ATTACHMENT OF *SALMONELLA* ON CANTALOUPE AND EFFECT OF ELECTRON BEAM IRRADIATION ON QUALITY AND SAFETY OF SLICED CANTALOUPE

A Dissertation

by

MANGESH PRAFULL PALEKAR

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

DOCTOR OF PHILOSOPHY

December 2004

Major Subject: Food Science and Technology

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ABSTRACT

Attachment of *Salmonella* on Cantaloupe and Effect of Electron Beam Irradiation on Quality and Safety of Sliced Cantaloupe. (December 2004)

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Increase in consumption of fresh produce over the past decade has resulted in a rise in incidents of foodborne outbreaks due to pathogens. Chemical sanitizers have been extensively used in the industry for decontamination of fresh produce. However, they are ineffective in certain commodities and under certain processing conditions, necessitating the evaluation of alternative technologies. Electron beam irradiated sliced cantaloupe were tested for 21 days of storage for total aerobic bacterial counts, texture, color and sensory parameters as a function of irradiation doses 0, 0.7 and 1.4 kGy and the wash treatments, water and 200 mg/L chlorine applied to the melons before cutting. Melons washed only with water prior to cutting had total aerobic bacterial counts of 4.0, 2.0 and 0.8 log cfu/g on day 0 at irradiation doses of 0, 0.7 and 1.4 kGy respectively. On day 0, melons washed with chlorine prior to cutting had total aerobic bacterial counts of 2.7, and 0.7 log cfu/g at irradiation doses of 0 and 0.7 kGy and below detection limit at 1.4 kGy. Texture measured as compression force was lower only for cantaloupe irradiated at 1.4 kGy. Irradiation did not affect objective color and descriptive attribute flavor and texture sensory attributes of cantaloupe. Irradiation reduced *Salmonella* Poona by 1.1 log

cfu/g at 0.7 kGy and 3.6 log cfu/g at 1.5 kGy. The D-value of *S.* Poona on irradiated sliced cantaloupe was found to be 0.211 kGy. Among the spoilage organisms, lactic acid bacteria and mold were reduced effectively by irradiation but there was no significant effect on reduction of yeasts. Our results show that electron beam irradiation in combination with chemical sanitizers is effective in decontamination of fresh-cut produce. Electron microscopy images provided valuable information on attachment sites of *S.* Poona on cantaloupe rind. The ineffectiveness of chemical sanitizers due to possible inaccessibility to pathogens in these attachment sites provides the basis for application of irradiation in decontamination of fresh produce.

DEDICATION

To my father, Prafull, mother, Usha and my sister, Nandita, for their sacrifice, support and prayers that helped me realize this dream.

To the memory of my grandparents who constantly motivated me to succeed in life.

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INTRODUCTION

Consumption of fresh fruits and vegetables has increased significantly over the past decade due to awareness of their health benefits among consumers. Rise in demand of year-round supply of fresh produce resulted in a boost to the produce processing sector. Increase in the market for fresh-cut minimally processed fruits and vegetables led to heightened pressure on the industry to deliver the safest possible product with extended shelf-life.

With the rise in consumption of fruits and vegetables rose incidents of foodborne disease due to pathogens associated with produce. Chemical decontamination is currently the approach most commonly employed by the industry for reduction of pathogens and spoilage organisms on fresh produce. However, research has exposed serious limitations in the efficacy of chemical sanitizers for pathogen reduction in fresh produce. This has spurred attention of researchers towards evaluating alternate intervention technologies that may be novel or being applied on other commodities like meats. Irradiation is one such technology that has been researched over the past century and been applied to foodstuffs for decades.

Gamma rays have been the most extensively studied form of irradiation and have been successfully applied to spices, tubers, grains and foods for the space program. However, consumer reluctance has limited its application over a broad range of

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This dissertation follows the style of the Journal of Food Science.

foodstuffs. As a result, alternate irradiation technologies such as electron beams and X-rays are attracting attention as possible decontamination tools. The potential for application of these novel irradiation technologies has not been studied extensively.

In this study, effectiveness of electron beam irradiation in pathogen reduction and extension of shelf-life of cantaloupe was evaluated. Cantaloupe has attracted attention due to its implication in several outbreaks as a carrier of foodborne pathogens. We evaluated the effectiveness of electron beam irradiation in combination with chlorine wash of pre-cut cantaloupes, in reduction of *Salmonella*, and extension of shelf-life of sliced cantaloupe. Effect of irradiation on sensory characteristics like color, texture, and flavor of cantaloupe was studied. We also propose a D-value for reduction of *Salmonella* on sliced cantaloupe using electron beam irradiation. Electron microscopy images of cantaloupe rind were taken to identify attachment sites of bacteria onto sites possibly inaccessible to chemical decontaminants and provide the basis for application of this technology for irradiation of whole cantaloupe.

REVIEW OF LITERATURE

Contamination of cantaloupe

Consumption of cantaloupe in the United States

The importance of fresh fruits and vegetables as a rich source of nutrients and their role in promoting good health has resulted in a rise in their demand over the past decade. The per capita consumption of fresh fruits and vegetables increased 6 percent between 1987 and 1995 and 8 percent between 1995 and 2000 (Kaufman and others 2003). Cantaloupe (*Cucumis melo* L var. *reticulatus*) is a popular fruit with an ever-increasing demand. It is a member of the Cucurbitaceae family which also includes honeydew, watermelons, cucumbers, pumpkins and squashes (Parnell and others 2003). Cantaloupes purchased in United States (US) are primarily grown in the US, Central America and Mexico. Cantaloupe consumption has grown significantly over the past decade (ERS, USDA 2003). The per capita consumption of cantaloupe has risen 27 percent between 1990-92 and 2000-02. One of the main reasons for this rise is increased health consciousness among consumers and year-round availability resulting from doubling of imports since 1990-92. Along with increase in consumption of whole fruits, demand for variety and convenience led to a huge potential for the fresh-cut produce industry. Branded packaged salads and fresh-cut fruit industry grew from a 1 percent share of total produce sales to a 15 percent share between 1987 and 1997 (Kaufman and others 2003). The increased market for these products has led to significant challenges for the fresh produce industry to improve quality, shelf-life and safety of their products.

Outbreaks of foodborne disease associated with cantaloupe

Increased consumption of fresh produce has paralleled a corresponding rise in the incidence of foodborne disease outbreaks. Many different bacteria, viruses, and protozoa have been linked epidemiologically to fresh produce-associated diseases. Most outbreaks with identified etiology were of bacterial origin; *Salmonella* spp. was most commonly reported (NACMCF 1999). A variety of commodities such as cantaloupe, mangoes, tomatoes, oranges, berries and parsley have been implicated in foodborne illnesses. In the case of cantaloupe, *Salmonella* has been found to be the pathogen most frequently associated with the commodity, leading to several outbreaks across the US and Canada (Ries and others 1990; Beuchat 1996; Mohle-Boetani and others 1999). In 1993 there was an outbreak (9 cases) of *Escherichia coli* O157:H7 related to consumption of cantaloupe in a restaurant in Oregon (Del-Rosario and Beuchat 1995).

