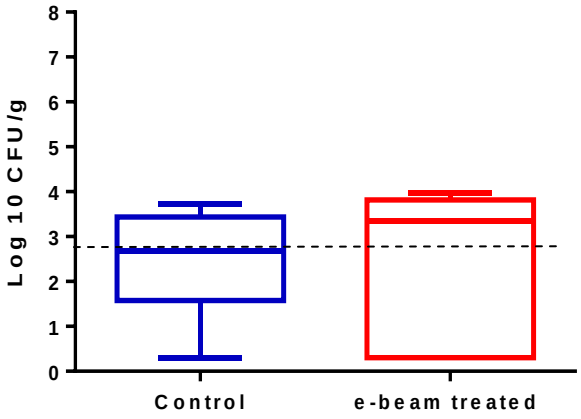


Figure 3.27 Effect of e-beam on the bioburden of watermelon stored for 3 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

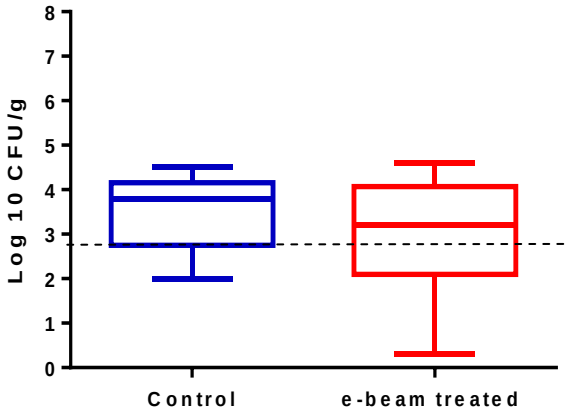
Bioburden analysis of watermelon in ambient atmosphere

Reduction of bioburden levels were seen with e-beam treated watermelon samples although the reduction was not as pronounced as in other fruits due to the overall low bioburden of watermelon compared to other fruits. Figures 3.25-3.30 show the effect of e-beam on fresh-cut watermelon over 21 days.



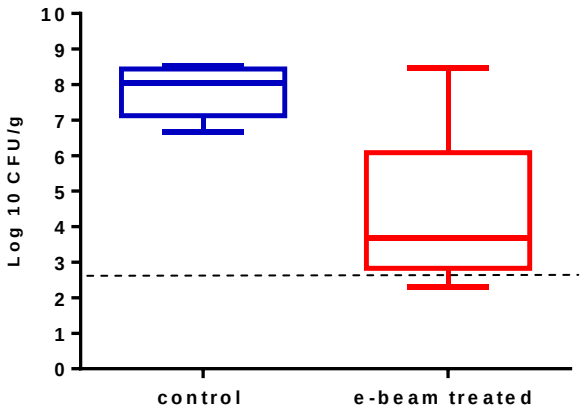
Day 7

Figure 3.28 Effect of e-beam on the bioburden of watermelon stored for 7 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g



Day 14

Figure 3.29 Effect of e-beam on the bioburden of watermelon stored for 14 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g



Day 21

Figure 3.30 Effect of e-beam on the bioburden of watermelon stored for 21 days at refrigerated temperatures. — control — e-beam treated - - -clean food diet upper limit = 500 CFU/g

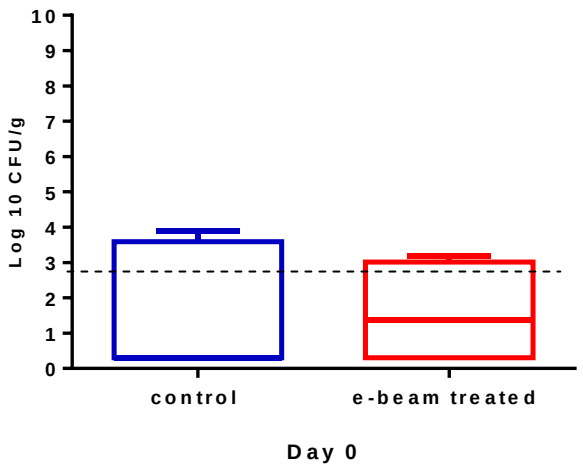


Figure 3.31 Effect of e-beam and MAP on the bioburden of watermelon stored for 0 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

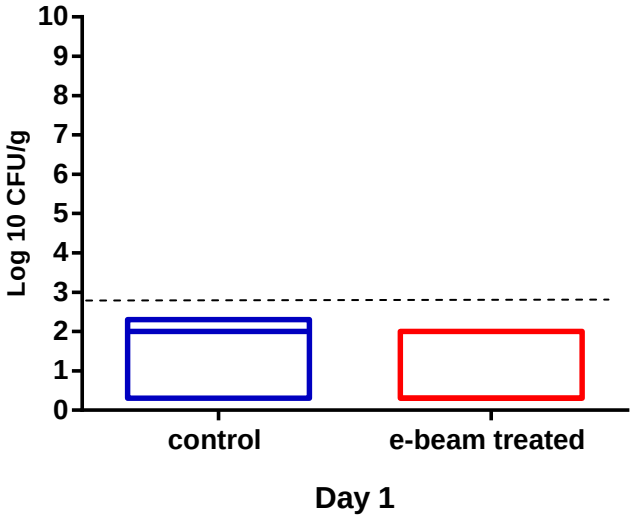


Figure 3.32 Effect of e-beam and MAP on the bioburden of watermelon stored for 1 day at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

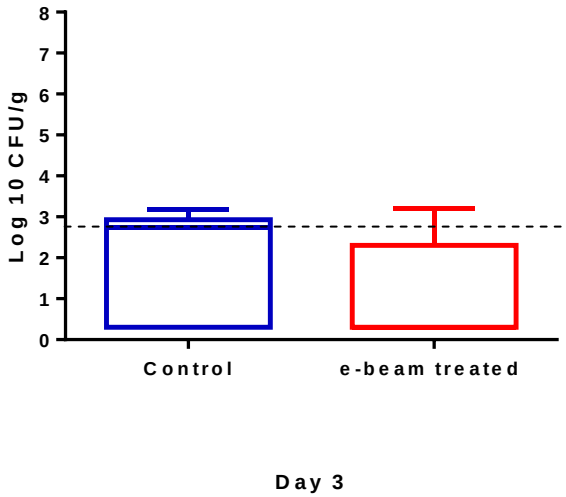


Figure 3.33 Effect of e-beam and MAP on the bioburden of watermelon stored for 3 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

Bioburden analysis of watermelon in modified atmosphere packaging

(5%O_2:10%CO_2:85%N_2)

E-beam treatment lowered the bioburden on modified atmosphere treated watermelon at all storage time points studied and they remained below the upper limit of the clean food guideline for 14 days (Fig 3.31-3.35). Notably, MAP-treatment alone did not reduce bioburden as samples not treated with e-beam steadily increased in bioburden measured after 3 days (Fig. 3.34-3.36).

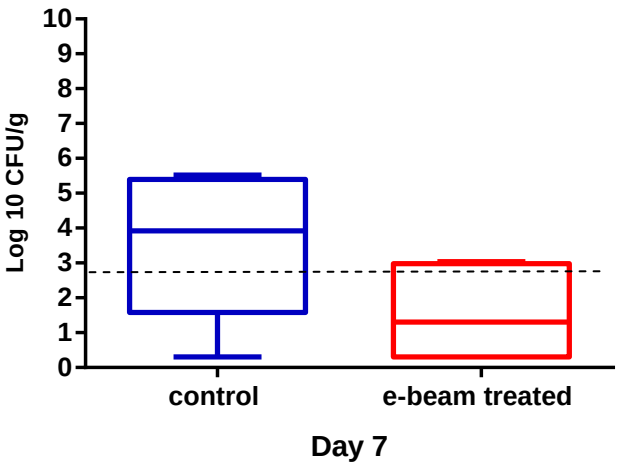


Figure 3.34 Effect of e-beam and MAP on the bioburden of watermelon stored for 7 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

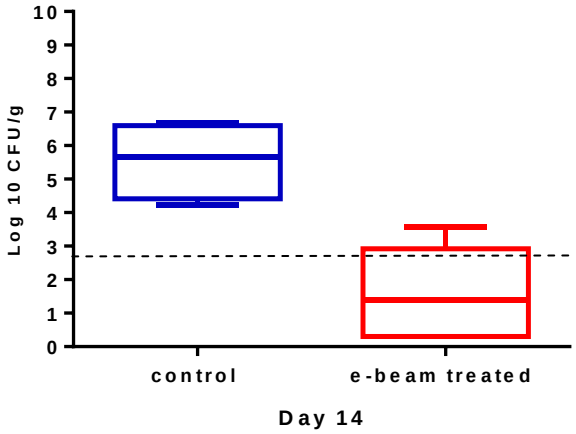


Figure 3.35 Effect of e-beam and MAP on the bioburden of watermelon stored for 14 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

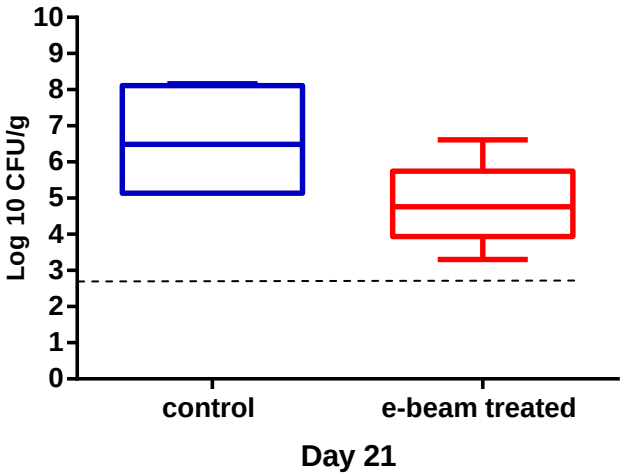


Figure 3.36 Effect of e-beam and MAP on the bioburden of watermelon stored for 21 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

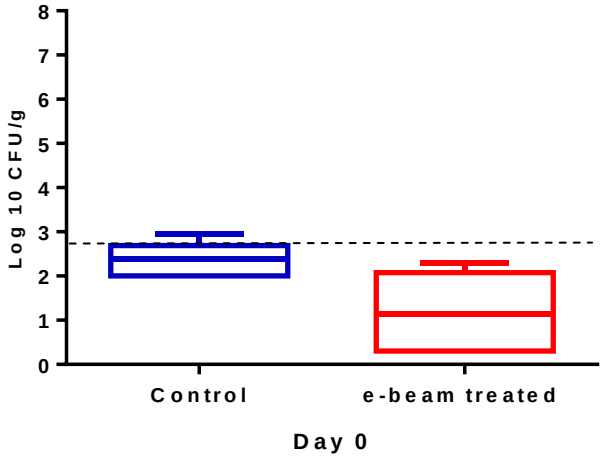


Figure 3.37 Effect of e-beam on the bioburden of grapes stored for 0 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

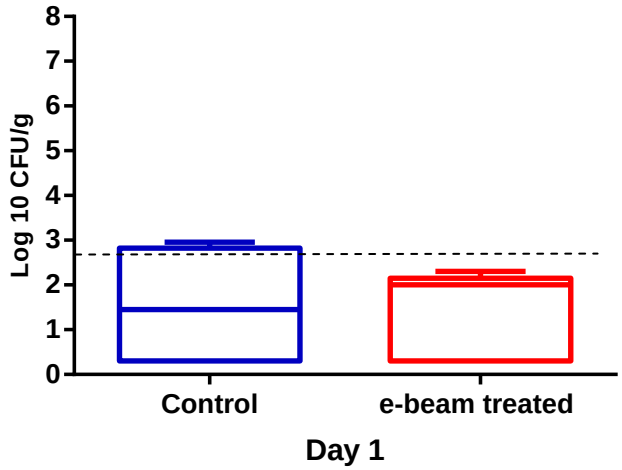


Figure 3.38 Effect of e-beam on the bioburden of grapes stored for 1 day at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

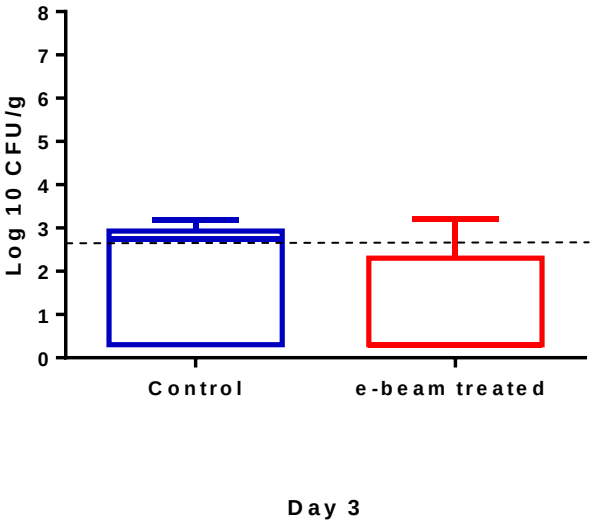


Figure 3.39 Effect of e-beam on the bioburden of grapes stored for 3 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

Bioburden analysis of grapes in ambient atmosphere

While some fruit had distinct patterns of reduction when processed with e-beam, other fruits did not. E-beam processed grapes had lower bioburden than control grapes (Figure 3.37-3.40 & Fig. 3.42), except at day 14 (Figure 3.41).

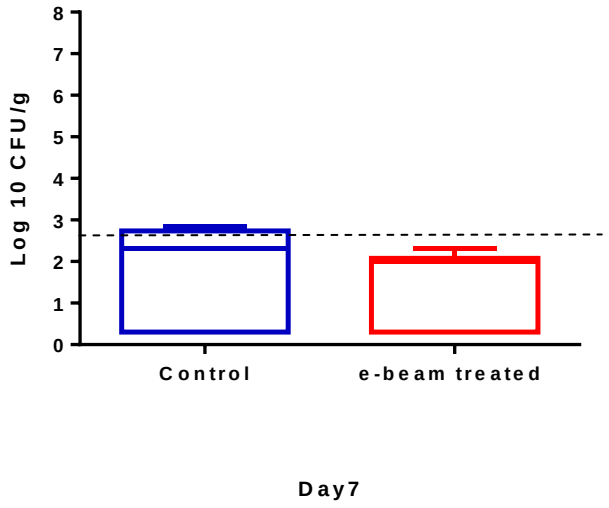


Figure 3.40 Effect of e-beam on the bioburden of grapes stored for 7 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

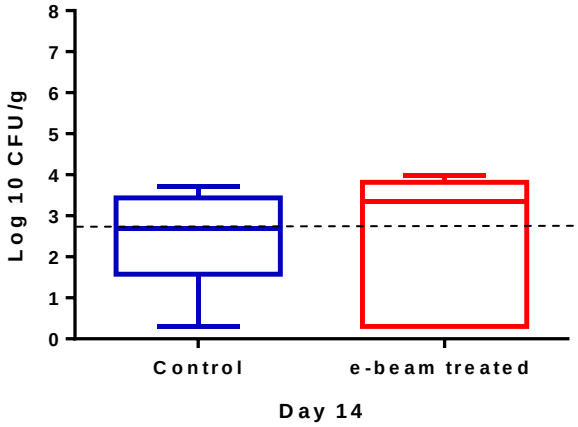


Figure 3.41 Effect of e-beam on the bioburden of grapes stored for 14 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

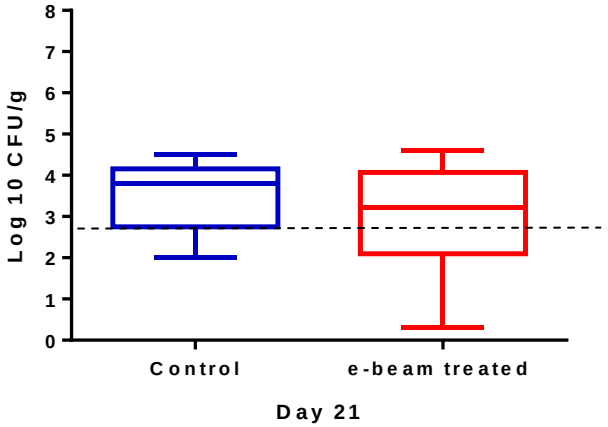
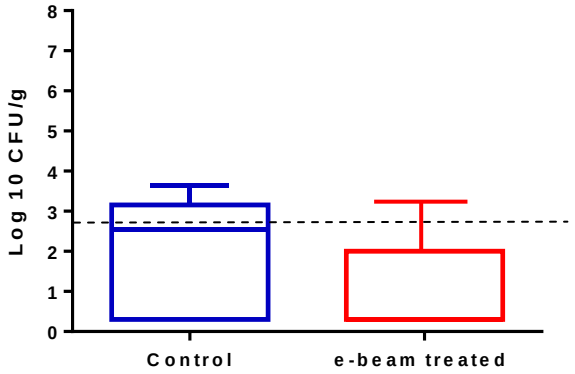
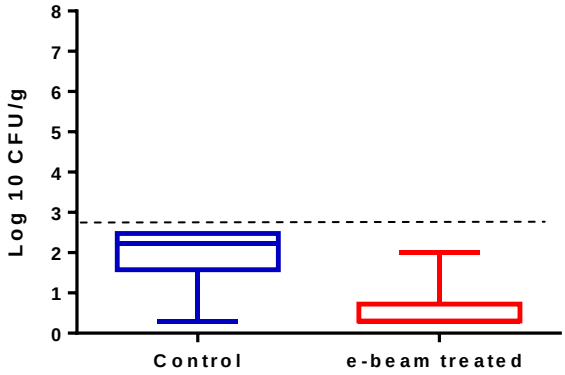


Figure 3.42 Effect of e-beam on the bioburden of grapes stored for 21 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g



Day 0

Figure 3.43 Effect of e-beam and MAP on the bioburden of grapes stored for 0 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g



Day 1

Figure 3.44 Effect of e-beam and MAP on the bioburden of grapes stored for 1 day at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

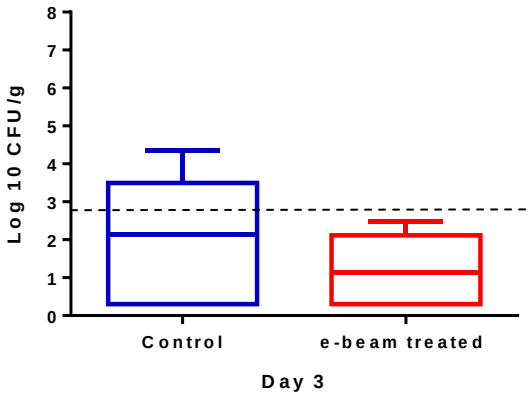


Figure 3.45Effect of e-beam and MAP on the bioburden of grapes stored for 3 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

Bioburden analysis of grapes in modified atmosphere packaging

(5%O_2:3%CO_2:92%N_2)

Irradiated grapes in modified atmosphere packaging had reduced bioburdens compared to non-irradiated grapes in MAP (Fig 3.43 – 3.47). On day 21 of storage, the bioburden of both ambient packaged and MAP-treated grapes were well below the clean food upper limit (Fig 3.48).