In the United States, a large multi-state outbreak (245 cases) of *Salmonella enterica* serotype Chester was traced to the consumption of cantaloupes in 1990 (Ries and others 1990). In another outbreak, more than 400 cases were reported from 23 states in the US and two provinces of Canada. The causative agent of the illness in this outbreak was *Salmonella enterica* serotype Poona linked to the consumption of contaminated cantaloupes produced in Texas (CDC 1991). Three multistate outbreaks of *S.* Poona infections associated with eating cantaloupe imported from Mexico occurred in the spring of 2000, 2001 and 2002 (CDC 2002). As a result, in October 2002, the US Food and Drug Administration (FDA) banned import of cantaloupes grown in Mexico

citing unsanitary conditions and the outbreaks as prime reasons (FDA 2002). However, a study conducted by Castillo and others (2004) found no difference in frequency of *Salmonella* on cantaloupes grown in Texas or Mexico.

Sources of pathogens in cantaloupe

The microflora of fresh fruits and vegetables is diverse, but is predominantly Gram-negative bacteria with varying levels of bacteria on plant surfaces in the field. There are several factors that can potentially contribute toward contamination of fresh produce. They can be broadly classified into preharvest and postharvest factors. Preharvest factors include the type and quality of manure used as fertilizer, fecal contamination from feral animals and farm workers, the use of contaminated irrigation water (Castillo and others 2004), the presence of feral or domestic animals, and human handling (NACMCF 1999). Many consumers prefer natural and organically cultivated produce, which could result in the increased use of manure rather than chemical fertilizers in food production. Improperly treated manure may contain enteric pathogens, such as *Salmonella* spp. and *E. coli* O157:H7 and may subsequently contaminate produce (NACMCF 1999). Cantaloupes grow at soil level which makes it susceptible to contamination from soil, water, and animals. Irrigation techniques that can subject the plant to direct contact with contaminated water can also increase the risk of contamination with pathogens. Spray irrigation would be expected to increase contamination in comparison to either drip irrigation or flooding. Irrigation water can become polluted either through the direct introduction of sewage, or through non-point

pollution sources such as ground water runoff (NACMCF 1999). Spray irrigation is most commonly applied in cantaloupe fields; however, drip irrigation is now getting increased attention among growers (University of Georgia 1999). Wild and domestic animals, including mammals, birds, reptiles, and insects, are other sources of pathogenic bacteria in agricultural environments (NACMCF 1999). Three outbreaks attributed to *S.* Poona-contaminated cantaloupe were traced to farms in Mexico and were reported to possibly originate from reptiles, such as iguanas, that fed on melon crops in the field and packing sheds. Subsequent contamination of process equipment and water used in the washing and cooling process might have spread the contamination (CDC 2002). Pollinating insects can serve as a means of transmitting enteric bacteria to flowers. Once contaminated, developing fruit may internalize the bacteria resulting in their inaccessibility to surface decontamination procedures (NACMCF 1999).

Postharvest factors include the use of contaminated wash water or ice, human handling, presence of animals or birds in the packing shed, the use of contaminated equipment or transportation vehicles, non-optimal processing, cross-contamination, and improper storage, packaging, and display temperatures (NACMCF 1999, Castillo and others 2004). Castillo and others (2004) isolated *Salmonella* from walls and floors of cooling rooms at a packing plant in Texas, indicating the significance of sanitation within packaging sheds. It is critical to wash whole produce prior to processing, utilize optimum sanitation and store at appropriate storage temperature to improve quality and prolong product life. Intact fruit have a protective covering that maintains the structure of the fruit, minimizes water loss, and prevents the inner tissue from infection or

contamination. However improper handling can result in injury to the surface, resulting in scars or cuts which serve as access points for possible internalization and proliferation of pathogens. Passing a knife through a contaminated surface may inoculate the newly exposed surfaces of cut produce (Lin and Wei, 1997). Shredding and slicing equipment are often hard to clean and identified as common points of accumulation of pathogens in processing environments.

The convenience of consuming packaged fresh-cut fruits resulted in these products becoming a rapidly growing segment of the retail and foodservice industry. Fresh-cut processing removes the fruit's natural cuticle and exposure of the tissue leads to increased respiration, ethylene production, greater susceptibility to water loss, microbial invasion, and proliferation with subsequent decay. All these lead to decreased shelf-life, off-color, tissue softening, and decay (Gorny and Beaulieu 2004). One of the issues of concern is the contamination of tissue with bacteria from the surface during cutting. Transmission of pathogens to the interior of the cantaloupe is possible while cutting unwashed melons (Beuchat 1996).

Cantaloupe as a medium for harboring organisms

The surface of a cantaloupe consists of an inedible rind made up of two different surface patterns. It consists of rough, raised netting over a yellow-skinned, relatively smooth background skin. Irregularities such as roughness, crevices and pits have been shown to result in bacterial adherence by increasing cell attachment and reducing the ability to remove cells (Austin and Bergeron 1995). According to Ukuku and others

(2002), surface structure and biochemical characteristics of bacteria and of substratum play a major role in how and where bacteria may attach. According to their discussion, bacterial attachment to surfaces is influenced not only by cell surface charge and hydrophobicity but also by the presence of bacterial surface appendages such as flagella and fimbriae, as well as extracellular polysaccharides. Plant surfaces and microbes both have negative surface potential, which results in electrostatic repulsion between the two surfaces. Surface appendages on the bacteria bridge the gap exerted by the electrostatic repulsion. The rind of a cantaloupe presents a variety of surfaces to which a bacterium may bind. The epidermal surface has a meshwork of raised tissue (net) that consist of lenticels and phellum (cork) cells which have hydrophobic suberized walls to reduce water loss and protect against pathogen ingress. These authors state that the hydrophobic nature to the outer surface of the cantaloupe is also imparted by the cuticle, composed of wax and cutin, that covers the epidermal cells and that hydrophilic components of plant cell walls and middle lamella may also be exposed because of cuticular cracks and injuries to the epidermal surface. The intact surface of a cantaloupe is hydrophobic in nature and *Salmonella,* with its highly hydrophobic surface, can bind to it well, as reported by Ukuku and others (2002). *Salmonella* also produces extracellular carbohydrate polymer cellulose and curli (aggregative fimbriae) as the two principle components of the extracellular matrix. These are thought to be responsible for biofilm formation and their strong attachment to the cantaloupe rind. However, both these components are repressed above 26-28 °C. Under natural conditions, when contamination occurs in the field the production of these two components may allow the

bacterium to bind to the melon surface and be highly resistant to removal by rain or by washing during processing (Ukuku and others 2002). The rind encompasses the orange colored edible tissue, which is rich in sugars, high water content and a pH close to neutrality (6.1 to 6.6). These properties make the edible tissue an ideal substrate for the proliferation of bacteria that may be introduced through the rind at low numbers during cutting or through cracks and crevices in the rind. This results in the need for efficient decontamination strategies for fruits, followed by optimum storage conditions for ensuring safety and maximizing shelf life. Most common strategies employed would include antimicrobial rinses (Ayhan and others 1998; Luna-Guzman and Barrett 2000; Sapers and others 2001; Barak and others 2003) and effective preservation technology to retard the natural changes of minimally processed fruits and vegetables during storage (Bai and others 2000; Lamikanra and others 2000).