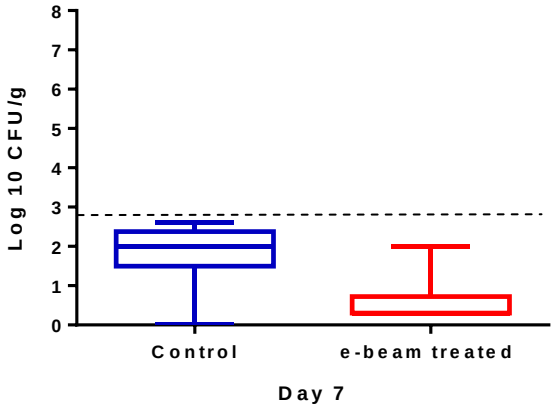


Figure 3.46 Effect of e-beam and MAP on the bioburden of grapes stored for 7 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

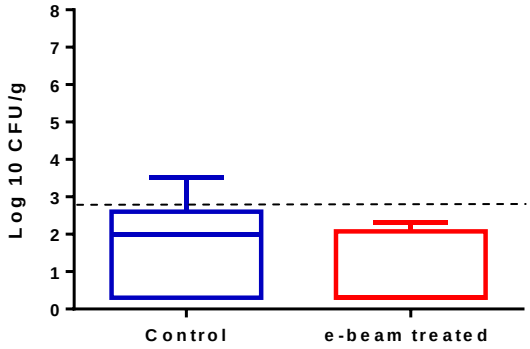


Figure 3.47 Effect of e-beam and MAP on the bioburden of grapes stored for 14 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

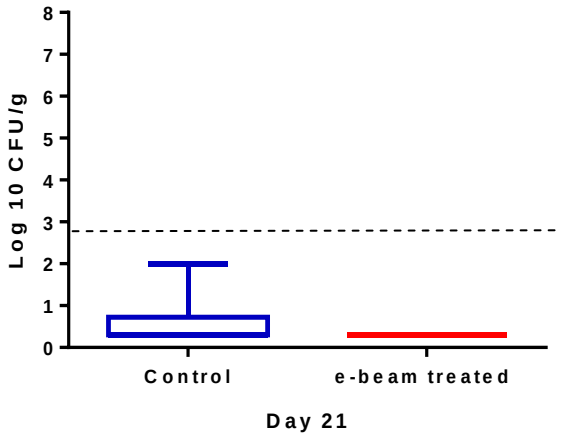


Figure 3.48 Effect of e-beam and MAP on the bioburden of grapes stored for 21 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

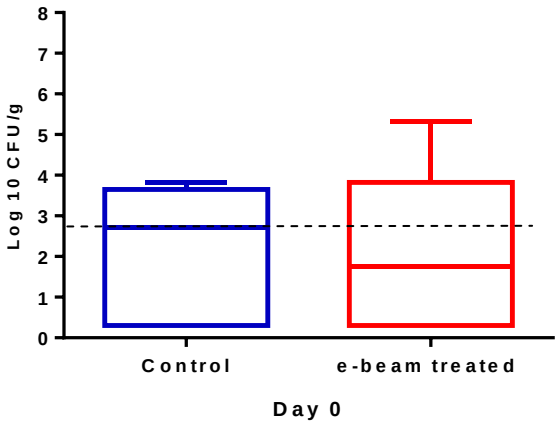


Figure 3.49 Effect of e-beam on the bioburden of tomato stored for 0 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

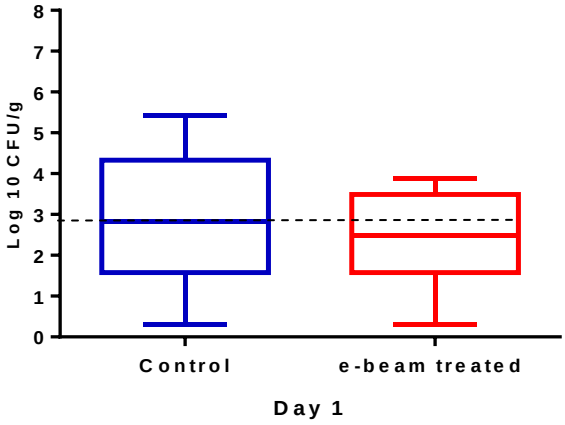


Figure 3.50 Effect of e-beam on the bioburden of tomato stored for 1 day at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

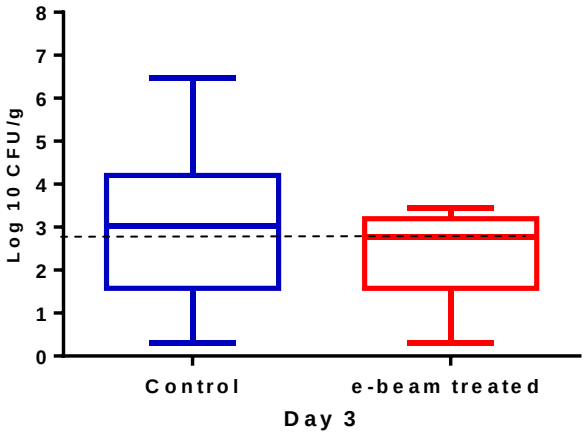


Figure 3.51 Effect of e-beam on the bioburden of tomato stored for 3 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

Bioburden analysis of tomato in ambient atmosphere

On each day of storage, except for day 0 (Fig. 3.49), irradiated tomatoes exhibited a reduced bioburden when compared to control samples (Fig 3.50-3.54).

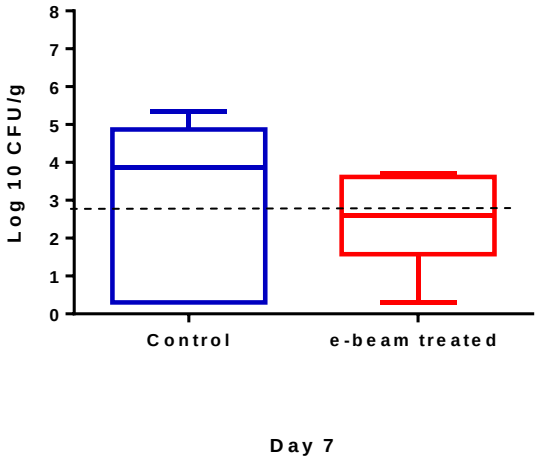


Figure 3.52 Effect of e-beam on the bioburden of tomato stored for 7 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

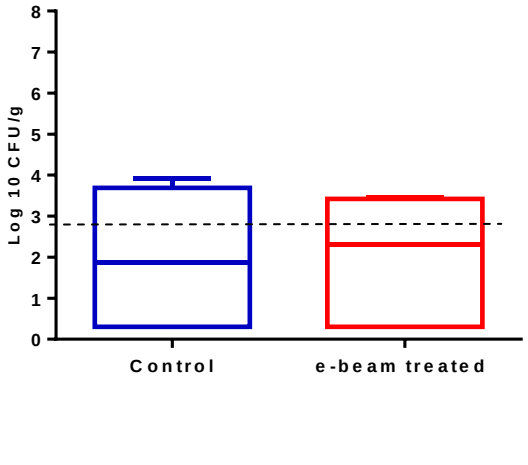


Figure 3.53 Effect of e-beam on the bioburden of tomato stored for 14 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

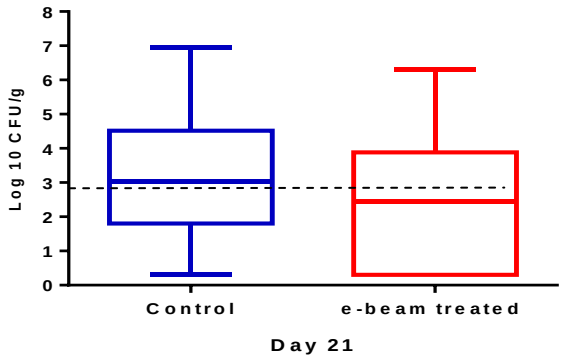


Figure 3.54 Effect of e-beam on the bioburden of tomato stored for 21 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

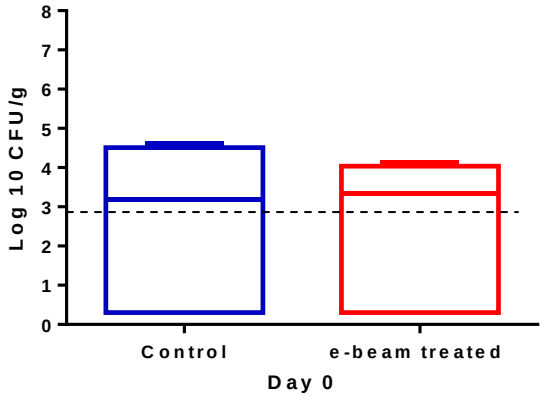


Figure 3.55 Effect of e-beam and MAP on the bioburden of tomato stored for 0 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

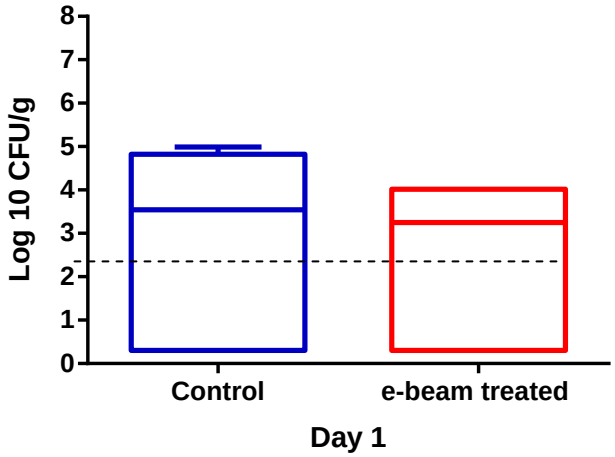


Figure 3.56 Effect of e-beam and MAP on the bioburden of tomato stored for 1 day at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

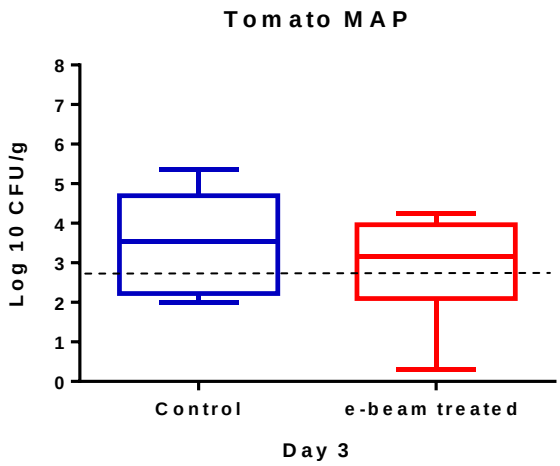


Figure 3.57 Effect of e-beam and MAP on the bioburden of tomato stored for 3 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

Bioburden analysis of tomato in modified atmosphere packaging (5%O_2:95%N_2)

Slight reductions were seen in e-beam treated MAP-processed tomato samples (Fig. 3.55 – 3.58 & 3.60) except for on day 14 (Fig 3.59).

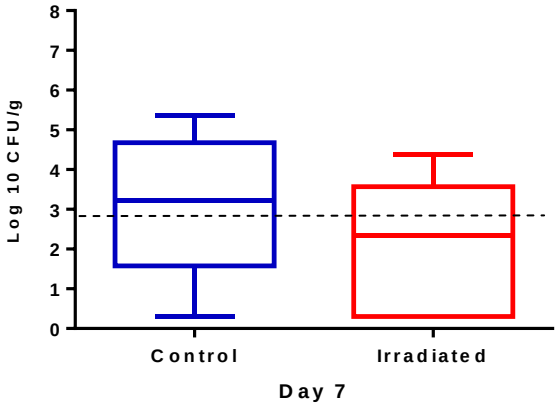


Figure 3.58 Effect of e-beam and MAP on the bioburden of tomato stored for 7 days at refrigerated temperatures. — control — e-beam treated - - - clean food diet upper limit = 500 CFU/g

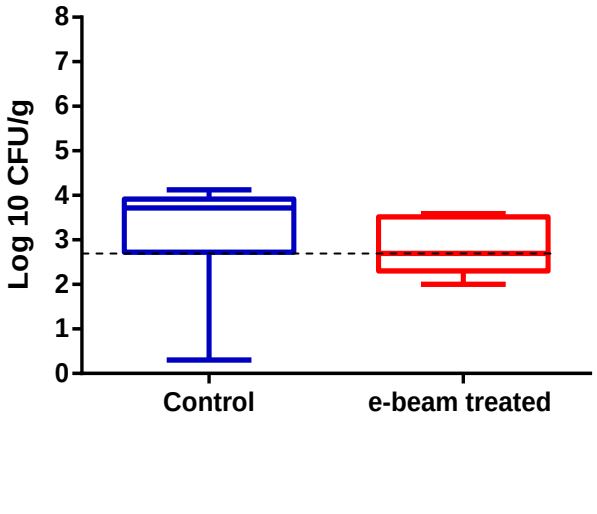


Figure 3.59 Effect of e-beam and MAP on the bioburden of tomato stored for 14 days at refrigerated temperature. —— control —— e-beam treated - - - clean food diet upper limit = 500 CFU/g

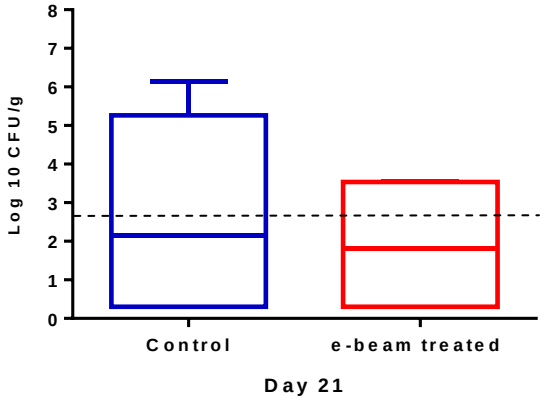


Figure 3.60 Effect of e-beam and MAP on the bioburden of tomato stored for 21 days at refrigerated temperatures. —— control —— e-beam treated - - - clean food diet upper limit = 500 CFU/g

Discussion

Low dose e-beam irradiation resulted in lower microbial counts in fresh fruit. Avocado, strawberries and watermelon samples had consistently reduced amounts of microorganisms throughout the 21 day storage period when treated with e-beam. On the other hand, bioburden reduction was more variable for irradiated grapes and tomato samples. This degree of variation could be attributed to the differing levels of native microorganisms initially found on the fruits. The varying levels of microbes depend on the commodity type, water activity, acidity and temperature, as well as other factors such as season harvested, farming method and weather. Even though the fruit was purchased from the same market and samples selected from bulk containers with matching lot numbers, there was still a chance for differing microbial exposures by individual pieces of fruit.

A recent study (76) aimed to characterize bacterial communities on different produce in order to get an idea of the diverse kinds of bacteria, other than pathogens, that thrive on produce. The authors concluded that microbiomes vary depending on the produce type itself since different commodities have varying chemical and physical parameters such as pH and water activity levels that favor different microbes (109). Grapes were found to have one of the most diverse populations of bacteria compared to strawberries. A wide variety of families were found to reside on grape surfaces (76). The study also showed that different farming practices, specifically conventional versus organic, tends to dictate the levels of microorganism that produce are exposed to (116,

117). Other than farming practices, location, storage and transportation conditions are other areas that are known to make a difference in bacterial populations (114, 118, 119)

These findings could help to explain the high variations seen with grape samples since Leff et al. found grapes to have one of the most highly assorted populations of bacteria compared to all of the other produce they tested. Although bioburdens were relatively low for grapes (< 500 CFU/g), a few were found to carry much higher bioburdens than other samples. In cases where e-beam grape samples were found to have higher bioburdens compared to non- irradiated samples, this did not indicate that e-beam was not effective at deactivating microbes or that the treatment somehow increased the microbial load. It simply suggests that the e-beam samples possibly started with a greater bioburden as this variable was not controlled in this series of studies. This also stresses the importance of good manufacturing practices. Irradiation is most useful on samples that have a low amount of microorganisms before processing; greater reductions can be achieved this way.

Another factor to consider when studying acidic foods are those bacteria that possess the ability to survive at low pH's. An example would be gram positive bacteria like *Listeria monocytogenes.* The glutamate decarboxylases (GAD) system has been shown to control pH of these microbes. In this mechanism, glutamate is internalized and changed into gamma-aminobutyrate which consumes a proton in the process. This increases the alkalinity of the cytoplasm. ATP can be produced from 3 cycles of decarboxylation and antiport through the F_1-F_o-ATPase proton pump (122).

E-beam treatment of strawberries consistently lowered the bioburdens compared to the non-irradiated samples. This is consistent with previous studies. Belli-Donini et al found that irradiation eliminated the growth of *Botrytis cinera* that causes fungal contamination in strawberries (97). They also observed that control (non-irradiated) berries had fungal growth after day 6 of refrigerated storage; 50% of berries were infected after 38 days. No contamination was seen in the irradiated strawberries.