Decontamination strategies for fresh produce

The FDA Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables defines "sanitize" as "to treat clean produce by a process that is effective in destroying or substantially *reducing the numbers of microorganisms* of public health concern, as well as other undesirable microorganisms, *without adversely affecting the quality* of the product or its safety for the consumer."(FDA 1998). Thus, effective sanitization requires a delicate balance between quality and microbiological safety of produce.

Decontamination strategies for fresh produce can be broadly classified in

chemical and physical treatments. The efficacy of either of the treatments used for pathogen reduction will be dependent on the following factors: Type of treatment chosen for the commodity of interest, characteristics of produce surfaces such as cracks, crevices, texture and hydrophobic tendency, the type and physiology of target microorganisms, exposure time and concentration of the sanitizer, pH and temperature of the produce as well as sanitizing agent (FDA 2001). Initial quality and subsequent handling of fresh fruits and vegetables strongly influence their microbiological safety (NACMCF 1999). Storage of produce at refrigerated temperatures is not reliable enough to contain pathogens. Farber and others (1998) demonstrated the ability of *Listeria monocytogenes,* a psychrotrophic pathogen, to grow on whole as well as cut produce stored at refrigerated temperatures. Proper sanitation at all levels in the fresh produce chain, from farm-to-table, is crucial.

Chemical methods for decontamination

Chlorine

Chlorine is the most widely used chemical decontaminant by the produce industry for sanitizing produce as well as surfaces in the processing facility. The most common forms of free chlorine include liquid chlorine and hypochlorites used in the range of 50-200 mg/L concentration with a product or surface contact time of 1-2 min (FDA 2001). In 1992, the produce industry implemented a voluntary Melon Quality Program which includes recommendations that hyperchlorinated water be used in all processing steps and only ice made from chlorinated water be used for transport

(NACMCF 1999). Free available chlorine in the form of hypochlorous acid causes bactericidal activity against a broad range of bacteria. However, factors such as temperature, organic matter, light, air and presence of metal compounds affect its concentration and hence effectiveness as an antimicrobial agent (FDA 2001). Fresh-cut cantaloupe cubes treated with 2000 mg/L chlorine resulted in less than 90% reduction of several strains of salmonellae (Beuchat and Ryu 1997). Apples, tomatoes and lettuce dipped in 2000 mg/L chlorine showed a maximum reduction of 2.3 log cfu/cm^2. (Beuchat 1998).

Other forms of chlorine like chlorine dioxide and acidified sodium chlorite have been explored as possible decontaminants for leafy produce, green pepper and oranges (Zhang and Farber 1996; Pao and Davis 1999; Han and others 2000). Park and Beuchat (1999) showed that acidified sodium chlorite was effective in reducing *E. coli* O157:H7 and salmonellae inoculated onto cantaloupes, honeydew and asparagus.

Though chlorine is commonly used to increase the efficacy of decontamination, it does not completely assure pathogen elimination (Wei and others 1995; Zhuang and others 1995; Beuchat 1996; Park and Beuchat 1999). Studies have shown that chlorinated water will reduce but not eliminate *Salmonella* once it is on the rind (Golden and others 1993). Crevices, cracks and fissures on the surface of produce, along with the waxy cuticle may prevent the chlorine from reaching microorganisms lodged in them (Seo and Frank 1999, Takeuchi and Frank 2000). Chlorine-washing systems may produce toxic by-products like trihalomethanes and chloramines and hence the need for alternative sanitizers (Dychdala 1991). Therefore, chlorine is only a risk reduction

factor, and other preventive strategies may be needed as a substitute or in combination with chlorine to make the decontamination process more effective.

Organic acids

The antimicrobial activity of organic acids is a function of reduction of pH in the microbial environment leading to disruption of membrane transport and permeability, accumulation of anions and lowering of intracellular pH within the cell by dissociation of hydrogen ions from the acid (FDA 2001). The most commonly studied organic acids are lactic acid, citric acid, acetic acid, tartaric acid, *p*-aminobenzoic acid (PABA) and orthophosphoric acid (Richards and others 1995; FDA 2001).

Lactic acid has been used extensively as sanitizer on food animal carcasses (Castillo and others 2002) and is a potential sanitizer for produce. It is generally recognized as safe (GRAS) for use in food products and has been found to be an effective antimicrobial sanitizer at elevated temperatures alone, or in combination with other chemicals (Sorrells and others 1989; Materon 2003). Lactic acid has been found to be more effective than chlorinated water in reducing pathogens on the surface of cantaloupes. Materon (2003) found that lactic acid at 35 °C drastically reduced *E. coli* O157:H7, by more than 7 log cfu/cm^2 on the surface of cantaloupes. Also, cantaloupe decontamination with (1.5%) lactic acid was found to be more effective than decontamination with chlorine or deionized water. A study conducted by Ibarra-Sanchez and others (2004) on the effect of chemical sanitizers on pathogen reduction in tomatoes showed that 2 % lactic acid spray at 55 °C reduced surface as well as internalized *S.*

Typhimurium and *E. coli* O157:H7. Citric acid was capable of reducing populations of *S.* Typhi on papaya cubes (Fernandez Escartin and others 1989) and *Campylobacter jejuni* on cubes of watermelon and papaya (Castillo and Escartin 1994). PABA was found to be a more potent antimicrobial compared to lactic, acetic, propionic, citric and formic acids against *L. monocytogenes, S.* Enteritidis, and *E. coli* (Richards and others 1995).

Numerous studies have concluded that total removal of pathogens was not possible using the existing chemical treatments. Washing will only remove a portion of the bacteria from the surface of produce but cannot assure complete removal of pathogens (Garg and others 1990; Beuchat 1996; Materon 2003).

Other treatments

Alkaline compounds have been studied for their potential as sanitizers on product as well as non-food surfaces (FDA 2001). Somers and others (1994) reported a 5- and 6-log reduction in *E. coli* O157:H7 at 10 °C and room temperature respectively when non-food surfaces were treated with 1% trisodium phosphate (TSP). Beuchat (1996) reported a 5.2 log cfu/cm^2 reduction in *S.* Montevideo when tomatoes were treated with 15% TSP for 15 s. Other high pH cleaning compounds like sodium hydroxide, potassium hydroxide, sodium bicarbonate and sodium orthophenylphenate led to significant reductions of *E. coli* on orange surfaces (Pao and Davis 2000). Concerns of environmental discharge of phosphates, coupled with the high pH (11-12) of alkaline compounds limits their application as sanitizers on produce (FDA 2001). Peracetic acid

at 40-80 mg/L is as effective decontaminant against salmonellae and *E. coli* O157:H7 on cantaloupe and honeydew melon surfaces (Park and Beuchat 1999). Hydrogen peroxide is a strong oxidant and generates hydroxyl radicals that can be cytotoxic and hence bactericidal (FDA 2001). Though it is effective on food contact surfaces in the industry, its effect on whole melons is not greater than hypochlorite or acidified sodium chlorite as was reported by Park and Beuchat (1999) from their study on whole cantaloupe, honeydew and asparagus treated with 1 % hydrogen peroxide. 5 % hydrogen peroxide caused bleaching of cantaloupe cubes.

Ozone has been used as an antimicrobial agent against *S.* Typhimurium, *Staphylococcus aureus*, *Yersinia enterocolitica* and *L. monocytogenes* for treatment of water (Peeters and others 1989; Korich and others 1990; Restaino and others 1995). Beuchat (1998) found reduction in microbial levels of produce such as apples, grapes, oranges, raspberries, strawberries and pears on treatment with ozonated water. Ozone also retarded ripening by oxidizing ethylene. However, its application is limited by its strong oxidizing activity which may cause physiological injury to produce and corrosion of metals in the processing equipment. Ukuku and others (2004) studied the effect of hot water and hydrogen peroxide on reduction of salmonellae on whole cantaloupe. They reported that water at 97 °C and 5 % hydrogen peroxide was effective in reducing *Salmonella* from the cantaloupe surface. Surface pasteurization of whole cantaloupe (76 °C for 2 to 3 min) resulted in a 5 log cfu/cm^2 reduction of *Salmonella* from the cantaloupe surface (Annous and others 2004).

Internalization of pathogens due to wash treatments

Immersion of warm fruit in cool wash solutions can induce infiltration of bacteria along with the solution into the fruit through openings on the surface or specific regions like the stem-end (Bartz and Showalter 1981; Zhuang and others 1995; Buchanan and others 1999 a; Burnett and others 2000). Some of the surface structures like stomata, lenticels and crevices or minute cracks occurring due to mishandling, insect wounds or hail damage can be access points for bacterial internalization. Iturriaga and others (2003) observed that deposition of *S.* Montevideo on the surface of tomatoes and tomatillos could result in attachment and subsequent colonization under suitable temperature and relative humidity of storage. These colonized bacteria could be introduced into the inner regions of the tomato during cutting. Bartz and others (1981) demonstrated that warm tomatoes immersed in a bacterial suspension at 20-22 °C caused penetration of cells in the stem tissue. Zhuang and others (1995) showed that a negative temperature differential of 15 °C caused the infiltration of *S.* Montevideo into the core of tomatoes. *E. coli* O157:H7 could penetrate into the core of warm apples immersed in a cool suspension of cells (Buchanan and others 1999 b) and through floral tubes and damaged tissue around puncture wounds of apples (Burnett and others 2000). Just as wash water has the potential of introducing pathogens into the fruit, an effective concentration of sanitizer should ideally reach the internalized pathogens and eliminate them. The challenge however, lies in selecting a sanitizer that would retain its antimicrobial activity just as effectively within the fruit tissue as it would on its surface. In a study conducted on tomatoes, water or chlorine wash alone did not significantly reduce the number of

internalized *S.* Typhimurium and *E. coli* O157:H7 but lactic acid spray reduced both pathogens to undetectable levels (Ibarra-Sanchez and others 2004). Research is currently being carried out to determine the effectiveness of sanitizers in elimination of internalized pathogens within whole fruit. Burnett and others (2004) studied the effect of water, chlorine and FIT Professional Line Antibacterial Cleaner on reduction of *L. monocytogenes* on lettuce leaves. They reported that though there was no significant difference between reductions caused by the two sanitizers, it was significantly different from reductions due to water wash.

Irradiation as an intervention strategy

History

Research on food irradiation dates back to the early 1900's when the first US and British patents for the use of ionizing radiation for killing bacteria were issued in 1905 (CCR, UC-Davis 2000). The technology gained significant momentum in the early 1950s as a result of the "Atoms for Peace" program established by President Eisenhower after which extensive research was carried out for irradiating meat and other foods as a potential for preserving food for the military (Olson and others 1994). In 1958, the FDA gained authority over the food irradiation process, thereby spurring its toxicological and microbiological evaluation, as well as testing for wholesomeness. The FDA has approved food irradiation processes for a variety of commodities including wheat, potatoes, pork, spices, poultry, fruits, vegetables and red meat (CCR, UC-Davis 2000). After long-term multigeneration studies on dogs, rats, and mice fed with irradiation-

sterilized meat, the joint Food and Agricultural Organization (FAO), International Atomic Energy Agency (IAEA) and World Health Organization (WHO) Committee on wholesomeness of irradiated food concluded in 1980 that irradiated foods are safe and wholesome at levels up to 10 kGy (Olson and others 1994). Irradiated foods have been a part of the National Aeronautics and Space Administration (NASA) Space Program for a long time now with astronauts consuming them since the Apollo program. In 1999, the World Health Organization determined that the limiting factor for irradiation at very high dose is palatability and not safety, and that irradiation should be considered parallel to cooking in all aspects of safety (CCR, UC-Davis 2000).

Ionizing radiation and its types

Ionizing radiation produces electrically charged particles or ions by removal of electrons from an atom. It has a higher energy than non-ionizing radiation like microwaves, radiowaves and light. There are three types of ionizing radiation that have been used for irradiating foods; gamma rays, x-rays and electron beams. Gamma rays are produced by radioactive isotopes such as cobalt-60 and cesium-137 and contain energy of about 1 to 2 MeV. Cobalt 60 is the most widely used radioisotope source in radiation facilities for use in radiotherapy, sterilization of medical products, and the irradiation of foods (Olson and others 1994). Electron beams are produced by linear accelerators which are powered by electricity and can generate and accelerate electrons to 99 % of the speed of light. When these high energy electrons penetrate a thin foil of metal such as tungsten, x-rays are produced. In terms of penetrating power, x-rays (up to 38 cm) are

slightly more penetrating than gamma rays. Both, gamma and x-rays have no mass and hence have higher penetrating power whereas electrons have a small mass and hence get slowed down as they enter the target matrix. Electron beams have a higher energy level of 10 MeV and higher but low penetration potential (3.5 inches, dual beam) and hence are best used for surface and subsurface irradiation of foods. The advantage of a linear accelerator over using radioisotope is the speed with which product can run over a conveyor through the beam as opposed to the slower process of exposure of the product to gamma rays that originate from a source has to be stored under water. Doses greater than 10 kGy essentially sterilize foods; medium doses between 1-10 kGy exert pasteurization effect leading to extension of shelf life; low doses less than 1 kGy are effective in controlling parasites, delaying senescence in fresh produce and in insect and pest control in grains and fruits (Olson and others 1994). The dose to be delivered to a product is controlled by the amount of time the food gets exposed to irradiation in case of gamma or the conveyor speed which moves the product under the beam of electrons or x-rays (Olson 1995). The longer the product gets exposed to gamma rays, higher will be the absorbed dose and slower the product is moved under the beam, higher the absorbed dose.

Dosimetry

In order to be able to implement irradiation as a decontamination tool, there is a need for a reliable and accurate method of measuring the dose absorbed by the irradiated product. According to Olson (1995), irradiation of foods causes release of free radicals

that are unstable and react with other chemical compounds in the food system to form stable radiolytic compounds. Such radiolytic compounds are also formed during other forms of food processing such as cooking and cannot be distinguished from those generated due to irradiation. As a result, absorbed radiation in a food sample cannot be measured by chemical methods. Dosimetry is the science of measuring the absorbed dose in an irradiated product using dosimeters made up of radiation sensitive material like alanine or radiochromic films. The radiochromic films change color when irradiated and can be quantified using a spectrophotometer. The amino acid alanine, when exposed to irradiation forms free radicals that can be measured and correlated to the absorbed dose using electron spin resonance spectrometry (Olson 1995). In order to validate an irradiation process, multiple dosimeters are placed at different geometric locations in the product to determine the maximum and minimum absorbed dose in the product. This generates a dose-distribution map for the product to be irradiated. Though the existing resources available for dosimetry enable a fairly accurate dose measurement, there is an urgent need for reliable and precise dosimeters that can measure extremely low doses between 10 to 1000 Gy.