Strawberries that were packaged with elevated CO_2 levels and e-beam processed carried fewer microorganisms than non-irradiated samples in MAP. The effects of the atmosphere seemed to be less effective at microbial reduction over storage than e-beam processing. It was reported by El-Kazzaz et al. that high CO_2 suppressed fungal growth on strawberries (especially *Botrytis* gray mold) and prohibited nesting (when fungal contamination spreads from contaminated berries to others close in proximity). In addition, this amount of CO_2 (15%) decreased C_2H_4 production for 9 days, thereby slowing fruit deterioration (120) . Strawberries in MAP did not appear to have a reduced bioburden than strawberries packaged with ambient atmosphere; however, a study would need to be performed comparing all of the treatment (modified and ambient) together to accurately assess the effects of both.

Although e-beam lowered the bioburden of strawberries, the final microbial levels were around 10^5 and still much higher than clean food diet limitations. The initial microbial load could be lowered if other processing steps were taken to clean the fruit. Washing strawberries before storage is not an option as they become water logged and rapidly lose quality. A more practical option may be to de-cap the strawberries before

storage and processing as the leaves may be harboring higher levels of microbes than the fruit itself.

Overall, e-beam treatment demonstrated the potential of the technology to aid in the development of clean foods. In certain fruits, bioburdens were below that of the upper limit of the Clean food diet criteria of <500 CFU/g food. Other fruits that did not reach this level and additional studies are needed to optimize their processing for purposes of enhanced sanitation and extended shelf life. Additional preservation techniques may be possible that can act to sensitize microbes on the fruit to e-beam treatment. E-beam treatment of fruit in MAP also showed lower microbial loads than non-irradiated fruit in MAP. In order to assess how the microbes were affected by both e-beam and MAP, it is suggested that treatments be carried out simultaneously since it has been shown that different batches of fruit carry differing loads of microorganisms.

CHAPTER IV

SENSORY ATTRIBUTES OF FRESH FRUITS AFTER E-BEAM PROCESSING AND MODIFIED ATMOSPHERE PACKAGING

Introduction

Post-harvest handling of fresh fruits and vegetables is an important factor in preserving its initial quality. Spoilage of fruit can occur at accelerated rates if the commodity is not stored under optimal conditions. Various processing techniques are employed such as cooking, freezing and drying to prevent spoilage and inactivate microorganisms. Several pathogenic strains of bacteria are associated with fresh produce contamination incidents. Since fresh fruits and vegetables are consumed in their raw state without a final kill-step, there remains a chance that these foods can harbor hazardous bacteria such as *E. coli* O157:H7, *Salmonella* species or *S. aeurus*. The safety of raw produce heavily relies to a large extent on Good Agricultural Practices (GAP). Particularly, hurdle technology is an approach used to ensure food safety and quality (109). Preservation techniques such as refrigeration, antioxidant addition and irradiation can be applied to fresh produce to collectively and synergistically preserve food freshness. Modified atmosphere packaging is another preservation method widely used in the fresh produce industry. Modified atmosphere packing systems are used to prolong shelf life of various food products including fresh fruits and vegetables. Packaging materials and gas mixtures for the system are chosen based on the commodity and various parameters associated with its respiration rate.

Electron beam irradiation is a non-thermal processing technique used on fresh produce to control pest, extend shelf life and eliminate the potential threat of pathogens found on fresh foods. However, application of this technology requires careful protocol development to insure both microbial safety and retention of important and vital sensory attributes.

Currently, various chemicals are used to disinfect fruits and vegetables from any disease causing organisms present on the surface. However, if pathogens are internalized, these approaches will not work.

I hypothesize that e-beam processing will not have a significant adverse effect on sensory quality measurements of fresh fruits; specifically, strawberry, tomato, grapes, avocado and watermelon. A parallel hypothesis is that consumers will find e-beam fruit acceptable in quality.

The main objective of this study was to evaluate the effects that electron beam treatment at FDA approved doses of < 1 kGy have on the sensory qualities of fresh fruit. Objective sensory testing was performed using instruments that measure moisture, color and texture as well as chemical changes within the fruit. A consumer study was also carried out to determine how untrained panelists would rate the irradiated fruit on 9 point hedonic scale. Fruit attributes of flavor, odor, color, firmness and overall liking of the appearance were examined. Thirty- eight consumers from the Texas A&M community participated in the study.

Materials and Method

Fruit samples

The following fruit samples: strawberry (*Fragaria ananassa*), grapes (*Vitis vinifera*), watermelon (*Citrullus lanatus*), cherry tomatoes (*Solanum lycopersicum var. cerasiforme*) and avocados (*Persea americana*) were purchased from a local farmers market. This farmers market was chosen because the fruit sold here (versus a supermarket) are claimed to be produced environmentally friendly without the use of excess pesticides. These fruit were assumed to therefore provide a better representation of the native microbial populations of fresh produce.

Fruit sample preparation for ambient atmosphere conditions

Gloves and knives were rendered sterile prior to use by disinfection with 10% bleach solution followed by distilled water rinses. Utensils were used to portion the fruit into 50 ± 1 gram samples. Samples were weighed in sterile weigh boats. Portioned samples were placed in plastic clamshells (Sambrailo, Santa Maria, CA), with triplicate clamshells prepared for each treatment to be tested.

Fruit sample preparation for modified atmosphere conditions

The fruit samples were portioned into clamshells in accordance with previous fruit preparation protocol. Briefly, the clamshells were then placed into poly nylon vacuum bags (S-15151, ULINE, Ontario, CA). The bags were flushed and filled with the appropriate gas mixture (Praxair, Danbury, CT) and then sealed using an Accu-Seal 635HP-S Validatable Vacuum Sealer (San Marcos, CA). Gas mixtures (Table 3.1) are shown.

Fruit sample irradiation

Samples were irradiated at the National Center for Electron Beam Research in College Station, TX. A 15kW, 10 MeV linear accelerator instrument (L3 Pulse Sciences) was used for irradiation. The conveyor belt speed was set to 58.5 feet per minute. Ten wooden boards were used to attenuate the beam in order to achieve the target dose of < 1kGy. The samples were irradiated from both sides. Upon completion of irradiation, all samples were stored at 4 ± 1˚C until analysis.

Dosimetery

L-α-alanine pellets (Gamma-Service Produkbestrahlung GmbH, Germany) traceable to ASTM standards and the E-scan electron paramagnetic resonance spectroscopy (Bruker,BioSpin., Billerica, Mass.) were used to measure the absorbed dose. Two alanine dosimeters were used for each speed check. One pellet was placed on top of the fruit while the second was attached to the bottom of the fruit. An X-acto knife was used to cut a hole into the fruit for dosimeter placement so that the pellets were an integral part of the fruit. Two speed checks were used for each irradiation run. Upon completion of irradiation, all samples were stored at 4 ± 1˚C until microbial analysis.

Color measurements

Surface color for tomatoes, strawberry, grapes and watermelon were measured using a Minolta Color Meter (Chroma Meter CR-310, Minolta, Tokyo, Japan). The instrument was calibrated using the white calibration plate (Calibration Plate CR-A43, Minolta Cameras, Osaka, Japan) before the measurement. Color values of L (whiteness-

gray), ± a (red-green) and b (yellow-blue) were measured four times for each sample. The internal color measurements were taken for avocado samples.

Moisture determination

The moisture of the samples were determined using the AOAC 90.15 method. Briefly, 5 gm of each fruit sample were weighed and placed on a glass petri dish. The oven was set to 105 ±1 ˚C and verified with a thermometer. Wet sample weight was taken before placing the fruit samples into the oven. Samples were dried for 3 hours or to a constant weight and re-weighed to determine moisture loss. To determine percent moisture of the sample, the wet sample weight was subtracted from the dry weight and this value was divided by the wet weight.

(Wet mass – Dry mass) / Wet Mass*100

Texture analysis

Fruit firmness and textural changes between treatments were measured using the a texture analyzer (model TA-XT2i, Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Survey, UK). Each test was carried out on the equatorial region of 10 samples. The modulus of deformation (N/mm), force (N), distance (mm) and work to rupture (N.mm) were recorded for all the fruit. These parameters are listed in Table 4.1. Description of the texture parameters are listed in Table 4.2.

Table 4.1 Texture analysis setup (for the TA.XT2i Texture Analyzer)

Fruit	Parameters							Trigger	
	Pre test speed (mm/s)	Test speed (mm/s)	Post test speed (mm/s)	Distance (mm)	Data acquisition rate (pps)	Load cell (kg)	Probe	Type	Force (N)
Watermelon	5	5	5	7	200	25	4 mm flat	Auto	0.05
Grape	5	1	5	5	200	25	2 mm flat	Auto	0.1
Strawberry	5	5	5	5	200	25	5 mm spherical	Auto	0.05
Tomato	5	1	5	7	200	25	2 mm flat	Auto	0.05
Avocado	5	5	5	7	200	25	4 mm flat	Auto	0.05

Table 4.2 Fruit texture analysis parameters measured

Parameter	Units	Description
Deformation of modulus	N/mm	Slope of the curve
Maximum force	N	Firmness. Hardness of the sample
Work	N.mm	Measure of energy. Area under the curve until rupture
Distance to rupture	Mm	Measure of the sample extensibility

Acidity and total soluble solids analysis

The titratable acidity of the fruit juice was determined in accordance to the reported method of Uckoo et al (2012). Each treatment juice solution was analyzed for acidity using a computer-controlled, automated pH titration system (Mettler Toledo DL50 Titrator, Schwerzenbach, Switzerland). The pH electrode (Mettler Toledo DG115 SE, Greifensee, Switzerland) was calibrated with pH buffers: 4.0, 7.0, and 10.0 (Fisher Scientific, Fair Lawn, N.J., U.S.A.). TSS was determined using a hand held refractrometer (BRIX50 model 137531L0, Leico Microsystems Inc., Buffalo, N.Y., U.S.A.).

Consumer study

The strawberries, watermelon, grapes and tomato samples were used for the consumer study. Irradiated and non-irradiated (control) samples from both ambient atmosphere and MAP samples were served. Samples were tested 24 hours after processing. Each treatment was assigned a random 3-digit code to reduce bias of sample treatments. Two- ounce (Solo-serve) plastic cups were labeled with the random 3-digit numbers in which the samples were placed. Fruit samples were prepared in the following ways:

Watermelon preparation: Watermelon were cut into 1 inch cubes, using only the central portion away from the rind.

Strawberry preparation: Strawberries were de-capped and cut into halves

Grape preparation: Grapes were removed from the bunch and 2 equal sized grapes were placed in each cup

Tomato preparation: One cherry tomato was placed into each cup.

Each sample was served to the consumer one at a time and they were given salt-less saltine crackers and double deionized water to cleanse their palate in between tastings. Thirty- eight untrained volunteers from the Texas A&M community participated in the taste tasting of irradiated fruits. They were asked to rate each fruit sample on the attribute of acceptability, odor, color, flavor and texture using a 9-point hedonic scale ranging from extremely dislike to extremely like. Extra space was included at the bottom of the ballot (Appendix) for comments.

This consumer study was carried with Texas A&M IRB approval. IRB Protocol # :2012-0463 The Office of Research Compliance and Biosafety's Human Subjects Protection Program is responsible for protecting humans used in research studies and ensuring that proper regulations and safety precautions are carried out. Therefore students and staff must get IRB approval before conducting a study.

Statistical analysis

The data was analyzed using the General Linear Model Procedure in Statistical Analysis System (SAS 9.3, 2001). Mean values were reported and the differences in the mean values were compared by ANOVA and LSD. Differences were considered significant when $P \leq 0.05$.

Results

Fruit sample absorbed dose for sensory analyses

Absorbed dose measured of the fruit used in objective sensory analyses ranged from 0.69 kGy to 1.01 kGy.

Absorbed dose measured for fruit used in consumer study ranged from 0.51 kGy to 0.84 kGy.

Strawberries: Effect of e-beam and modified atmosphere on color

Neither e-beam nor MAP treatments significantly ($p<0.05$) affected the redness (a-value) of strawberries throughout storage; however, on day 21 the MAP processed strawberries, independent of e-beam treatment, were significantly redder than ambient atmosphere packaged strawberries (Table 4.3). MAP seemed to have a stronger effect on redness of strawberries than e-beam irradiation since these values tended to be higher when subjected to this treatment.

Lightness (L-values) of strawberries was not significantly affected by e-beam or MAP treatments (Table 4.4). Only on day 14, the e-beam treated strawberries in modified atmosphere packaging (E-beam + MAP) was significantly lighter ($p< 0.05$) than control strawberries (Table 4.4).

Table 4.3 Effect of e-beam irradiation and modified atmosphere packaging (MAP) on strawberry redness across 21 days of storage.

Treatment	Day 0		Day 3		Day 7		Day 14		Day 21	
Air	36.12[a]	± 9.32	44.33[a]	± 6.87	25.44[b]	± 6.83	27.23[a]	± 1.49	23.17[b]	± 10.17
EB	38.39[a]	± 10.32	35.27[ab]	± 3.18	21.88[b]	± 10.80	26.78[a]	± 4.09	28.41[b]	± 6.91
MAP	43.25[a]	± 8.23	41.26[ab]	± 2.09	32.84[a]	± 13.44	35.16[a]	± 3.60	37.95[a]	± 8.36
EB+MAP	43.20[a]	± 4.67	26.45[b]	± 13.24	33.11[a]	± 10.41	36.20[a]	± 9.17	40.77[a]	± 8.33

Each value is the mean of 4 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
Means in same column not followed by no letter = no significance $P>0.05$
±SD

Table 4.4 Effect of e-beam irradiation and modified atmosphere packaging (MAP) on strawberry lightness across 21 days of storage

Treatment	Day 0		Day 3		Day 7		Day 14		Day 21	
Air	45.01[a]	± 5.23	45.22[a]	± 7.00	41.49[a]	± 2.60	33.89[b]	± 3.42	36.58[a]	± 7.93
EB	41.29[a]	± 9.98	37.49[a]	± 8.95	46.44[a]	± 8.31	33.08[b]	± 1.79	39.12[a]	± 8.39
MAP	42.80[a]	± 6.11	39.73[a]	± 6.58	40.41[a]	± 4.52	39.75[b]	± 3.82	39.60[a]	± 6.31
EB+MAP	44.69[a]	± 6.58	43.49[a]	± 1.22	40.05[a]	± 2.71	44.90[a]	± 8.00	45.76[a]	± 6.12

Each value is the mean of 4 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
Means in same column not followed by no letter = no significance $P>0.05$
±SD

Effect of e-beam and modified atmosphere on strawberry texture

E-beam processing did not significantly affect the deformation measurements of the strawberries. Modulus of deformation is the force to deformation ratio (N/mm) and can be used to determine fruit elastic behavior (how well fruit return to their original shape after deformation). This can be used to indicate sample firmness. Table 4.5 shows that strawberries stored in modified atmosphere packaging (MAP and EB + MAP) had higher values (meaning they were more firm) than those samples in ambient atmosphere. Means were significantly different ($p<0.05$) on days 3-21 where the MAP strawberries were the most elastic (most firm).

Skin firmness was measured in Newtons and represents the amount of force used to puncture the fruit; it is a factor of skin toughness and firmness of the flesh. Strawberry skin was significantly affected ($p < 0.05$) by e-beam processing as less force was needed to rupture the skin for these samples (e-beam and e-beam +MAP) than the control. The strawberries subjected to only modified atmosphere packaging (MAP) required significantly more force to break the skin early on during storage (on day 0 and 3) than the force used for the control (non-irradiated air) strawberries.

Distance to rupture (mm) is a measure of the fruit extensibility (not to be confused with elasticity). Extensibility is the capability of fruit to be stretched or extended. All of the strawberries treated with e-beam or modified atmosphere packaged, were significantly less ($p<0.05$) extensible or able to be stretched than control samples on most days of storage.

Table 4.5 Effect of e-beam irradiation and modified atmosphere packaging (MAP) on strawberry firmness

Deformation (N/mm)

Treatment	Day 0		Day 3		Day 7		Day 14		Day 21	
Air	6.27[a]	±1.73	5.01[bc]	±1.50	2.97[b]	±2.06	1.58[b]	±0.61	1.84[b]	±1.44
EB	6.70[a]	±3.03	4.73[c]	±1.36	3.19[b]	±1.68	2.13[b]	±1.09	0.96[c]	±2.70
MAP	7.07[a]	±1.10	6.97[a]	±1.40	6.41[a]	±1.33	6.03[a]	±0.95	3.38[a]	±1.19
EB+MAP	6.62[a]	±1.10	6.57[ab]	±3.02	3.09[b]	±0.47	2.41[b]	±0.70	1.56b[c]	±1.95

Each value is the mean of 10 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
Means in same column not followed by no letter = no significance $P>0.05$
±SD

Effect of e-beam and modified atmosphere packaging on strawberry moisture

Strawberry moisture content was not adversely affected by e-beam treatment during the 21 storage period (Table 4.6). After 14 days of storage, modified atmosphere packaged strawberries had significantly higher moisture than control samples. The average strawberry moisture content is 92 % as found in the literature (121). The strawberries in modified atmosphere packaging were comparable to this amount (92.17% and 91.58%) in EB + MAP and MAP strawberries respectively.