Mode of action of ionizing radiation

Irrespective of the source, all forms of ionizing radiation exert their destructive effect on bacterial cells by the same process. Ionizing radiation causes single or double stranded breaks in strands of the RNA and DNA double helix. This nucleic acid damage is brought about by direct impact of the high energy electrons from the beam or

indirectly when adjacent molecules are ionized which in turn impact the genetic material (Pillai 2004). In electron beam irradiation the incident high energy electrons from the linear accelerator are the primary electrons while those produced as a result of ionization of molecules within the matrix of the product are the secondary electrons. Both types contribute towards damage to pathogens as well as non-pathogens. Water is most often the source of these secondary electrons. Ionizing radiation causes water molecules to lose an electron and form hydroxyl radicals and hydrogen peroxide, both of which are highly reactive with nucleic acids and cause breakage of bonds within the nucleic acid strands as well as those that hold the strands together (Pillai 2004). Microorganisms vary in their sensitivity to irradiation. Bacterial spores have a higher chance of surviving irradiation than vegetative cells. However, low-dose irradiation of food does not increase the risk from sporogenous bacteria (Olson and others 1994). Also, some microorganisms are susceptible to irradiation at low doses when irradiated during the logarithmic phase of growth than during the stationary phase (Olson and others 1994). One of the reasons, according to Pillai (2004), is that bacterial cells in logarithmic phase have multiple copies of their genomes in one cell. As a result, they have a better chance of survival despite damage to a portion of their nucleic acid.

Role of food in deciding irradiation dose

Some of the factors that determine the irradiation dose for foods include the physical and chemical composition of the food being irradiated, its handling or possible processing post-irradiation, its intended use and the target consumers (Olson and others

1994). Composition of the food system plays a significant role in determining the irradiation dose. Foods high in lipids tend to get rancid at high doses. This can be countered to some extent by vacuum packaging where oxygen is removed and hence radicals will not be formed from oxygen, water and lipid molecules. Freezing can also serve the purpose by reducing the amount of free water available for radical formation (Olson 1995). Foods with low water activity contribute to greater resistance of microorganisms against irradiation. This may be due to the formation of fewer secondary electrons and free radicals due to low water content. However, foods with low water activity also reduce the ability of surviving bacteria to recover and proliferate to large numbers. Most of the research related to food irradiation in the past has focused on meats and commodities like spices, grains or tubers. Most of the research pertaining to fresh produce was focused on the area of disinfestations and delaying the ripening process. However, irradiation of fresh fruits and vegetables is gaining increasing attention due to the need for novel intervention strategies to compliment the existing chemical compounds used for pathogen reduction. Studies completed on irradiated fruits provide information on the tolerance of these products to ionizing radiation. Yu and others (1995) found that irradiating strawberries with doses of 1 and 2 kGy of electrons suppressed fungi on stored berries and increased storage time. Buchanan and others (1998) found that the D-value for *E. coli* O157:H7 in apple juice at 2 °C was dependent on the level of suspended solids and ranged from 0.26 to 0.35 kGy. In a later study, they discovered that acid adaptation of *E. coli* O157:H7 increased its resistance to radiation (Buchanan and others 1999 a). In a study on shredded carrots irradiated at 2 kGy,

Chervin and Boisseau (1994) found that growth of aerobic and lactic acid bacteria on the shredded carrots was inhibited by irradiation, and sensory analysis did not result in any adverse effect of irradiation on carrots at that dose. They also compared irradiation with chlorination followed by spin-drying and found that the former treatment was more effective in reduction of bacteria. One of the key limiting factors in the application of irradiation to fresh produce is its deleterious effect on texture, flavor and other sensory attributes at medium or high doses. Electron beam irradiation of orange juice at 3 kGy resulted in 5 log reduction of inoculated *E. coli.* However, unacceptable sensory characteristics prevent the commercial application of such high doses to orange juice (FDA 2001).

The susceptibility of bacteria to irradiation varies with the composition of food matrix in which they exist. *L. monocytogenes* had a D-value of 0.35 kGy in a bacteriological medium and a value of 0.77 kGy in chicken (Murano 1995). Temperature of the food plays an important role in determining the lethality of irradiation dose. Research has shown that certain bacteria are resistant to irradiation at low temperatures. *Y. enterocolitica* has a D-value of 0.2 kGy in ground beef irradiated at 25 °C and 0.4 kGy when irradiated in ground beef at -30 °C (Murano 1995). According to Murano (1995), the reason for this difference in lethality is the non-availability of free water at lower temperatures for formation of indirect radical formation, resulting in lower overall antimicrobial activity. A similar observation was reported by Mulder (1984) who pointed out that irradiation under frozen and anaerobic condition was most favorable for survival of *Salmonella.* The D-value for *S. typhimurium* in poultry meat at

22 °C was 0.4 kGy, at 0 °C was 0.58 kGy, at 4 °C was 0.7 kGy and at -20 °C was 0.73-0.79 kGy. Irradiation has been found to affect the sensitivity of bacteria to subsequent heat treatments (Olson and others 1994). Thayer and others (1991) studied the effect of heat (60 °C for 3 min) and gamma irradiation on *S.* Typhimurium inoculated in mechanically deboned chicken meat (MDCM). They found that irradiation of MDCM followed by heat treatment resulted in increases sensitivity of *Salmonella* to thermal death. However, heating MDCM prior to irradiation did not result in increased sensitivity of *Salmonella* to irradiation. This phenomenon can be explored in treatment of foods that may be sensitive to processing at high temperature.

Irradiation of fresh-cut produce

The International Fresh-cut Produce Association defines a fresh-cut product as fruits or vegetables that have been trimmed and/or peeled and/or cut into 100% usable product that is bagged or pre-packaged to offer consumers high nutrition, convenience and flavor while still maintaining freshness (www.fresh-cuts.org). Fresh-cut processing involves removal of the fruit's natural cuticle which acts as a barrier to gas diffusion and microbial invasion. Disruption of tissue while cutting results in increased respiration, water loss, ethylene production and microbial activity. The outcome of such processing contributes to decreased shelf-life compared to the whole fruit in terms of browning, color change, softening, flavor change resulting from enzymatic spoilage and microbial decay (Gorny and Beaulieu 2004). In fresh-cut cantaloupe, with pH close to neutral, bacteria are the main contributors towards spoilage. Thus, it is imperative to reduce