Effect of e-beam and modified atmosphere packaging on total soluble solids (˚Brix) and titratable acidity of strawberry

˚Brix measurements are often used to assess fruit quality. The higher the brix value, the sweeter the sample (i.e. more soluble solids is an indicator of more sugar molecules). The brix content was not significantly affected ($p < 0.05$) by e-beam treatment but was found to be significantly lower in modified atmosphere packaged strawberries (both EB+MAP and MAP) (Fig 4.1). Overall, brix values decreased over 21 days in samples stored in the ambient atmosphere (air and EB strawberries) while strawberries in the modified atmosphere (both irradiated and non-irradiated) significantly increased ($p<0.05$) in brix values. For soluble solids content, the normal levels range from 4.8-10.9% as found in literature for ripe strawberries (122, 123)

Table 4.6 Effect of e-beam irradiation and modified atmosphere packaging (MAP) on the moisture content of strawberries stored for 21 days

Day	Air		EB		MAP		EB+MAP	
0	87.83[a]	±0.73	88.76[a]	±9.31	89.63[a]	±1.83	88.47[a]	±0.17
3	89.65[a]	±1.34	89.62[a]	±1.54	89.64[a]	±1.55	90.26[a]	±0.09
7	91.05[a]	±2.00	90.51[a]	±0.55	92.54[a]	±0.09	92.52[a]	±0.19
14	86.34[c]	±0.05	88.42[bc]	±0.96	92.49[ab]	±0.03	90.21[ab]	±0.15
21	87.07[a]	±1.28	85.06[a]	±1.90	91.58[a]	±0.66	92.17[a]	±1.40

Each value is the mean of 4 samples.
Means in row with different superscript (a,b,c) represent significant difference ($P<0.05$)
Means in same row followed by the same letter are not significantly different at $P<0.05$
Means in same row not followed by no letter = no significance $P>0.05$
±SD

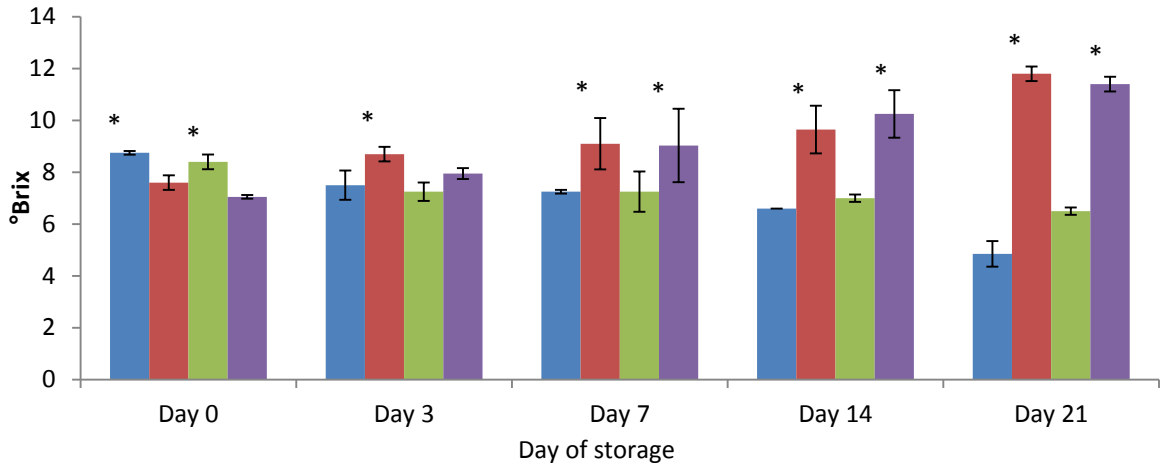


Figure 4.1 Effect of irradiation and modified atmosphere on brix values of strawberries over 21 days at refrigerated storage EB+MAP EB MAP air
* represents significant difference (P<0.05)

Titratable acidity (TA) is expressed in terms of citric acid equivalents since this is the main organic acid found in strawberries. E-beam treatment did not significantly affect ($p<0.05$) titratable acidity of strawberry samples as irradiated strawberries stored in the ambient atmosphere were similar to control samples (Table 4.7). Modified atmosphere did significantly ($p<0.05$) decrease titratable acidity values over time.

Tomato: Effect of e-beam and modified atmosphere packaging on color

E-beam processing alone (EB samples) significantly ($p<0.05$) increased red color in tomatoes on day 3 (Table.4.8). From day 7 of storage to day 14, irradiation and modified atmospheres did not significantly ($p<0.05$) affect redness of tomatoes. However on day 21, irradiated strawberries in a modified atmosphere (EB+MAP) samples were significantly higher in red color than the control (air) tomatoes.

Table 4.7 Effect of e-beam irradiation and modified atmosphere packaging (MAP) on titratable acidity of strawberry stored over 21 days

	Day 0		Day 3		Day 7		Day 14		Day 21	
Air	0.83^{b}	±0.09	0.86^{b}	±0.02	1.02^{a}	±0.05	1.32^{a}	±0.08	0.95^{a}	±0.07
EB	0.85^{b}	±0.05	0.90^{a}	±0.02	1.03^{a}	±0.01	1.23^{a}	±0.05	1.15^{a}	±0.07
MAP	1.08^{a}	±0.24	0.79^{c}	±0.04	0.75^{b}	±0.03	0.72^{b}	±0.09	0.76^{b}	±0.06
EB+MAP	0.90^{ab}	±0.11	0.68^{d}	±0.02	0.78^{b}	±0.06	0.73^{b}	±0.09	0.69^{b}	±0.08

Each value is the mean of 4 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
Means in same column not followed by no letter = no significance $P>0.05$
±SD

Table 4.8 Effect of e-beam treatment and modified atmosphere on a-values of tomato samples stored for 21 days

Treatment	Day 0		Day 3		Day 7		Day 14		Day 21	
Air	31.99^{b}	±1.85	32.11^{ab}	±2.26	33.24^{a}	±1.42	32.07^{a}	±1.09	31.40^{ab}	±3.02
EB	38.50^{a}	±2.52	35.46^{a}	±0.70	33.85^{a}	±1.60	34.23^{a}	±0.77	30.81^{b}	±3.38
MAP	40.11^{a}	±0.46	31.62^{b}	±1.59	33.93^{a}	±1.75	33.86^{a}	±2.10	31.56^{ab}	±2.33
EB+MAP	33.92^{b}	±3.17	31.28^{b}	±2.97	33.02^{a}	±0.73	32.94^{a}	±3.46	35.05^{a}	±1.06

Each value is the mean of 10 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
Means in same column not followed by no letter = no significance $P>0.05$
±SD

L-values (lightness) of tomato were not significantly affected by e-beam irradiation during storage ($p<0.05$). Both modified atmosphere packaged tomatoes (MAP and EB+MAP) were significantly darker than control tomatoes on days 3, 7 and 14 (Table 4.9).

Effect of e-beam and modified atmosphere packaging on tomato texture

Tomato firmness (turgor) was not affected by either e-beam or MAP treatments consistently through storage as there was no distinct trend or pattern (Table 4.10).

Skin firmness of tomato samples were affected by e-beam treatment ($p<0.05$) as they were found to be significantly softer than the control. The tomato samples treated only with e-beam required the most force to puncture the skin out of each treatment on all storage days.

Distance to rupture (turgor) was not affected by e-beam treatment as they were found to be similar to control samples. After 14 days of storage, MAP samples (MAP and EB+MAP) were significantly more turgid than the control, meaning they were not able to be extended or stretched as much as other tomato samples.

Table 4.9 Effect of e-beam irradiation and modified atmosphere packaging on lightness of tomato samples stored for 21 days

Treatment	Day 0		Day 3		Day 7		Day 14		Day 21	
Air	36.96^{b}	±1.45	36.18^{a}	±1.88	37.42^{a}	±0.87	37.84^{a}	±1.06	36.73^{a}	±0.73
EB	38.87^{ab}	±1.80	35.73^{ab}	±0.97	36.39^{ab}	±1.21	36.85^{ab}	±0.32	34.61^{b}	±2.04
MAP	37.62^{a}	±0.66	33.51^{b}	±0.92	35.10^{b}	±1.52	35.54^{b}	±1.70	36.67^{ab}	±1.39
EB+MAP	39.65^{ab}	±2.05	33.61^{b}	±2.10	35.27^{b}	±1.30	35.27^{b}	±2.39	35.39^{a}	±0.64

Each value is the mean of 4 samples.
Means in row with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same row followed by the same letter are not significantly different at $P<0.05$
Means in same row not followed by no letter = no significance $P>0.05$
±SD

Table 4.10 Effect of e-beam irradiation and modified atmosphere packaging on tomato firmness over 21 days of storage

	Deformation (N/mm)									
Treatment	Day 0		Day 3		Day 7		Day 14		Day 21	
Air	1.42^{a}	±0.19	1.15^{a}	±0.21	1.06^{a}	±0.23	0.90^{a}	±0.27	1.06^{a}	±0.24
EB	1.01^{b}	±0.15	1.10^{ab}	±0.22	0.80^{b}	±0.16	0.70^{b}	±0.22	1.01^{a}	±0.24
MAP	1.30^{a}	±0.21	1.21^{a}	±0.20	0.83^{b}	±0.16	0.78^{ab}	±0.17	1.00^{a}	±0.12
EB+MAP	1.08^{b}	±0.11	0.96^{b}	±0.11	0.91^{ab}	±0.15	0.77^{ab}	±0.11	0.88^{a}	±0.20

Each value is the mean of 10 samples.
Means in row with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same row followed by the same letter are not significantly different at $P<0.05$
Means in same row not followed by no letter = no significance $P>0.05$
±SD

Effect of e-beam and modified atmosphere packaging on tomato moisture content

The moisture content in tomatoes was not significantly ($p<0.05$) affected by e-beam treatment over 21 days of storage at 4 ˚ C (Table 4.11). Moisture was also unaffected by modified atmosphere packaging.

Effect of e-beam and modified atmosphere packaging on tomato soluble solids content and titratable acidity

On day 0, e-beam treated tomatoes (in ambient atmosphere) were the only treatment significantly ($p<0.05$) higher in total soluble solids (TSS) content than control tomatoes (Fig. 4.2). On day 7 and thereafter, TSS values were generally not significantly different between treatments.

Titratable acidity (TA) was expressed in citric acid equivalents since it is the predominate organic acid found in tomatoes. TA was not significantly ($p<0.05$) affected by e-beam treatment or modified atmosphere packaging.

Table 4.11 Effect of e-beam treatment and modified atmosphere packaging (MAP) on the moisture content of tomatoes stored for 21 days

Treatment	Day 0		Day 3		Day 7		Day 14		Day 21	
Air	94.97[a]	±0.62	91.52[a]	±0.63	91.29[a]	±0.11	89.70[a]	±0.58	90.82[a]	±0.70
EB	93.71[a]	±0.42	94.58[a]	±0.68	91.90[a]	±0.43	90.70[a]	±0.43	91.00[a]	±0.12
MAP	94.14[a]	±0.83	91.70[a]	±2.74	91.99[a]	±1.19	90.23[a]	±0.11	90.13[a]	±0.88
EB+MAP	93.42[a]	±0.54	91.46[a]	±0.37	91.41[a]	±1.59	91.07[a]	±0.86	91.04[a]	±1.48

Each value is the mean of 6 samples.
Means in row with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same row followed by the same letter are not significantly different at $P<0.05$
Means in same row not followed by no letter = no significance $P>0.05$
±SD

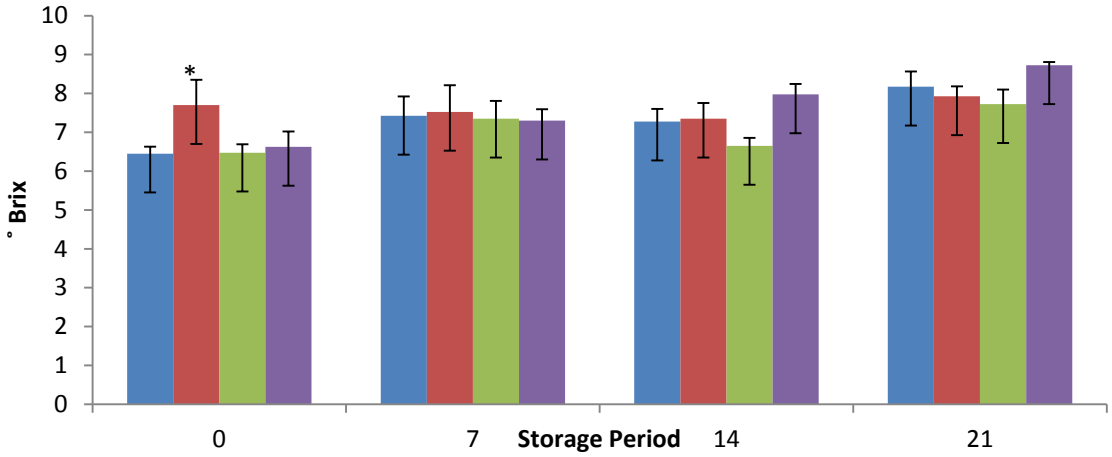


Figure4.2 Effect of e-beam processing and MAP on brix (TSS) values of cherry tomatoes stored for 21 days EB+MAP EB MAP air
* represents significant differences ($P<0.05$)

Table 4.12 Effect of e-beam and modified atmosphere on titratable acidity of tomato samples stored over 21 days

Treatment	Day 0		Day 7		Day 14		Day 21	
Air	0.71[a]	±0.05	0.63[a]	±0.05	0.49[a]	±0.22	0.90[a]	±0.11
EB	0.72[a]	±0.04	0.63[a]	±0.04	0.68[a]	±0.04	0.77[a]	±0.03
MAP	0.73[a]	±0.02	0.67[a]	±0.11	0.58[a]	±0.11	0.80[a]	±0.27
EB+MAP	0.66[a]	±0.05	0.69[a]	±0.19	0.49a	±0.21	0.64[a]	±0.04

Each value is the mean of 4 samples.
Means in row with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same row followed by the same letter are not significantly different at $P<0.05$
Means in same row not followed by no letter = no significance $P>0.05$
±SD

All samples on each storage day had similar titratable acidity values (Table 4.12).

Avocado: Effect of e-beam and modified atmosphere packaging on color

Avocado L-values (lightness) were not significantly affected by e-beam irradiation over storage (Fig. 4.3). The atmospheric conditions did however affect the lightness of the samples. Avocados that were vacuum sealed remained lighter in appearance over 21 days of refrigerated storage regardless of e-beam treatment. Those avocados stored in 5 % oxygen atmospheres however experienced a darkened color (turned grey) over the storage period.

The greenness of avocado (negative a value) was not significantly ($p<0.05$) affected by e-beam treatment for days 0 -14 of storage (Fig.4.4). However, on day 21, both e-beam samples (EB and EB+MAP) were significantly less green than the control. E-beam and MAP (EB+MAP) together did adversely affect avocado greenness particularly after day 14 and 21 of storage and a significant ($p<0.05$) interaction was seen. This combination of treatments caused a grey color to develop during storage.

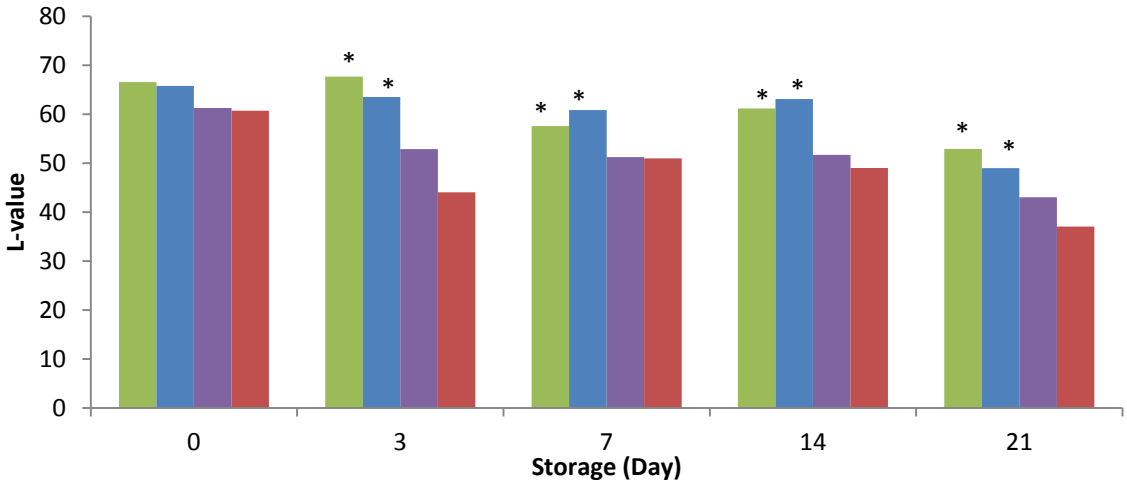


Figure4.3 Effect of e-beam processing and modified atmosphere packaging on the lightness (L-values) of avocado samples stored for 21 days

* represents significant differences (P<0.05) air EB MAP EB+MAP

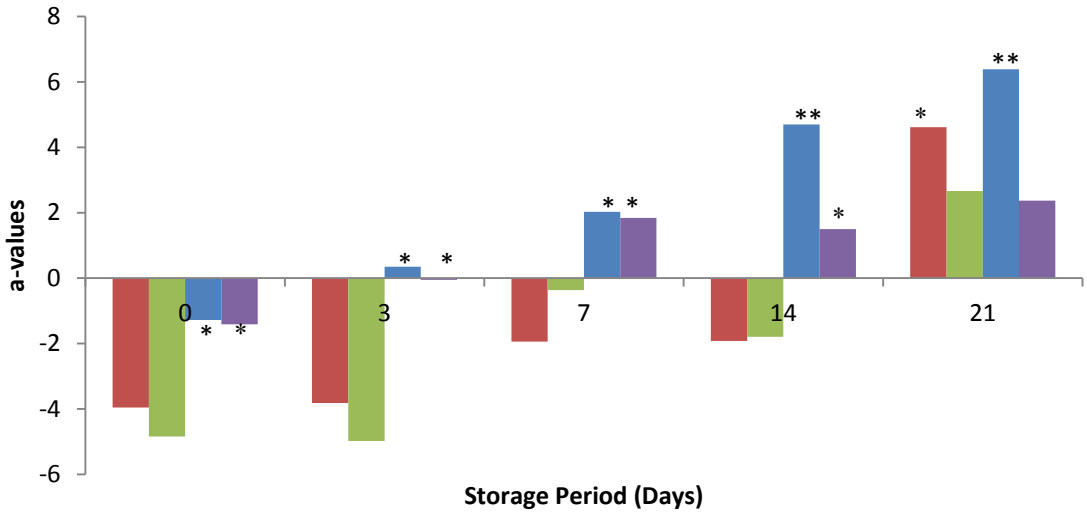


Figure4.4 Effect of e-beam processing and modified atmosphere packaging on the greenness (negative a-values) of avocado samples stored for 21 days
* represents significant differences (P<0.05) EB air EB+MAP MAP