microbial load of fresh-cut products before packaging and maintaining it then on under conditions that would limit their proliferation. Fresh-cut products are subjected to low temperatures (0-5 °C) through storage and distribution till it reaches the consumer. Low temperatures help in reducing the respiration rate, microbial growth and some enzymatic deterioration. Shelf-life of fresh-cut vegetables is generally 10-14 days and slightly less for fresh-cut fruits. Radiation at doses less than 2 kGy can extend shelf-life of several fresh-cut products by 2-12 days without affecting sensory characteristics (Prakash and Foley 2004). Modified atmosphere packaging is extensively used in the fresh-cut industry. The principle behind the technology is that high levels of carbon dioxide and low levels of oxygen would create an environment that would control the proliferation of bacteria and at the same time keep the product fresh. Fresh fruits and vegetables are commonly packed in plastic film bags or containers over-wrapped with films, which create a modified atmosphere with time. The material chosen for packages has an optimum permeability for gases and storage at low temperature lowers the respiration rate of fruit. A low level of oxygen in the package may retard browning and spoilage and maintain fresh appearance of the fruit; however, it can also cause off-flavors (Cameron and Smyth 1997). A very low level of oxygen or a high level of carbon dioxide in the package may inhibit the growth of spoilage microorganisms but may allow or stimulate the growth of foodborne pathogens like *L. monocytogenes*, *Y. enterocolitica* and *Aeromonas hydrophila* (Farber 1991; Prakash and Foley 2004). Irradiation is a potential technology that can be applied to reduce the microbial load of fresh-cut products to a level that would result in greater shelf-life of the packaged product. The higher water

content of fresh produce results in lower D-values for most pathogens in irradiated fruits and vegetables than other commodities (Prakash and Foley 2004). The investigators reported that the level of pathogens in produce is usually low (= 3 log cfu/g) and hence low doses (< 1 kGy) can eliminate the risk in most conditions. According to Gorny and Beaulieu (2004), use of chemical treatments like chlorine washes may not be advisable for certain fresh-cut products due to the concern of increased water activity and washing away of desirable flavor attributes. Some chemical treatments may leave residues or cause color change of the product. Also, the centrifugation and spinning process employed to remove the excess water may be damaging to the tissue structure. The limitation of using irradiation as the sole intervention step for fresh-cut produce is that, at high doses it causes a change in sensory quality especially the flavor and texture of most fruits and vegetables. Studies have shown that firmness of Roma tomatoes irradiated at 0.5 kGy decreased by 30 % and of cut romaine lettuce irradiated at 0.35 kGy decreased by 10 % (Prakash and Foley 2004). Tissue softening may be caused by partial depolymerization of cell wall polysaccharides, mainly cellulose and pectins (D'Amour and others 1993) and by damage to cell membranes (Voisine and others 1993) which leads to loss of intracellular water and cell turgescence. Cantaloupe chunks in packages containing high levels of oxygen, irradiated at 3 kGy, showed bleaching effect on the color as a result of oxidative bleaching of carotenoids (Prakash and Foley 2004). Thus, a combination of chemical sanitization followed by irradiation of the final product can be used to achieve the required microbial reduction without affecting the characteristics of the product.

OBJECTIVES

The specific objectives of this study were as follows:

1. To determine the effectiveness of low dose electron beam irradiation on the microbiological and sensory characteristics of sliced cantaloupe over 21 days of storage.
2. To study of the effect of electron beam irradiation on the reduction of inoculated *S.* Poona and indigenous microflora on sliced cantaloupe.
3. To determine the D-value for *S.* Poona on sliced cantaloupe irradiated with electron beam.
4. To determine the effect of the shape of cantaloupe pieces on uniformity of dose distribution over its entire surface.
5. To identify attachment sites for *S.* Poona in the rind of cantaloupe using electron microscopy.

MATERIALS AND METHODS*

Quality and shelf-life of irradiated sliced cantaloupe

Package designing

A preliminary study was conducted to determine the optimum material for packaging sliced cantaloupe. Cantaloupe pieces were cut as described further and amounts of 100, 200, 250 and 300 g were packed in 2 types of 15 cm x 19 cm polyethylene bags with varying thickness of material. The 2 bags under consideration were Whirl-Pak™ and Ziploc bags. The bags were double sealed and stored at 5 °C. At the end of 8 days, a gas sample was withdrawn from the bags using an airtight syringe and analyzed for percentage of oxygen using an oxygen analyzer (S-3A/I AEI Technologies Inc. Pittsburgh, PA) and carbon dioxide using an infrared gas analyzer (Horiba Model PIR-2000 Irvine, CA). The package and fruit weight combination that had the lowest oxygen permeation rate at the same time preventing anaerobic fermentation of the fruit was chosen for the irradiation and storage of cantaloupe pieces.

Fruit preparation and packaging

Cantaloupes were purchased from a major supplier. The fruit were unwashed and boxed in the field. All melons were washed in the laboratory with distilled water along with gentle scrubbing using a brush for 2 min, after which half of the fruits were further

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further dipped in a solution with 200 mg/L sodium hypochlorite for 3 min. Excess chlorine from the surface of melons was washed off with distilled water rinses before setting them to dry for 12 h at 21 °C. The fruits were then separated into 2 groups depending on the wash treatment. Each cantaloupe was sliced and cored into cylindrical pieces with a diameter of 2.54 cm using a stainless steel corer. All materials used for cutting and handling were continually sanitized using 70% ethanol and flame to prevent cross contamination. The cylindrical pieces of fresh-cut cantaloupe were packed in 15-cm x 19-cm polyethylene bags. Each bag contained 100 g of fruit and was double sealed using an Impulse Heat Sealer (American International Electric, New York, NY) to ensure hermetic conditions. Preliminary experiment data, as described above, determined the optimum packaging film and the exact weight of fruit to be packed so that the gas permeation rate of the film balances the respiration rate of the fruit at 5 °C.

Dosimetry

Extensive dose mapping of the cantaloupe cylinders was carried out using alanine pellets (Harwell Dosimeters, UK) at the National Center for Electron Beam Food Research at Texas A&M University. A dual beam of electrons, from the top and bottom of the sample, was used for this experiment to minimize the dose variation throughout the sample. High density polyethylene (HDPE) sheets were used as attenuators for reducing the energy of incident electrons in order to achieve the low target doses of 0.7 and 1.4 kGy on the cantaloupe pieces. The alanine pellets placed on the cantaloupe pieces were analyzed for absorbed dose using Electron Paramagnetic Resonance (EPR)

spectroscopy (Bruker EMS 104 EPR Analyzer, Bruker Instruments, Germany). Multiple runs of dosimetry were carried out to finalize the exact attenuation scheme and conveyor speed required to achieve the target doses for the experiment.

Irradiation treatment

Cantaloupe packets were placed in a single layer inside cardboard boxes on a conveyor and exposed to either 0.7 or 1.4 kGy electron beam irradiation. The dose of 0.7 kGy corresponds to the highest reported D-value for non-sporeforming pathogens according to Olson (1998) and a higher dose of 1.4 kGy was also chosen in this study to investigate its effect on quality of cantaloupe at double dose intensity. The irradiation treatment was carried out at the National Center for Electron Beam Food Research at Texas A&M University. A non-irradiated set of packets served as the control. Upon irradiation, the 0 day bags were immediately analyzed and the remaining bags, along with the control were transferred to the cold room for storage at 5 °C for up to 21 days. Periodically, packets were removed for microbiological, texture and color analysis as well as sensory evaluation by a trained sensory panel.