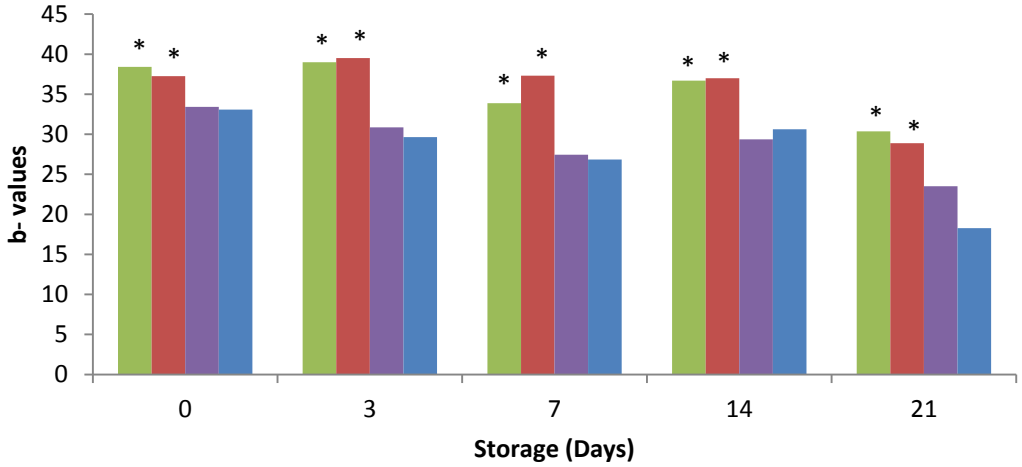


Figure 4.5 Effect of low dose e-beam and modified atmosphere packaging on the yellowness (b-values) of avocado samples stored for 21 days.
* represents significant differences ($P<0.05$) air EB MAP EB+ MAP

B-values (yellowness) were significantly lower ($p<0.05$) in avocado samples packaged in 5% O_2 atmospheres versus vacuum sealed packages (Fig. 4.5). This trend was seen on all storage time points. Irradiation did not have an effect on avocado yellowness. Oxygen excluded packages (control and EB samples) were more yellow than avocado samples with oxygen (MAP and EB+MAP).

Effect of e-beam and modified atmosphere packaging on avocado texture

Avocado flesh texture was relatively unaffected by e-beam processing in any of the texture parameters except for firmness on day 3 where force required to break the flesh was found to be significantly ($p<0.05$) lower in the vacuum sealed irradiated avocado samples than the non-irradiated vacuum-sealed avocados. However, after 21 days of storage, these control samples were less firm than samples treated with e-beam or in gas filled packages (Table 4.13).

Effect of e-beam and modified atmosphere packaging on avocado moisture content

The moisture content in avocado was not affected by irradiation. MAP samples did have slightly higher moisture retention although it was not significant (Table 4.14).

Table 4.13 Effect of e-beam irradiation and modified atmosphere packaging (MAP) on texture of avocado samples stored for 21 days

Firmness (N)										
Treatment	Day 0		Day 3		Day 7		Day 14		Day 21	
Air	1.73^{b}	±0.41	1.43^{ab}	±0.33	1.28^{b}	±0.43	0.09^{b}	±0.02	0.59^{c}	±0.13
EB	2.07^{b}	±0.59	1.09^{c}	±0.25	0.86^{b}	±0.21	0.12^{a}	±0.02	0.86^{b}	±0.18
MAP	1.96^{b}	±0.19	1.66^{a}	±0.21	1.43^{a}	±0.31	0.13^{a}	±0.05	1.69^{a}	±0.58
EB+MAP	2.68^{a}	±1.00	1.24^{bc}	±0.14	1.41^{a}	±0.16	0.08^{b}	±0.02	1.05^{b}	±0.12

Each value is the mean of 10 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
±SD

Table 4.14 Effect of e-beam treatment and modified atmosphere packaging (MAP) on the moisture content of avocado

Treatment	Day 0		Day 3		Day 7		Day 14	
Air	75.48[a]	±0.72	69.14[a]	±2.70	75.32[a]	±3.15	73.02[a]	±3.36
EB	73.53a	±4.24	73.17[a]	±7.33	67.80[a]	±3.75	73.02[a]	±6.11
MAP	74.94[a]	±0.84	72.93[a]	±7.58	76.76[a]	±1.00	74.67[a]	±0.48
EB+MAP	76.92[a]	±0.62	76.65[a]	±5.69	73.41[a]	±8.05	77.04[a]	±0.86

Each value is the mean of 6 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$

Table 4.15 Effect of e-beam and modified atmosphere packaging on grape redness over 21 days of storage.

Treatment	Day 0		Day 3		Day 7		Day 14		Day 21	
Air	7.33	±0.57	8.78	±0.61	9.55	±0.83	7.76	±0.47	6.48	±0.40
EB	12.20	±1.59	10.58	±1.23	10.36	±1.11	7.21	±0.76	7.14	±0.81
MAP	9.67	±0.66	10.04	±0.42	9.82	±0.30	9.59	±1.03	10.38	±0.59
EB+MAP	10.92	±1.72	11.18	±0.83	8.72	±0.31	7.36	±0.47	11.22	±1.23

Each value is the mean of 4 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
±SD

Grape: Effect of e-beam and modified atmosphere packaging on color

E-beam irradiated grapes were significantly more red ($p<0.05$) than control grapes (Table 4.15) on day 0 until day 7 of storage. modified atmosphere (MAP) grapes were also significantly higher in red values during 21 days of storage than control grapes.

Effect of e-beam and modified atmosphere packaging on grape texture

Fruit firmness (Modulus of deformation) was not significantly affected by e-beam treatment on any day of storage. Modified atmosphere also did not have a significant effect on the modulus of deformation except for on days 7 and 21 were fruit were more elastic or turgid than the other grape samples (Table 4.16).

Significantly less force ($p<0.05$) was needed to rupture irradiated grape skin (e-beam and EB+MAP samples) during all days of storage compared to the control, except on day 21. Modified atmosphere packaging itself did not affect grape skin firmness on days 0 and 21.

Distance to rupture (capability of being stretched) was greatest in control grapes at all storage times. E-beam processing did not adversely affect the distance to rupture measurements in grape samples but rather maintained grape skin integrity. Modified atmosphere also significantly ($p<0.05$) decreased distance to rupture measurements and aided in preserving grape skin tautness.

Table 4.16 Effects of e-beam irradiation and modified atmosphere packaging (MAP) on grape texture stored over 21 days.

Modulus of deformation (N/mm)

Treatment	Day 0		Day 3		Day 7		Day 14		Day 21	
Air	0.70^{a}	±0.18	0.49^{b}	±0.13	0.46^{b}	±0.14	0.16^{a}	±0.10	0.18^{c}	±0.09
EB	0.79^{a}	±0.14	0.53^{b}	±0.17	0.50^{b}	±0.15	0.15^{a}	±0.08	0.79^{b}	±0.24
MAP	0.72^{a}	±0.29	0.84^{a}	±0.18	0.69^{a}	±0.14	0.19^{a}	±0.08	1.02^{a}	±0.22
EB+MAP	0.62^{a}	±0.13	0.62^{b}	±0.18	0.46^{b}	±0.23	0.17^{a}	±0.04	0.26^{b}	±0.13

Each value is the mean of 10 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
Means in same column not followed by no letter = no significance $P>0.05$
±SD

Table 4.17 Effect of e-beam irradiation and modified atmosphere packaging on the moisture content of grapes stored for 21 days

Treatment	Day 0		Day 3		Day 7		Day 14		Day 21	
Air	83.67[a]	±2.33	77.81[a]	±3.16	77.73[a]	±4.31	67.09[a]	±3.61	74.88[ab]	±2.51
EB	79.92[a]	±1.65	78.48[a]	±2.77	77.01[a]	±2.69	71.28[a]	±3.61	73.42[b]	±2.18
MAP	81.48[a]	±2.01	80.10[a]	±1.98	79.22[a]	±2.91	73.39[a]	±3.41	79.64[a]	±0.24
EB+MAP	79.61[a]	±1.98	78.83[a]	±1.36	78.98[a]	±0.54	80.58a	±1.81	79.14[ab]	±0.05

Each value is the mean of 10 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
Means in same column not followed by no letter = no significance $P>0.05$
±SD

Effect of e-beam and modified atmosphere packaging on grape moisture content

E-beam processing did not significantly affect moisture content in grape samples over the 21 day storage period. Moisture content of grapes were significantly higher ($p<0.05$) in samples stored in a modified atmosphere after 14 days of storage (Table 4.17). The average grape water content in is 81 % (108).

Effect of e-beam and modified atmosphere packaging on grape soluble solids content (˚Brix) and titratable acidity

Total soluble solids content (TSS) was measured in ˚Brix. E-beam processing did not have a significant effect ($p<0.05$) on the brix values of grapes after day 0. Modified atmosphere packaging had an effect on brix values at days 7, 14 and 21 of storage. The grape samples MAP and EB+MAP had significantly higher soluble solids values than ambient atmosphere samples (control and e-beam) (Fig.4.6).

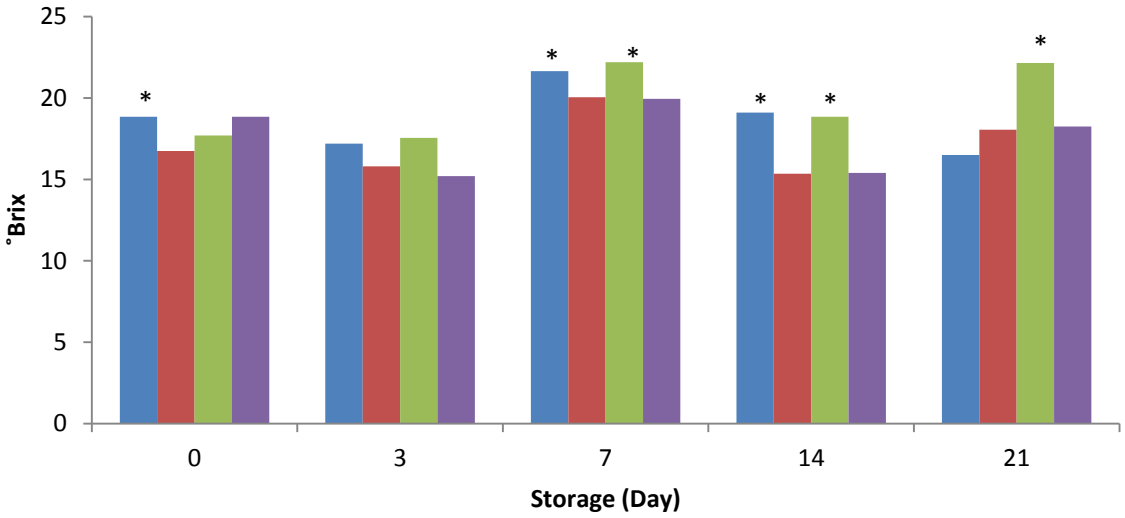


Figure 4.6 Effect of low dose e-beam and modified atmosphere packaging on brix values of grapes stored for 21days. air EB+MAP EB MAP
* represents significant differences ($P<0.05$)

Watermelon: *Effect of e-beam and modified atmosphere packaging on color*

Redness (a-values) of watermelon was significantly ($p<0.05$) higher in e-beam treated ambient atmosphere samples (EB) immediately after processing on day 0. On day 7, e-beam treatment and modified packaging both alone and together significantly increased red values compared to the control watermelon. On day 14, e-beam samples were significantly more red than the non-irradiated watermelon (Fig 4.7). After 21 days of storage all treatments were similar to the control (air).

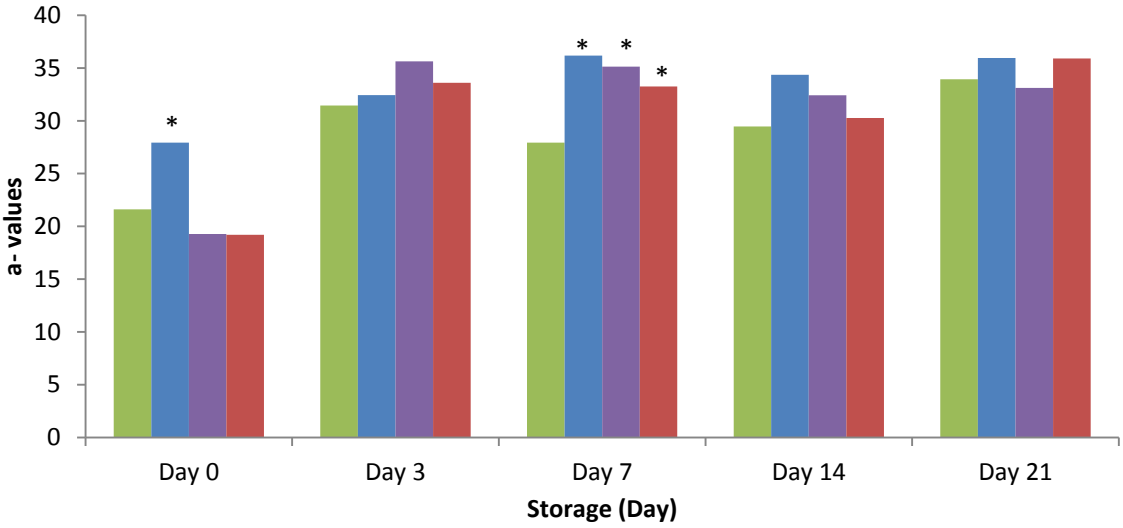


Figure 4.7 Effect of low dose e-beam and modified atmosphere packaging on the redness (a-values) of tomato samples stored for 21 days
* represents significant differences ($P<0.05$) control EB EB+MAP MAP

Table 4.18 Effect of e-beam irradiation and modified atmosphere packaging (MAP) on texture of fresh cut watermelon samples stored for 21 days

Treatment	Day 0		Day 3		Day 7		Day 14		Day 21	
Air	2.45[c]	±0.22	3.32[a]	±0.80	3.57[b]	±0.48	0.35[b]	±0.10	3.52[a]	±0.84
EB	3.35[b]	±0.30	4.01[a]	±1.79	4.62[a]	±0.64	0.59[a]	±0.14	3.82[a]	±1.42
MAP	3.84[a]	±0.68	3.79[a]	±0.91	3.62[b]	±0.30	0.39[b]	±0.05	3.57[a]	±0.61
EB+MAP	3.04[b]	±0.36	3.60[a]	±0.52	3.26[b]	±0.25	0.40[b]	±0.09	3.28[a]	±0.54

Each value is the mean of 10 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
Means in same column not followed by no letter = no significance $P>0.05$
Measured in Newtons (Firmness)
±SD

Effect of e-beam and modified atmosphere packaging on watermelon texture

E-beam treatment did not adversely affect watermelon firmness. On days 0, 7 and 14, e-beam treated samples (ambient atmosphere) were significantly more firm ($p<0.05$) than the control watermelon samples. Modified atmosphere did not have a significant effect on firmness except for day 0, when these watermelon were more firm than the control or any other treatment (Table 4.18).

Effect of e-beam and modified atmosphere packaging on watermelon total soluble solids and titratable acidity

Total soluble solids content was measured in ˚Brix and is an estimate of the sweetness of the sample. Overall, e-beam and MAP treatments did not have a significant effect ($p<0.05$) on the brix values of watermelon sample over 21 days (Figure 4.8).

Titratable acidity (TA) was express in citric acid equivalents as this is the major organic acid in watermelon. TA was not significantly affected by e-beam processing and was similar to the control samples (Table 4.19).

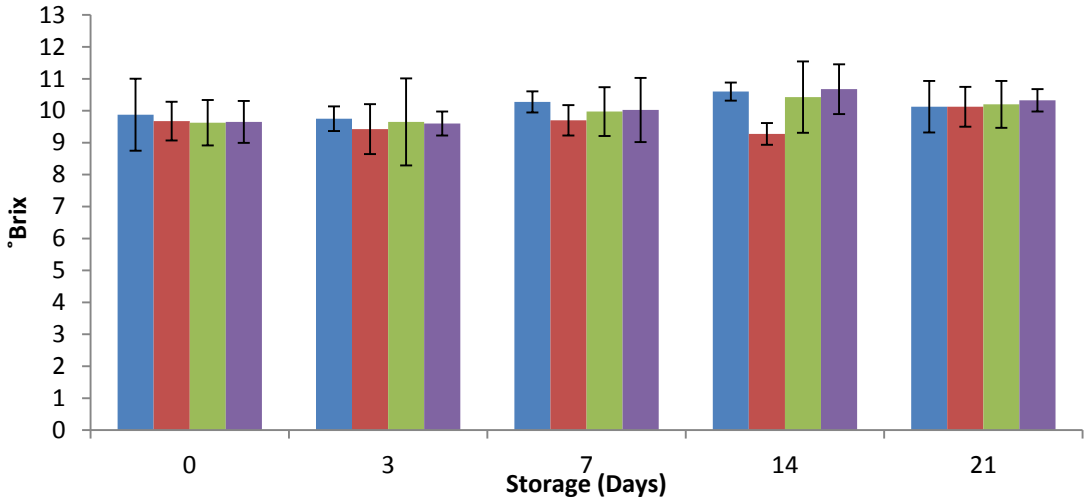


Figure 4.8 Effect of e-beam and MAP on ˚brix of watermelon samples stored for 21 days

Table 4.19 Effect of e-beam processing and modified atmosphere packaging (MAP) on the titratable acidity of watermelon samples stored for 21 days

Treatment	Day 0		Day 3		Day 7		Day 14		Day 21	
Air	0.14[a]	±0.01	0.14[a]	±0.01	0.13[a]	±0.01	0.14[a]	±0.01	0.17[a]	±0.01
EB	0.15[a]	±0.01	0.14[a]	±0.02	0.13[a]	±0.01	0.13[a]	±0.01	0.13[a]	±0.01
MAP	0.14[a]	±0.01	0.13[b]	±0.01	0.12[a]	±0.02	0.14[a]	±0.01	0.15[a]	±0.01
EB+MAP	0.14[a]	±0.02	0.14[ab]	±0.01	0.13[a]	±0.02	0.13[a]	±0.01	0.16[a]	±0.02

Each value is the mean of 4 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
Means in same column not followed by no letter = no significance $P>0.05$
±SD

Table 4.20 Mean values of consumer scores on grape acceptability

Treatment	Appearance		Odor		Color		Firmness		Flavor	
Air	7.49[a]	±1.12	6.20[a]	±1.28	7.06[a]	±1.78	7.89[a]	±1.02	7.89[a]	±1.49
EB	7.29[a]	±1.56	6.17[a]	±1.60	7.37[a]	±1.44	7.77[a]	±1.11	7.60[a]	±1.17
MAP	6.29[b]	±2.16	5.74[a]	±1.22	6.09[b]	±2.12	7.43[a]	±1.46	7.63[a]	±1.31
EB+MAP	7.34a	±1.43	5.94[a]	±1.57	7.09[a]	±1.40	7.74[a]	±1.63	7.86[a]	±1.35

Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
Means in same column not followed by no letter = no significance $P>0.05$
±SD

Consumer study

Grapes

Consumers rated grapes as acceptable (score of 5 or more) in all sensory attributes for both e-beam treated and MAP samples (Table 4.20). Most of the attributes were not significantly different ($p<0.05$) from the control grapes or any other treatments. Eighty-eight percent of consumers scored MAP grapes samples as a 7, 8 or 9 (moderate - extremely like); this was higher than any other treatment (Fig.4.9). EB+MAP grapes however did receive more perfect scores (9-extremely like) than other treatments.

Tomato

Overall appearance and odor in tomato samples were all rated similarly and as acceptable by consumers (Table 4.21). Irradiated MAP tomato samples were scored significantly ($P<0.05$) lower in color and perceived firmness than control tomatoes. Non-irradiated MAP tomato samples were rated the lowest in flavor (5.72) compared to e-beam treated tomatoes (7.32) that had a significantly higher score

Watermelon

Consumers rated overall appearance and firmness of watermelon samples equally across treatments. Irradiated ambient watermelon was given a significantly ($p<0.05$) higher score in the odor attribute (7.42) and was the highest rated in flavor (6.03) (Table 4.22).

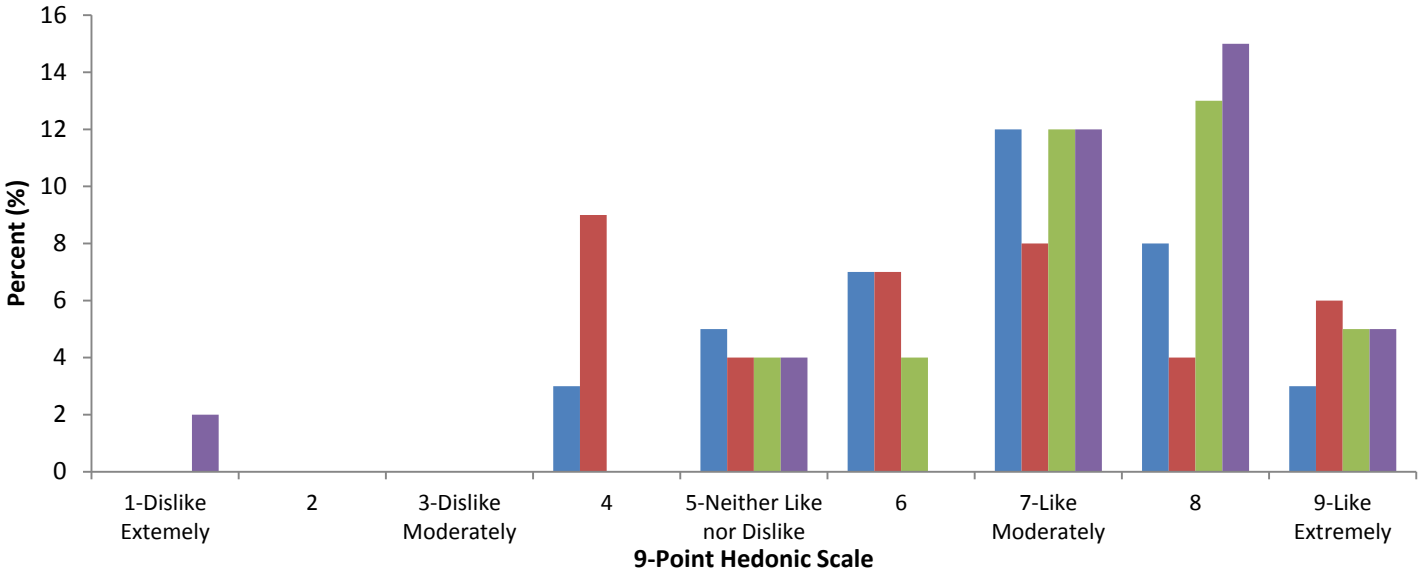


Figure 4.9 Consumer scores on a 9-point hedonic scale for overall acceptance of grape appearance.

Table 4.21 Mean values of consumer scores on tomato acceptability

Treatment	Appearance		Odor		Color		Firmness		Flavor	
Air	8.12[a]	±0.97	6.44[a]	±1.45	8.32[a]	±0.95	7.32[a]	±1.31	7.20[ab]	±1.41
EB	7.44[a]	±1.16	6.04[a]	±1.43	7.68[ab]	±1.11	6.20[ab]	±2.20	7.32[a]	±1.31
MAP	7.52[a]	±1.61	5.96[a]	±1.51	7.68[ab]	±1.55	6.36[ab]	±1.89	5.72[c]	±1.99
EB+MAP	7.68[a]	±1.91	5.88[a]	±1.74	7.20[b]	±1.71	5.84[b]	±2.62	6.40[ba]	±1.73

Means in column with different superscript (a,b) represent significant difference (P<0.05)
Means in same column followed by the same letter are not significantly different at P<0.05
Means in same column not followed by no letter = no significance P>0.05
±SD

Table 3.22 Mean values of consumer scores on watermelon acceptability

Treatment	Appearance		Odor		Color		Firmness		Flavor	
Air	7.08[a]	±1.23	7.42[a]	±1.42	7.11[a]	±1.37	6.47[a]	±1.81	6.03[a]	±1.03
EB	6.75[a]	±1.27	6.61[b]	±1.73	6.36[b]	±1.13	6.31[a]	±1.69	5.14[b]	±1.38
MAP	6.94[a]	±1.51	6.58[b]	±1.46	6.89[ab]	±1.63	6.69[a]	±1.75	5.56[ab]	±1.50
EB+MAP	6.42[a]	±2.14	6.31[b]	±1.43	6.50[ab]	±1.72	6.33[a]	±1.88	5.22[b]	±2.01

Means in column with different superscript (a,b) represent significant difference (P<0.05)
Means in same column followed by the same letter are not significantly different at P<0.05
Means in same column not followed by no letter = no significance P>0.05
±SD

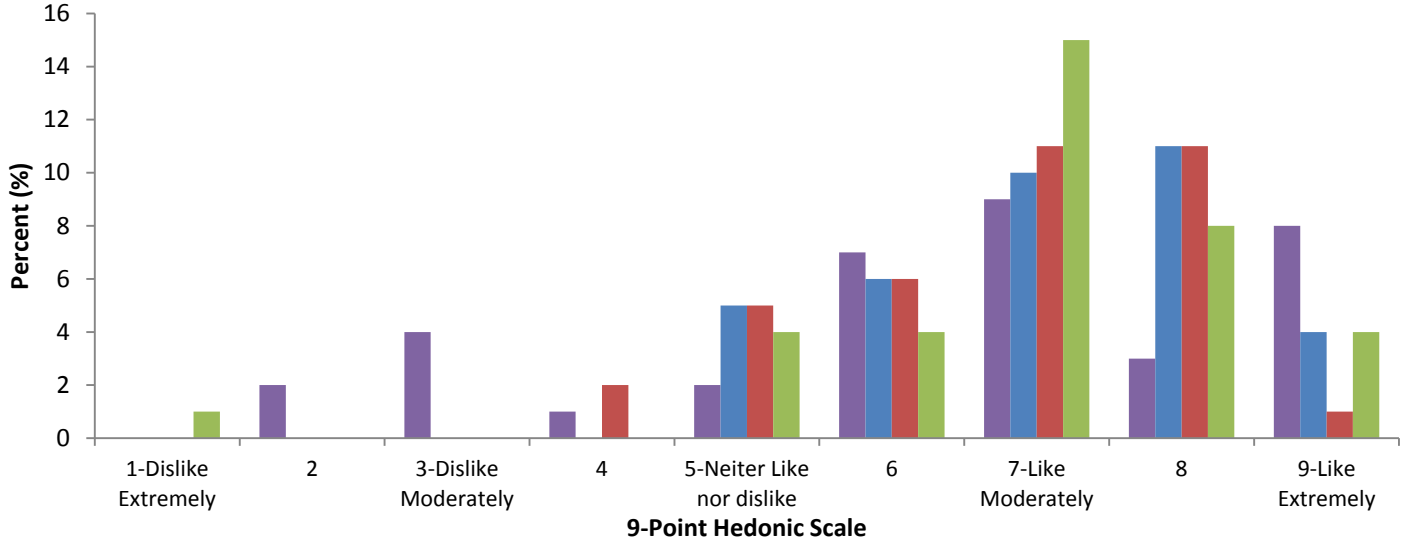


Figure 4.10 Consumer scores for overall acceptance of watermelon appearance

air MAP EB+MAP EB

The e-beam treated watermelon in ambient atmosphere (e-beam) were highly liked by consumers in overall appearance (69% scored them 7-9) only second to control samples (Fig 4.10). All treatments were rated high for this attribute (69% 7-9).

Strawberry

Control (air) strawberries were rated higher for each attributes but it was only significantly higher in flavor and color scores ($p<0.05$). Irradiated MAP strawberries were rated the lowest for all categories but were still rated above acceptable for each sensory attribute (Table 4.23).

Discussion

Overall, low dose e-beam irradiation did not have as much of an effect on the sensory qualities of fruit like modified atmosphere packaging.

Fruit moisture was not affected by irradiation. MAP samples did have slightly higher moisture retention as the packaging and gas mixtures are designed to slow moisture loss of the food product. The numbers were usually not significantly higher, but a trend was seen.

This pattern was also observed when investigating how these treatments affected the color of the fruit samples. E-beam did not have a great affect whereas MAP did. The redness of strawberries, tomatoes and watermelon were not degraded by e-beam treatment. Only EB+MAP strawberries on day 3 of storage, were significantly ($p<0.05$) less red than control (air) strawberries. This could mean that a combination of the two treatments potentially degraded red pigments like anthocyanins in strawberries compared to the other treatments that better maintained them. However since the interaction

Table 4.23 Mean values of consumer scores for strawberry acceptability

Treatment	Appearance		Odor		Color		Firmness		Flavor	
Air	7.29[a]	±1.16	7.74[a]	±1.43	7.61[a]	±1.24	7.53[a]	±1.33	7.53[a]	±1.50
EB	6.68[ab]	±1.38	7.16[a]	±1.13	6.74[b]	±1.33	6.84[a]	±1.31	6.45[b]	±1.55
MAP	7.13[a]	±1.82	7.24[a]	±2.22	7.58[a]	±2.16	7.00[a]	±2.45	6.53[b]	±2.48
EB+MAP	6.32[b]	±1.76	7.08[a]	±1.46	6.39[b]	±1.50	5.95[b]	±1.72	6.45[b]	±1.69

Means in column with different superscript (b,c,) represent significant difference (P<0.05)
Means in same column followed by the same letter are not significantly different at P<0.05
Means in same column not followed by no letter = no significance P>0.05
±

between the two treatments were not significant ($P>0.05$), this result could possibly be due to random fruit variations as different batches of strawberries tended to vary widely despite a higher n value. Some strawberries may have been harvested at different times thus contributing to the variation. MAP did not affect strawberry redness until day 21 where strawberries were significantly ($p<0.05$) more red than control strawberries. The modified atmosphere possibly had an effect on preserving strawberry color. The MAP environments provide an acidic environment for compounds like anthocyanins that would otherwise degrade at higher pH's (12).

In tomatoes, both MAP treatments (MAP & EB+MAP) tomatoes were darker than control (air) tomatoes for most of the storage time. MAP potentially preserved tomato color by slowing the rate of deterioration and degrading of lycopene, a compound that gives tomatoes red color. Also, e-beam processed tomatoes were initially more red for 3 days. Shurong et al. (114) also found that samples irradiated at doses above 0.5 kGy had a significantly higher red color during days 2 and 4 of storage. This could be due to an increase in lycopene experienced in tomatoes that undergo irradiation.

Watermelon redness was also significantly higher in e-beam treated ambient atmosphere samples (EB only samples) on day 0. This could be due to a situation similar to the tomatoes where an increase in lycopene content is seen when fruit undergo stressful environments even at low levels (111). Also on day 14, e-beam samples were significantly more red than control samples.

E-beam grapes were redder shortly after processing and storage. The same trends were seen in grapes as what was reported for the effects of e-beam on strawberry color

which could be the influence of e-beam on the anthocyanin content that gives both of these fruit their red color.

The color changes in avocado stored in 5% O_2 can be attributed to the available oxygen acting as a substrate in enzymatic reactions (PPO or PAL) that use oxygen to oxidize phenolic compounds and form colorful polymers (melanins: brown colored pigments). The presence of oxygen in MAP avocados, (unlike the vacuum sealed samples) greatly increased lipid oxidation in the avocado (16). Also, this oxygen can be used by spoilage microbes to support their growth. Irradiation had no effect on color for the first 14 days of storage as irradiated vacuum sealed samples were similar to the control. MAP with 5% O_2 had greater effects on avocado lightness, greenness and yellowness. These observations show that oxygen is a larger factor on the influence of changes in avocado color over storage than irradiation.

Fruit texture was another quality parameter that was marginally affected by e-beam irradiation. The modulus of deformation was used to evaluate firmness. It is a measurement of fruit elasticity and assesses how well the fruit is able to recover from an applied stress; thus being a widely used parameter for fruit firmness. Modified atmosphere did significantly ($p<0.05$) affect the deformation of fruit allowing fruit to maintain elasticity through storage. Skin firmness (measured in Newtons) was taken but was not necessarily used to assess texture quality in whole fruit because it is heavily influenced by the size of the fruit, maturity level, and the day it was harvested (122). It is also a factor of skin toughness and firmness of the flesh underneath (124) thus not portraying the turgor of the fruit unlike deformation measurements. E-beam did

significantly ($p<0.05$) soften fruit skin while MAP did not have an effect. Although the skin of the fruit itself (strawberries, tomatoes and grapes) was less firm in irradiated samples, the fruit integrity (as measured by the modulus of deformation) was not affected.

Strawberry firmness (elasticity) was not significantly ($p<0.05$) affected by e-beam processing. Modified atmosphere packaging did affect strawberry firmness on days 3-21 where the MAP strawberries were the most elastic. As previously mentioned, strawberry skin was softened by irradiation and was less firm for all days of storage compared to control samples. This was also reported by Ahmed et al. (125) The reduction in skin firmness (from 6N – 2N) however did not make the strawberries mushy or unacceptable as they were still with in normal ranges of strawberry firmness. Hietaranta et al. (124) reported strawberry firmness measurements that ranged from 1.41 – 0.5 N. Another study assessing skin firmness in different strawberry maturity levels reported firmness values from 3.8-10.8 N (123). Modified atmosphere alone (without e-beam) affected skin firmness early during storage, having higher values. Increased levels of CO_2 (15%) has shown to decrease ethylene production for 9 days in strawberries, thereby slowing fruit deterioration and maintaining firmness in the samples (120). The modified atmosphere (15% CO_2; 5 % O_2; 85% N_2) packed strawberries were initially more firm than control samples, but this preservation may only last for a certain period. During prolonged storage, the high carbon dioxide content present in the package dissolves into the tissues causing sogginess in strawberry fruit. Furthermore, MAP combined with e-beam (EB+MAP) resulted in increasingly less skin firmness

strawberries after 7 days of storage compared to MAP only samples. This occurrence can be minimized with possibly choosing a different package material that allows higher gas transmission. Irradiation tends to increase the respiration rate in produce. Therefore if higher CO_2 levels accumulate in the package, this could increase the sogginess of strawberries as seen in this study. These texture changes seen in e-beam strawberries are most likely due to their effect on tissue integrity and solubilization of the cell tissue. Pectin is one of the main cell wall constituents that when broken down result in fruit softening (126). Yu et al (96) reported that irradiation (1 and 2 kGy dose) initially slightly increase strawberries firmness (when stored at 2˚C storage) and then the berries became less firm over time stored. Firmness was correlated to the oxalate-soluble pectin content which decreased on day 0 and 1 after irradiation. Thus could explain the softer strawberries. The softness however was not seen as a negative attribute in the consumer study where all samples were rated as acceptable.

Firmness (turgor) of tomatoes was not affected by either e-beam or modified atmosphere packaging. However, MAP did maintain the extensibility of the tomatoes after 14 days of storage, meaning that the MAP tomatoes were less able to be stretched or protruded. This can be contributed to the protective barrier the packaging provided for the fruit. Consumers were accepting of all treatments of the fruit and although e-beam softened tomato skins significantly ($p<0.05$), consumers still rated the tomatoes as acceptable in firmness.