Microbiological analysis

Aerobic plate count (APC) was carried out to determine the microbial load of the sample. At each sampling time, packets were opened aseptically using scissors flame sterilized after dipping in 70% ethanol. A 25-g sample was weighed out from each packet and placed into a stomacher bag with 0.1% peptone water (Difco Laboratories,

Detroit, MI). This mixture was pummeled in a laboratory blender (Stomacher 400, Seward, London, UK) at high speed for 2 min. Appropriate serial dilutions were made from this homogenate and inoculated on Petrifilm^{TM} Aerobic Count Plates (3M Microbiology Products, St.Paul, MN). These plates were incubated at 25 °C for 48 h and the developing red colored colonies were reported as colony forming units (cfu). Colony counts were calculated as cfu/g and then converted into log value for statistical analysis.

Analysis of texture and color

Texture in the form of firmness was measured as the force (N) required for a puncture effect on the flat side of each melon cylinder. Three pieces per packet were analyzed on a Texture Analyzer (TA.XT2i, Texture Technologies Corp. Scarsdale, New York) with a 25-kg load cell. A 5-mm diameter flat-headed stainless steel cylindrical probe traveled 30 % of the height of the cylinder at 1mm/s and the first peak force value obtained during the test was recorded (Luna-Guzmán and others 1999). Objective color was determined by taking three pieces of fruit per packet and measuring the color using a Hunter colorimeter (Hunter Assoc, Reston, VA; McGuire, 1992). The L*, a* and b* color space values were recorded and hue and chroma values were calculated. Hue was calculated using the formula arctan(b*/a*) and chroma was calculated using the formula $[(a^*)^2 + (b^*)^2]^{1/2}$.

Sensory evaluation

The sensory analysis of cantaloupe was carried out by an expert, trained 5-member descriptive attribute sensory panel at the Sensory Testing Facility at Texas A&M University. Panelists were selected as described by American Meat Science Association (AMSA; 1995) and Meilgaard and others (1999). Training and ballot development sessions were conducted to determine color, odor, flavor, basic tastes and texture attributes of cantaloupe. Products for training represented cantaloupe from the treatments and storage times defined in the study. Terms from Civelle and Lyon (1996) and Meilgaard and others (1999) were used to assist in identifying attributes. For color, paint cards ranging from yellow to dark orange were obtained. A color reference card was developed so that 1 = light orange and 8 = dark orange. Color standards used were, SW6888, SW6889, SW6890 and SW6891 (Sherwin-Williams Co. Cleveland, OH). Attributes were identified as fermented, earthy, soured, nutty, musty and fruity odors and flavor aromatics, sweet, sour and bitter basic tastes and hardness and firmness textures. Descriptive attributes were evaluated on the 15-point Universal Spectrum ^{TM} scale where 0 = none and 15 = extremely intense. After training, panelists evaluated 18 samples per day while seated in individual booths separated from the sample preparation area. Samples were identified with random 3-digit codes and served by treatment in random order. Each sensory day, panelists conducted a warm-up sample from the control to standardize the panelists within a day. Panelists were provided double distilled, deionized water, saltless saltine crackers and ricotta cheese to cleanse their palates between samples. Samples were served no less than 4 minutes apart to reduce halo

effects and taste bud fatigue. Panelists were given 6 samples at a time with a 20 min break between each set. On each day of analysis, the samples were removed from the refrigerator at least 1 h prior to serving to allow them to equilibrate at room temperature (20 °C). The panelists were served the pieces of cantaloupe in transparent glass bowls covered with a concave glass lid. Panelists first slightly shook the container with the lid intact, moved the lid slightly and evaluated cantaloupe odors. Then the panelists bit into a cantaloupe piece and evaluated flavor aromatics, basic tastes and texture attributes.

Reduction of *S*. Poona and native flora on irradiated sliced cantaloupe

Media preparation

Enumeration of salmonellae was carried out on Tryptic Soy Agar (TSA; Difco) plates containing rifampicin (100 mg/L; Sigma Chem. St. Louis, MO) and cycloheximide (100 mg/L; Sigma Chem.). Rifampicin served as the selective agent for the rifampicin-resistant *S.* Poona, which was inoculated into the cantaloupe. Cycloheximide served as an antifungal agent to prevent fungi from the sample from spreading onto the solid medium before bacterial colonies could develop. 0.1 g of rifampicin dissolved in 5 ml methanol as well as 0.1 g of cycloheximide dissolved in 5 ml sterile distilled water was added to 1 L of sterilized TSA after adequate cooling and then poured into petri plates. Enumeration of lactic acid bacteria was carried out using deMan, Rogosa and Sharpe agar plate (MRS; Difco) with an overlay of All Purpose Tween agar (APT; Difco) adjusted to pH 4.0 with 10% tartartic acid (Mallinckrodt

Chemical Works, St. Louis, MO; APHA 2001).

Bacterial cultures

Rifampicin-resistant strain of *S.* Poona was obtained for this study from Dr. Linda Harris at University of California at Davis (Davis, CA). The strains were stored at -80 °C on Protect™ Bacterial Preservers (Key Scientific Products, Round Rock, TX). Whenever needed, the frozen cultures were revived in tryptic soy broth (TSB) and incubated for 24 h at 37 ºC. A loopful of the broth culture was plated on TSA. The TSA plates were incubated at 37 ºC for 24 h. A single colony was picked up from the plate and transferred to TSA slant. Further incubation of the slant at 37 ºC for 24 h yielded confluent growth.

Inoculum preparation

The rifampicin-resistant *S.* Poona maintained on TSA slant was grown in a flask containing 300 ml of TSB and incubated at 37 ºC for 24 h. The cells were then harvested by centrifugation (Centrifuge B4i, Jouan, Winchester, VA) at 3000 rpm in sterile tubes and then washed 3 times with equal volume of sterile 0.1% peptone water. The resulting suspension in peptone water was found to be 8 log cfu/ml and was used for dipping the cantaloupe pieces.

Fruit preparation and packaging

Cantaloupes were purchased from a major supplier and were cut and cored into

cylinders 2.54-cm in diameter as described in the previous section. Each piece was dipped one at a time in the 8 log cfu/ml suspension of *S*. Poona and left at 25 ºC for 1 h on a sterilized rack to allow the excess suspension to drain off. The inoculated cylindrical pieces of fresh-cut cantaloupe were packed in 15-cm x 19-cm polyethylene bags as in the previous experiment. Each bag contained 100 g of fruit and was double sealed using an Impulse Heat Sealer to ensure hermetic conditions but not vacuum packaging.

Dosimetry

Dosimetry data from the previous experiment was used as a starting point since the sample and packet specifications for this experiment were identical to the previous study. Additional dosimetry was carried out to confirm the parameters that had been established for the previous irradiation process. This was done to account for any variation that may have risen from changes in the beam or wear and tear of conveyor belts with time. The process was fine tuned and all required parameters were established to achieve 0.7 and 1.5 kGy in the final run.

Irradiation treatment

Cantaloupe packets were placed in a single layer in cardboard boxes on a conveyor and exposed to either 0.7 or 1.5 kGy electron beam irradiation. The irradiation treatment was carried out at the National Center for Electron Beam Food Research at Texas A&M University. The post-irradiation handling and storage process was similar to

the one in the previous experiment. On day 0, 3, 6, 9, 12, 15, 18 and 21, packets were removed from the cold room for microbiological and pH analysis.