Avocado fruit were cut into 1 cm cylinders to assess flesh firmness since this is the portion of fruit that is eaten. The texture was not overall affected by e-beam processing or modified atmospheres through storage.

MAP alone maintained grape integrity as these samples were more durable and able to return to their original shape than the other treated grapes. E-beam irradiation did not have an effect on the grape elasticity. Control grapes were significantly more easily stretched and wrinkled in appearance (less turgid) through storage compared to all of the other treated grape samples. Modified atmosphere (5% O2, 3% CO2 and 92%N2) significantly aided in preserving grape qualities. MAP provides a protective barrier from moisture loss in samples as well as slowing the respiration rate thus prolonging fruit deterioration (31). Skin firmness tended to lower in e-beam treated grapes but this did not however affect consumer scores of overall acceptance of samples.

Watermelon texture was not adversely affected by e-beam treatment or MAP as measurements of flesh firmness (N) were relatively close for each sample throughout the storage period. This fruit like the avocado was cut into 1cm cylinders and only the flesh texture was assessed.

The next fruit quality measured was total soluble solids. This is an indicator of the amount of sugars in a sample. Modified atmosphere packaging affected total soluble solids in strawberry samples as these values were significantly lower than control (air) strawberries. This is consistent with findings by Caner et al. (126) and Almenar et al. (127) who reported that TSS decreased in MAP strawberries during storage. The range for TSS (4.8-10.9%) was found in literature for ripe strawberries (122, 123). Irradiation

alone did not significantly affect TSS. TSS increased in ambient atmosphere and was also observed by Nunes et al. (128) where 'Oso Grande' strawberries increase in brix value by 10, 6.5 and 7 % during storage at 1 ˚C. It is thought that soluble solid increases in postharvest strawberry is not caused by the breaking down of starch to soluble sugars because they contain such small amounts of starch while developing (129). One explanation for the increase observed in this current study and by Huber et al. could be due to the increase seen in titratable acidity of ambient atmosphere strawberries because acids contribute to TSS. The increases could also be due to the solubilization of polyuronides in the cell wall and hemicelluloses (130).

Titratable acidity of strawberries was not affected by e-beam, but rather by modified atmosphere packaging. MAP samples had lower titratable acidity values than the ambient atmosphere strawberries. As fruits age, titratable acidity normally decreases while pH increases. This is due to the breaking down of the predominate organic acid (citric acid) found in strawberries over storage time. This increase in TA could be tied to the high CO_2 which dissolves in the fruit tissue and the carbonic acid that is produce acidifies the fruit which could lead to lower quality as pH is increased (126). For ambient atmosphere strawberries, TA increased slightly over time which is consistent with the observations of Nunes et al. (128128).

The tomato sample TSS and TA seemed to be more robust to treatments. On day 0, the e-beam treated tomatoes (e-beam only sample) had higher brix values. This could possibly be due to the irradiation treatment breaking down polysaccharides into more soluble solids (12). Overall, brix values increase with storage time. This is normal

because as fruit ripens, starches are broken down into sugars (23). E-beam did not adversely affect brix values and MAP did not have a significant effect. Neither e-beam nor MAP significantly affected titratable acidity.

Watermelon titratable acidity and total soluble solids were similar to tomato in that these samples were neither affected by irradiation of MAP packaging as all values were similar to the control (air) samples.

CHAPTER V

CONCLUSIONS AND FUTURE WORKS

E-beam processing (at FDA approved doses) was able to reduce the bioburden of fresh fruit. This technology was more "successful" at treating some fruits over others. This is primarily a result of the varying microbial populations that fresh produce may have on their surfaces. Some bacteria may also possess the ability to resist treatment by mechanisms that allow them to survive in acidic environments. Grapes, fresh cut watermelon and avocado were fruits that when irradiated exhibited reductions in bioburden levels to that of clean food diet standards. This demonstrates the usefulness of the technology in processing raw foods. Application for this technology is not only useful in hospitals for immunocompromised patients but could be valuable for children and school lunches as well. Other fruits tested, like strawberries and tomatoes, did have reductions when irradiated; however due to the high levels of bacteria on the fruit to begin with, the level of reduction was not achieved using small doses. Coupling this technology with other hurdle technologies such as sanitization and good worker hygiene are some ways in which e-beam can be applied at the end of a production line to receive the maximum microbial reduction. Other sensitizers that can be used to enhance e-beam reduction include adding antioxidants and/or using a modified atmosphere system. In this study, irradiated MAP fruit resulted in reduced bioburdens compared to samples that were non-irradiated in MAP. Bacterial counts were not necessarily found to be lower on fruit in MAP trials than ambient atmosphere trials; however a separate study running all

treatments together would allow a more accurate comparison since different batches of fruit behave differently.

Objective sensory qualities of fresh fruit were not affected by e-beam processing in moisture, soluble solids or titratable acidity levels. Slight differences were seen in the greenness of avocado that had been stored for 14 days. Redness of watermelon and strawberry was slightly affected as these values were higher early on during storage. Also, e-beam processed fruit samples were softer than control samples when measured by a texture analyzer. These results given from the compression tests could be dependent on the parameters set (probe, test speeds, sample size). TPA (texture profile analysis) may be a better measurement of true texture differences in fruit. Although textural differences were measured using a textural analyzer instrument, subjective measurements by consumers showed that softer fruit (that had been irradiated) were liked just as much as non-irradiated fruit (with higher measurements in skin firmness).

Modified atmosphere packaging seemed to have more of an effect on various sensory attributes than e-beam processing. For example, because of the elevated CO_2 levels, it increased fruit pH and resulted in higher TA and lower TSS values. These measurements are important to fruit quality as lower TSS values can be seen as undesirable (less sweet food product). One way to combat this issue would be to test different packaging materials.

E-beam has proven to be a value adding technology that shows promise to develop clean foods. This technology could prevent unnecessary food borne outbreaks that plague communities and keep food safe for at risk populations. Furthermore, e-beam

does not affect important sensory attributes of fresh fruit, especially at low doses (<1kGy). Any differences seen in sensory attributes were not found undesirable by consumers.

Various follow up studies are suggested to gain further knowledge and insight on the effects of e-beam irradiation on these fruit samples. One follow up study suggested would be to perform an inoculated or spiked study. A known amount of bacteria could be introduced to the fruit and multiple doses could be applied to the samples. This would allow a better understanding of the killing efficiency of e-beam on various selected microorganisms and at what doses are the most effective. Currently, the day 0 samples could be used as a baseline; however, a spiked study could give a more accurate measurement of the reduction seen when fruit are treated with low dose e-beam. The current approved dose is 1kGy; however, this was set to treat insects that do not require high doses to sterilize and inactivate on fresh foods. Some bacteria may need higher doses and an inactivation kinetics study could be useful information to prove (or disprove) this thought.

If higher doses were used, texture studies would need to be carried out to evaluate the effect that higher doses may have on fresh fruit. Also, exploring different texture tests like Texture Profile Analysis (TPA) would be worth the investigation. This could be a better indicator of fruit firmness than the puncture tests used. Puncture tests done on whole fruit like grapes may be influenced by skin thickness or toughness and not account for the turgor of the fruit. TPA carries out multiple tests to determine not

only firmness but springiness, cohesiveness and adhesiveness of the sample, which may be more descriptive of fruit samples.

Furthermore, additional consumer studies done over a longer storage period would allow more insight into how fruit samples are accepted over time (1 or 2 weeks stored). After day 1 of treatment, the affects that the treatments may have on the fruit may not be apparent until further storage. For example, strawberries that had been stored in MAP after 1 day were not much different from those in ambient atmosphere but after 14 days, the MAP strawberries had a more firm appearance. More consumer studies could answer these questions.

Another important aspect to investigate are the various films that are available for use. In this study, polypropylene (PP) with nylon layered on the outside film was used for all fruit. Nylon is a high barrier film that holds in gasses and moisture. By layering the film with an additional polymer film like PP may allow a more breathable package. This could alleviate some of the CO_2 accumulation and also reduce condensation inside the bag that was often observed. This condensation build up may have also contributed to some of the sogginess seen in strawberry samples packaged with this film. MAP proved to be efficient at holding in fruit moisture. Both gas mixtures and the film itself are important to achieve this goal. Different fruit may thrive better in different packaging films as well. For instance, tomatoes were packaged in a lower permeable film, but since the respiration rate is higher than that of grapes, a different film may have worked better for these fruit. Furthermore, the packaged tomatoes exhibited an off flavor when opened. A more permeable film could work to dissipate the

odor if designed properly. Gas mixtures were also a challenge to customize with each fruit. Tomatoes for example may be sensitive to CO_2 and this could cause severe damage to the fruit and the metabolic processes ultimately leading to an accelerated loss of quality. While most studies recommend lowering oxygen content in the package, others have shown that high oxygen atmospheres are efficient at prolong shelf life as well. Many intricate and important parameters go into properly designing a successful MAP system, especially if used along with irradiation and although the system would never be perfect, there are endless options to explore.

An additional factor to consider when looking at the effects of e-beam on food is the influx of electrons that e-beam provides to the foods. This process is different from other irradiation sources like gamma or x-ray. Therefore, the effects of oxidation could be different and possibly less apparent in samples treated with e-beam. For example, the avocados that were treated with e-beam were more so affected by the presence of oxygen than e-beam irradiation. When oxygen was excluded, the e-beam samples were the same greenness as the non-irradiated avocado samples. Studies looking at these effects of lipid oxidation of a food item could prove useful.

Lastly, different storage temperatures would be worth investigating as some fruit were subjected to chill injury during storage. This may have been more of an influence on the quality changes than the treatments themselves. For instance, tomatoes are sensitive to low temperatures which was apparent in the ambient atmosphere samples. These were stored in clamshells and exposed to the environment versus the MAP that had a protective film. This factor could have accelerated the fruit decay in ambient

samples since the optimal storage for this fruit is around 10˚C and these were stored at 4˚C. Also, avocado which are tropical fruits tend to rapidly decay at cold temperatures and so these fruit may have deteriorated sooner than would be expected at slightly higher temperatures.

These various studies would provide more detailed insight as to techniques that optimize the process of e-beam irradiation and storage of fresh fruit. Although the process would never be perfect because of uncontrollable factors, the current state of fresh fruit could be drastically improved from a safety and quality standpoint.

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APPENDIX

Bioburden Analysis of Strawberry

Trial I

Effect of e-beam processing on the microbial counts of strawberries over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)*		Yeast & Molds (Log 10 CFU/g)*	
	Control	E-beam	Control	E-beam
0	4.37	3.73	4.61	3.79
1	4.61	3.85	4.60	3.84
3	4.40	3.53	4.61	3.81
7	4.28	3.56	4.44	3.89
14	5.09	4.38	5.12	4.60
21	4.81	4.47	4.92	4.56

*These values were calculated from the median CFU/g count out of 3 rep

Percentage of control values of e-beam processed strawberries stored for 21 days

Day	Aerobic bacteria	Yeast & Molds
0	22.93	15.31
1	17.20	17.42
3	13.65	15.84
7	18.90	28.15
14	19.51	30.53
21	45.00	43.61

Each value was calculated using median values out of 3 reps

Trial II

Effect of e-beam processing on the microbial counts of strawberries over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Molds (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	5.39	4.91	4.96	4.68
1	5.52	4.98	4.84	4.60
3	5.96	5.33	5.55	4.44
7	5.73	4.92	5.29	4.65
14	6.08	5.14	5.32	4.60
21	5.60	4.95	5.28	4.88

These values were calculated from the median CFU/g count out of 3 reps

Percentage of control values of e-beam processed strawberries stored for 21 days

Day	Aerobic bacteria	Yeast & Molds
0	32.79	52.75
1	29.09	58.33
3	23.04	7.83
7	15.37	23.20
14	11.37	19.19
21	22.25	39.37

These values were calculated from the median CFU/g count out of 3 reps

Bioburden Analysis of Grapes

Trial I

Effect of e-beam processing on the microbial counts of grapes over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Mold (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	2.00	2.00	2.00	BD
1	2.78	2.00	2.48	2.00
3	2.85	BD	2.48	BD
7	2.70	2.00	2.30	2.00
14	2.78	3.69	2.60	3.72
21	4.01	3.89	4.79	3.93

These values were calculated from the median CFU/g count out of 3 reps
BD : Below detection at 10^2 dilution

Trial II

Effect of e-beam processing on the microbial counts of grapes over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Molds (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	2.48	BD	BD	2.30
1	BD	BD	2.00	3.18
3	BD	BD	BD	BD
7	BD	BD	BD	BD
14	2.00	BD	BD	2.00
21	3.00	2.70	3.08	3.00

These values were calculated from the median CFU/g count out of 3 reps
BD : Below detection at 10^2 dilution

Bioburden Analysis of Tomato

Trial I

Effect of e-beam processing on the microbial counts of cherry tomatoes over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Molds (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	3.59	3.32	3.57	3.23
1	3.97	3.36	3.76	3.34
3	3.45	2.78	3.46	3.04
7	4.29	3.59	3.88	3.36
14	3.61	3.41	3.68	3.36
21	3.71	3.08	3.45	3.23

These values were calculated from the median CFU/g count out of 3 reps

Percentage of control values of e-beam processed tomatoes stored for 21 days at 4 ˚C

Day	Aerobic bacteria	Yeast & Molds
0	53.85	45.95
1	24.73	38.6
3	21.43	37.93
7	20.16	30.67
14	63.41	47.92
21	23.53	60.71

These values were calculated from the median CFU/g count out of 3 reps

Trial II

Effect of e-beam processing on the microbial counts of cherry tomatoes over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Mold (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	BD	BD	BD	BD
1	2.00	2.00	2.00	2.00
3	2.00	2.00	BD	BD
7	BD	2.00	BD	BD
14	BD	BD	BD	BD
21	2.30	BD	2.00	BD

These values were calculated from the median CFU/g count out of 3 reps
BD : Below detection at 10^2 dilution

Bioburden Analysis Avocado Vacuum-Sealed

Trial I

Effect of e-beam processing on the microbial counts of vacuum-sealed avocado over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)*		Yeast & Molds (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	5.47	2.00	4.48	2.30
1	3.30	2.00	3.54	2.00
3	4.90	2.90	4.81	3.28
7	3.66	2.30	3.77	2.48
14	4.05	2.70	3.64	2.00
21	3.51	2.48	3.00	2.60

*Log 10 CFU/g median values taken from 3 samples

Percentage of control values of e-beam processed vacuum sealed avocado trial I stored for 21 days at 4 ˚C

Day	Aerobic	Yeast & Molds
0	0.034	0.667
1	5.000	2.857
3	1.000	2.923
7	4.348	5.085
14	4.464	2.273
21	9.375	40.000

These values were calculated from the median CFU/g count out of 3 reps

Trial II

Effect of e-beam processing on the microbial counts of vacuum-sealed avocado over 21 days of storage at 4 ± 1 ˚C

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Mold (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	5.26	2.85	3.87	2.70
1	3.66	2.00	3.34	2.00
3	4.04	2.00	4.03	2.30
7	4.36	2.30	3.46	BD
14	3.70	2.00	3.30	BD
21	3.89	2.00	3.46	2.00

BD : Below detection at 10^2 dilution

These values were calculated from the median CFU/g count out of 3 reps

Percentage of control values of e-beam processed vacuum sealed avocado trial II stored for 21 days at 4 ˚C

Day	Aerobic bacteria	Yeast & Molds
0	0.39	6.76
1	2.17	4.55
3	0.91	1.89
7	0.88	0.034
14	2.00	0.05
21	1.30	3.45

These values were calculated from the median CFU/g count out of 3 reps

Bioburden Analysis Watermelon

Trial I

Effect of e-beam processing on the microbial counts of watermelon over 21 days of storage at 4 ± 1 ˚C

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Molds (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	BD	2.00	BD	BD
1	2.30	BD	BD	BD
3	BD	2.00	BD	BD
7	2.70	2.00	2.30	BD
14	5.23	6.29	4.49	6.09
21	8.06	3.43	8.07	3.56

These values were calculated from the median CFU/g count out of 3 reps

Trial II

Effect of e-beam processing on the microbial counts of watermelon over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Mold (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	4.02	2.30	3.15	BD
3	3.90	2.00	3.73	BD
7	5.83	3.30	5.72	2.90
14	6.48	4.00	6.46	3.98
21	8.05	3.95	8.12	4.23

These values were calculated from the median CFU/g count out of 3 reps

Percentage of control values of e-beam processed watermelon stored for 21 days

Day	Aerobic bacteria	Yeast & Molds
0	1.92	0.07
3	1.25	0.02
7	0.30	0.15
14	0.32	0.33
21	0.01	0.01

These values were calculated from the median CFU/g count out of 3 reps

Bioburden Analysis Strawberry MAP

Trial I

Effect of e-beam processing on the microbial counts of MAP strawberries over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Mold (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	5.36	4.98	4.78	4.62
1	5.46	5.04	5.16	4.45
3	6.18	4.67	5.42	4.25
7	6.00	5.11	5.39	4.70
14	6.42	5.31	5.33	4.77
21	5.76	5.00	4.97	4.61

These values were calculated from the median CFU/g count out of 3 reps

Percentage of control values of e-beam processed strawberries trial I stored for 21 days in a modified atmosphere (5%O_2, 10% CO_2, and 85% N_2)

Day	Aerobic bacteria	Yeast & Molds
0	41.67	69.33
1	37.93	19.14
3	3.09	6.77
7	12.96	20.49
14	7.93	27.76
21	17.24	44.09

These values were calculated from the median CFU/g count out of 3 reps

Trial II

Effect of e-beam processing on the microbial counts of MAP strawberries over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Mold (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	4.87	3.89	4.79	3.89
1	4.99	4.00	4.92	3.93
3	4.88	3.93	4.81	4.03
7	4.92	4.00	4.69	4.04
14	4.45	3.18	4.38	3.15
21	3.90	3.11	3.81	2.90

These values were calculated from the median CFU/g count out of 3 reps

Percentage of control values of e-beam processed strawberries stored for 21 days in a modified atmosphere (5%O_2 ,15% CO_2, 80% N_2)

Day	Aerobic bacteria	Yeast & Molds
0	10.54	12.62
1	10.10	10.12
3	11.47	16.56
7	12.17	22.24
14	5.33	5.86
21	16.25	12.31

These values were calculated from the median CFU/g count out of 3 reps

Bioburden Analysis of Grapes MAP

Trial I

Effect of e-beam processing on the bioburden load of MAP grapes over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Mold (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	BD	2.00	BD	BD
1	2.48	BD	2.60	BD
3	2.30	2.00	BD	BD
7	2.00	BD	BD	BD
14	2.30	BD	BD	BD
21	BD	BD	BD	BD

BD : Below detection at 10^2 dilution
These values were calculated from the median CFU/g count out of 3 reps

Trial II

Effect of e-beam processing on the microbial counts (median CFU/g) of MAP grapes over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Molds (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	3.00	BD	2.78	BD
1	2.00	BD	BD	BD
3	2.00	BD	BD	BD
7	2.30	BD	BD	BD
14	2.00	BD	BD	BD
21	BD	BD	BD	BD

BD : Below detection at 10^2 dilution
These values were calculated from the median CFU/g count out of 3 rep

Percentage of control values of e-beam processed grapes stored for 21 days in a modified atmosphere (5%O_2 ,3% CO_2, 92% N_2)

Day	Aerobic	Y & M
0	0.10	0.17
1	1.00	100.00
3	1.00	100.00
7	0.50	100.00
14	1.00	100.00
21	100.00	100.00

These values were calculated from the median CFU/g count out of 3 reps

Bioburden Tomato MAP

Trial I

Effect of e-beam processing on the microbial counts of MAP cherry tomatoes over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Molds (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	BD	BD	BD	BD
1	BD	BD	2.00	BD
3	2.30	2.48	BD	BD
7	2.00	BD	BD	BD
14	3.79	2.00	3.41	2.00
21	BD	BD	2.00	BD

These values were calculated from the median CFU/g count out of 3 reps
BD : Below detection at 10^2 dilution

Trial II

Effect of e-beam processing on the microbial counts of MAP cherry tomatoes over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Mold (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	4.48	3.58	4.40	3.72
1	4.76	4.02	4.76	3.98
3	4.47	3.30	4.68	3.11
7	4.44	3.30	4.40	3.08
14	3.65	2.70	3.68	3.04
21	4.97	3.53	5.07	3.40

These values were calculated from the median CFU/g count out of 3 reps

Percentage of control values of e-beam processed cherry tomatoes stored for 21 in a modified atmosphere (5%O_2 ,95% N_2)

Day	Aerobic bacteria	Yeast & Molds
0	12.62	20.59
1	17.93	16.84
3	6.70	2.71
7	7.19	4.73
14	11.11	22.92
21	3.62	2.12

These values were calculated from the median CFU/g count out of 3 reps

Bioburden Avocado MAP

Trial I

Effect of e-beam processing on the microbial counts of MAP avocado over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Molds (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	4.18	2.00	3.89	2.30
1	3.48	2.70	3.23	BD
3	3.48	BD	2.30	BD
7	4.59	BD	4.02	BD
14	4.51	BD	3.76	BD
21	5.45	2.00	3.77	2.00

BD : Below detection at 10^2 dilution
Median values were taken from triplicate reps

Percentage of control values of e-beam processed avocado stored for 21 days in a modified atmosphere (5%O_2, 10 % CO_2, 95% N_2)

Day	Aerobic bacteria	Yeast & Molds
0	0.654	2.564
1	16.67	0.06
3	0.03	0.50
7	0.003	0.010
14	0.003	0.018
21	0.036	1.70

These values were calculated from the median CFU/g count out of 3 reps

Trial II

Effect of e-beam processing on the microbial counts MAP avocado over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Molds (Log 10 CFU/g)	
	Control	E-beam	NI-SD	Irr-SD
0	2.95	2.30	2.90	1.15
1	3.28	BD	3.26	BD
3	3.59	2.30	3.34	2.85
7	3.28	2.00	3.36	2.48
14	4.11	2.00	4.12	2.30
21	4.06	2.00	3.69	2.00

BD : Below detection at 10^2 dilution
These values were calculated from the median CFU/g count out of 3 reps

Percentage of control values of e-beam processed avocado stored for 21 days in a modified atmosphere (5%O_2, 10 % CO_2, 95% N_2)

Day	Aerobic bacteria	Yeast & Molds
0	22.2	1.75
1	0.05	0.06
3	5.13	31.82
7	5.26	13.04
14	0.78	1.53
21	0.86	2.04

These values were calculated from the median CFU/g count out of 3 reps

Bioburden Analysis Watermelon MAP

Trial I

Effect of e-beam processing on the microbial counts of MAP watermelon over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Aerobic count (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	BD	BD	BD	BD
1	2.00	BD	BD	1.52
3	BD	BD	BD	BD
7	2.00	BD	BD	1.52
14	4.58	BD	4.38	BD
21	5.14	6.14	4.86	4.72

BD : Below detection at 10^2 dilution
These values were calculated from the median CFU/g count out of 3 reps

Trial II

Effect of e-beam processing on the microbial counts of MAP watermelon over 21 days of storage

Storage (Days)	Aerobic count (Log 10 CFU/g)		Yeast & Molds (Log 10 CFU/g)	
	Control	E-beam	Control	E-beam
0	3.90	2.95	3.38	BD
3	4.17	2.48	4.17	2.00
7	5.36	2.95	5.46	2.48
14	6.52	2.70	6.68	2.30
21	8.07	4.65	7.91	4.77

BD : Below detection at 10^2 dilution
These values were calculated from the median CFU/g count out of 3 reps

Percentage of control values of e-beam processed watermelon stored for 21 days in a modified atmosphere (5%O_2, 10 % CO_2, 95% N_2)

Day	Aerobic bacteria	Yeast & Molds
0	11.25	0.04
3	2.04	0.68
7	0.39	0.10
14	0.02	0.00
21	0.04	0.07

These values were calculated from the median CFU/g count out of 3 reps

Texture Analysis of e-beam Treated Fruit

Tomato

Texture measurements of tomato stored for 21 days

Treatment	Day 0	Day 3	Day 7	Day 14	Day 21
Firmness (N)					
EB	5.16^{c}	5.03^{c}	4.25^{d}	5.34^{b}	5.03^{b}
EB+MAP	6.04^{b}	5.70^{b}	5.00^{c}	5.38^{b}	4.91^{b}
Air	7.05^{a}	6.77^{a}	6.47^{a}	6.43^{a}	6.42^{a}
MAP	6.95^{a}	6.75^{a}	5.69^{b}	6.22^{a}	6.36^{a}
Distance to rupture (mm)					
EB	5.26^{c}	5.85^{b}	6.00^{ab}	7.93^{a}	8.80^{a}
EB+MAP	6.24^{a}	6.47^{a}	5.63^{b}	6.48^{b}	6.57^{b}
Air	5.69^{bc}	6.37^{a}	6.38^{a}	7.71^{a}	9.10^{a}
MAP	5.90^{ab}	5.39^{c}	6.15^{b}	6.17^{b}	6.64^{b}
Work (N.mm)					
EB	11.89^{b}	12.12^{c}	10.90^{c}	16.45^{b}	13.94^{b}
EB+MAP	15.89^{a}	15.12^{b}	12.33^{c}	14.05^{c}	13.42^{b}
Air	17.41^{a}	17.23^{a}	17.36^{a}	18.56^{a}	20.22^{a}
MAP	17.60^{a}	16.51^{ab}	15.52^{b}	16.25^{b}	17.92^{a}

Each value is the mean of 10 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
Means in same column not followed by no letter = no significance $P>0.05$

Avocado

Texture measurements of avocado flesh stored for 21 days

Treatment	Day 0	Day 3	Day 7	Day 14	Day 21
Distance to rupture (mm)					
EB	3.64[a]	4.50[a]	2.95[a]	3.79[a]	3.86[a]
EB+MAP	3.68[a]	3.15[a]	4.19[a]	2.63[ab]	3.64[a]
Air	2.93[a]	2.88[a]	2.89[a]	2.94[ab]	3.27[a]
MAP	4.20[a]	3.91[a]	2.89[a]	2.01[b]	2.81[a]
Work (N.mm)					
EB	4.79[a]	3.28[a]	1.55[b]	0.33[a]	2.11[ab]
EB+MAP	5.55[a]	2.48[a]	3.99[a]	0.13[b]	2.68[ab]
Air	3.20[a]	2.59[a]	2.15[b]	0.19[ab]	1.34[b]
MAP	5.12[a]	4.62[a]	2.56[ab]	0.15[b]	3.03[a]

Each value is the mean of 10 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
Means in same column not followed by no letter = no significance $P>0.05$

Grapes

Texture measurements of grapes stored for 21 days

Treatment	Day 0	Day 3	Day 7	Day 14	Day 21
Firmness (N)					
EB	1.64^{c}	2.08^{b}	2.00^{b}	1.33^{b}	1.74^{b}
EB+MAP	1.79^{c}	1.67^{c}	1.92^{b}	1.18^{b}	1.34^{c}
Air	2.57^{b}	2.68^{a}	2.78^{a}	1.78^{a}	1.92^{b}
MAP	3.31^{a}	2.60^{a}	1.99^{b}	1.62^{a}	2.58^{a}
Distance to rupture (mm)					
EB	2.16^{c}	2.93^{b}	2.88^{b}	2.09^{b}	2.34^{b}
EB+MAP	2.83^{b}	2.22^{c}	2.48^{b}	1.60^{c}	1.66^{c}
Air	3.74^{a}	4.39^{a}	4.02^{a}	2.82^{a}	3.91^{a}
MAP	3.88^{a}	2.74^{bc}	2.89^{b}	2.33^{b}	2.26^{b}
Work (N.mm)					
EB	2.05^{c}	3.09^{b}	3.00^{b}	1.64^{bc}	2.33^{b}
EB+MAP	2.81^{c}	1.89^{c}	2.40^{b}	1.13^{c}	1.33^{c}
Air	5.07^{b}	5.86^{a}	5.49^{a}	2.72^{a}	3.86^{a}
MAP	6.48^{a}	3.85^{b}	3.21^{b}	2.11^{b}	3.17^{ab}

Each value is the mean of 10 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
Means in same column not followed by no letter = no significance $P>0.05$

Watermelon

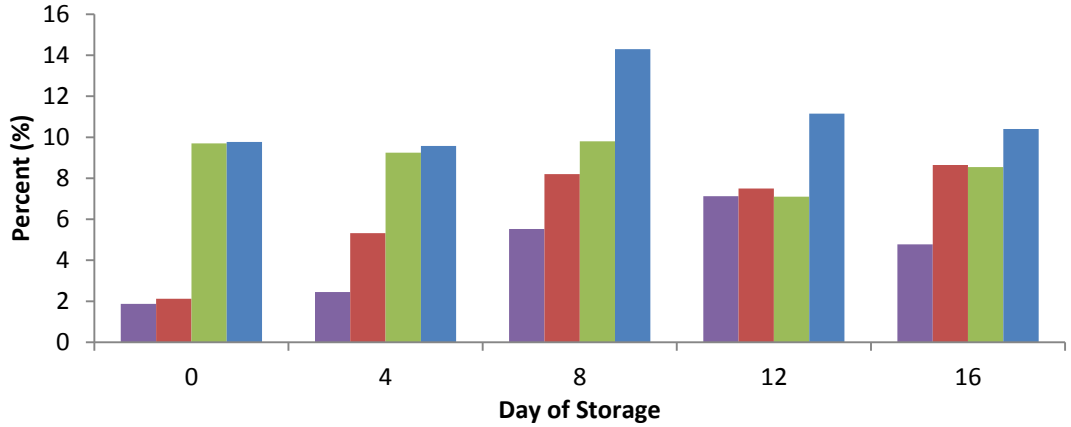
Texture measurements of fresh-cut watermelon stored for 21 days

Treatment	Day 0	Day 3	Day 7	Day 14	Day 21
Work(N.mm)					
EB	8.52[a]	11.77[ab]	11.94[a]	1.70[a]	8.27[ab]
EB+MAP	8.93[a]	12.29[a]	9.59[a]	1.14[ab]	7.28[ab]
Air	7.11[a]	7.59[b]	10.37[a]	0.84[b]	5.58[b]
MAP	9.28[a]	9.50[ab]	7.78[a]	084[b]	9.76[a]
Distance to rupture (mm)					
EB	3.75[a]	5.01[a]	4.24[a]	4.66[a]	4.22[ab]
EB+MAP	4.26[a]	5.16[a]	4.31[a]	4.27[a]	3.61[b]
Air	4.24[a]	3.83[a]	4.67[a]	3.88[a]	3.45[b]
MAP	3.74[a]	3.83[a]	3.46[a]	3.88[a]	4.63[a]

Each value is the mean of 10 samples.
Means in column with different superscript (a,b) represent significant difference (P<0.05)
Means in same column followed by the same letter are not significantly different at P<0.05
Means in same column not followed by no letter = no significance P>05

Headspace Gasses of e-beam Treated Fruit

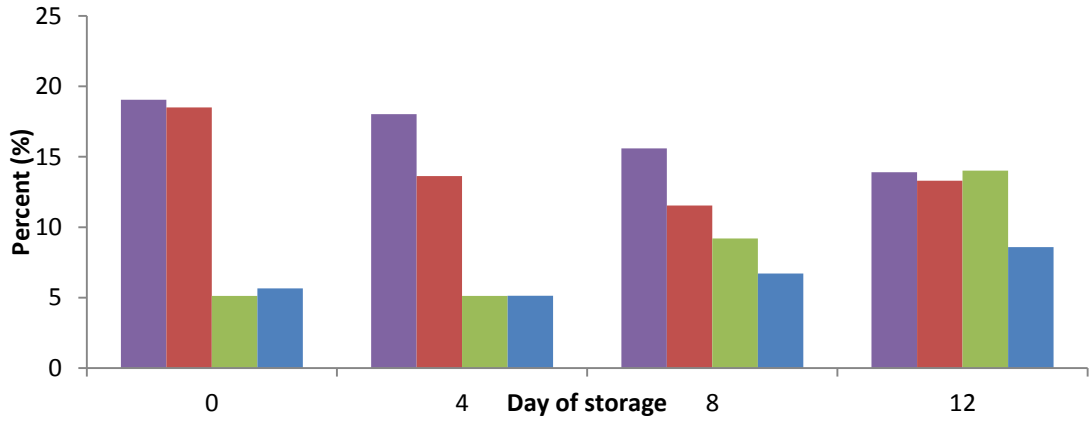
CO_2 headspace gas of fresh cut watermelon



Effect of e-beam processing and modified atmosphere packaging (MAP) on CO_2 headspace gas in watermelon stored for 16 days at refrigerated temperatures.

Air EB MAP EB+MAP

O_2 headspace gas of fresh cut watermelon



Effect of e-beam processing and modified atmosphere packaging (MAP) on oxygen headspace gas in watermelon stored for 16 days at refrigerated temperatures.

Air EB MAP EB MAP

Effect of e-beam and MAP on Fruit Color

Tomato

Effect of e-beam irradiation and modified atmosphere packaging (MAP) on yellowness (positive b-values) of tomato samples stored for 21 days

Day	Air	EB	MAP	EB+MAP
0	21.97^{b}	25.91^{a}	27.13^{a}	22.46^{b}
3	22.28^{ab}	23.69^{a}	19.36^{c}	20.09^{bc}
7	22.31^{a}	21.82^{b}	23.55^{a}	23.67^{a}
14	22.30^{a}	21.60^{b}	23.47^{a}	22.79^{a}
21	24.12^{ab}	21.62^{b}	23.96^{ab}	25.25^{a}

Each value is the mean of 4 samples.
Means in column with different superscript (a,b) represent significant difference ($P<0.05$)
Means in same column followed by the same letter are not significantly different at $P<0.05$
Means in same column not followed by no letter = no significance $P>0.05$

Grape

Effect of e-beam irradiation and modified atmosphere packaging (MAP) on color of grape stored for 21 days

Treatment	Day 0		Day 3		Day 7		Day 14		Day 21	
	L	b	L	b	L	b	L	b	L	b
MAP	25.83	3.33	24.40	3.63	23.53	3.73	24.39	3.91	24.44	4.49
Air	25.91	1.41	25.20	2.25	26.17	3.30	25.33	1.72	23.78	0.79
EB+ MAP	27.49	3.13	25.11	4.89	23.35	3.06	25.90	2.71	27.22	5.64
EB	31.06	6.05	26.69	4.17	26.85	2.95	24.39	1.76	24.18	1.77

Each value is the mean of 4 samples

Sensory Evaluation of Fresh Fruit

Date: _____

Type of sample:_____ Sample # _____

Please evaluate each sample and circle the number for each quality attribute that best describes your feeling.

1. Circle the rating of your **OVERALL LIKE** or **DISLIKE** of the appearance of the sample.

1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither like nor dislike				Like Extremely

2. Circle the rating of your **LIKE** or **DISLIKE** of the **ODOR** of the sample.

1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither like nor dislike				Like Extremely

3. Circle the rating of your **LIKE** or **DISLIKE** of the **COLOR** of the sample.

1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither like nor dislike				Like Extremely

4. Circle the rating of your **LIKE** or **DISLIKE** of the **FIRMNESS** of the sample.

1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither like nor dislike				Like Extremely

5. Circle the rating of your **LIKE** or **DISLIKE** of the **FLAVOR** the sample.

1	2	3	4	5	6	7	8	9
Dislike Extremely				Neither like nor dislike				Like Extremely

Comments: _____

_____