Microbiological analysis

A 25-g sample was taken from each packet and placed into a sterile stomacher bag with 225 ml of 0.1% peptone water. All materials used for cutting and handling were sterilized using 70% ethanol and flame to prevent cross contamination. The mixture was pummeled in a laboratory blender at high speed for 2 min. Appropriate serial dilutions were made from this homogenate and spread-plated onto TSA plates containing rifampicin (100 mg/L) and cycloheximde (100 mg/L). The plates were incubated at 37 °C for 24 h and colonies were counted and recorded as cfu of *Salmonella* / g and then converted into log cfu/g.

For enumeration of lactic acid bacteria, appropriate dilutions were spread-plated onto MRS agar plates and then overlaid with molten, acidified APT agar (pH 4.0). The overlaid agar was allowed to set after which, the plates were incubated at 35 °C for 3-5 days. Colonies of lactic acid bacteria embedded between the 2 agar layers were counted and calculated as cfu/g.

Yeasts and molds were enumerated by inoculating appropriate dilutions onto Yeast and Mold Petrifilm™ (3M Microbiology Products, St. Paul, MN) and incubating the petrifilm plates for 5 days at 25 °C. The observed growth after incubation was recorded separately as yeast and mold counts.

Confirmation of isolates

For each round of sample analysis, 10 representative colonies of *Salmonella* were picked off the TSA plate for further confirmation. Confirmation was carried out biochemically using triple sugar iron (TSI; International Bioproducts, Bothell, WA) and lysine iron (LIA; International Bioproducts) agar slants and serologically using the agglutination reaction with *Salmonella* O Poly A-I and Vi antiserum (Becton Dickinson, Sparks, MD). On TSI slant, typical *Salmonella* displayed an alkaline slant (red) and acid butt (yellow) with H_2S production in the form of blackening at the base of the slant. On LIA, *Salmonella* had an alkaline reaction (purple) with H_2S production. The serological test was carried out by adding a drop of 0.85% saline solution to a glass slide. Bacterial growth was emulsified with the saline and 1 drop of *Salmonella* O Poly A-I and Vi antiserum was added and mixed well. Agglutination in the drop indicated positive reaction for *Salmonella*.

For each round of sample analysis, 15 characteristic lactic acid bacteria colonies were randomly picked from the MRS + APT agar plates and transferred to TSA slants for further confirmation using gram staining, catalase reaction and fermentative metabolism (O-F Glucose; O-F Basal Medium + Glucose + 0.1%Yeast Extract, Difco) test.

Measurement of surface pH of cantaloupe

pH on the surface of cantaloupe pieces from each package was measured immediately after opening and prior to conducting the microbiological analysis.

Markson portable pH meter Model 612 (Markson Science, Inc., Phoenix, AZ) with a flat bulb design electrode (Markson Science, Inc) was used for the measurement. The pH meter was calibrated and sanitized prior to use on each day of analysis. Three pH readings were taken for each sample and average was calculated.

D-value for *S.* Poona on irradiated sliced cantaloupe

Sample preparation and inoculation

Cantaloupes were purchased from a major supplier and cut and cored into 2.54-cm cylindrical pieces as in previous experiments. 4-mm thin sections of cantaloupe were sliced from each cylindrical piece and placed on a petri dish. Six discs of cantaloupe were placed per petri plate and they served as replicates for the particular dose. The rationale behind using thin discs of melon was to ensure a very precise dose delivery to the sample and achieve multiple irradiation doses with small increments. Rifampicin-resistant *S.* Poona was grown in a flask containing 300 ml TSB and incubated at 37 ºC for 24 h. The cells were then harvested and washed with 0.1% peptone water as described above. The final inoculum was a suspension of *S.* Poona at a cell density of 8.6 log cfu/ml. 0.1 ml of this suspension was inoculated on each cantaloupe disc and the petri plate was covered using saran wrap and transported to the National Center for Electron Beam Food Research facility for irradiation.

Dosimetry

Alanine pellets were used as dosimeters for dose mapping in this experiment.

The dose mapping was aimed at establishing a range of doses between 0 and 1 kGy at target increments of 0.1 kGy. Cantaloupe discs of thickness 4-mm were cut for this study. The rationale behind this size was to match it with the 4 mm thickness of alanine dosimeters so that the absorbed dose on the dosimeter would be an accurate measure of the dose that would be absorbed by the cantaloupe disc, all other parameters being constant. The dosimeters were placed in holes punched in the center of cantaloupe discs placed in a petri dish. This was done to take into account the effect of cantaloupe mass surrounding the alanine, in dose absorption. HDPE sheets were used as attenuators as in the previous study. Extensive dosimetry work along with various combinations of attenuation led to the finalization of the following doses for the experiment: 0, 0.12, 0.19, 0.29, 0.41, 0.54, 0.63, 0.79, 0.85, and 1.06 kGy.

Irradiation treatment

Ten petri dishes containing six cantaloupe discs each were prepared and inoculated with *S.* Poona. Each plate was covered with Saran wrap and placed in a secondary packaging of Whirl-Pak™ bags to prevent any leakage and contamination. The plates were then irradiated at the doses mentioned above. After irradiation, the samples along with the non-irradiated control were immediately analyzed for microbial counts.

Microbiological analysis

After irradiation of samples, each 4-mm disc of cantaloupe was mixed separately

with 10 ml sterile 0.1 % peptone water in Whirlpak^{TM} bags, 7.6 x 12.7 cm in size and pummeled at high speed for 2 min in the lab stomacher. An aliquot of the homogenate was serially diluted 10-fold and spread-plated onto TSA containing rifampicin (100 mg/L) and cycloheximide (100 mg/L). Developing colonies were reported as cfu/g and then converted to log cfu/g. The counts were reported as an average of six replicates for each dose of irradiation.

Confirmation of isolates

Five representative colonies were transferred from each plate onto TSA slants and confirmation for *Salmonella* was carried out according to the procedure described in the previous section.

Dose-mapping of cantaloupe cylinders and cubes

The objective of this experiment was to compare the circular shape of cantaloupe cylinders with the flat plane of a cantaloupe cube as a function of uniformity of dose absorbed over their entire surface. The objective was to dose-map each point over the entire cylindrical surface using alanine dosimeters. Paper-thin alanine dosimeter films (Kodak Biomax, New York) were chosen instead of pellets for this study for their ease of handling. Three cantaloupe cylinders of 2.54-cm diameter were cored and covered with a thin layer of Saran wrap as a barrier between the moisture from the cantaloupe and the alanine films. From the 3 cantaloupe pieces, the entire surface of 2 cylinders was covered with 20 alanine films and secured with a thin elastic band in their respective

numbered positions relative to their clockwise orientation. The 2 cylindrical pieces with dosimeters on them were placed adjacent to each other and the third cantaloupe piece without any dosimeters served as a source of cantaloupe mass on one side of the piece at the center. Thus, the set-up was designed to enable dose-mapping of cylinders located at the center and at the sides in a packet. Cantaloupe cubes were cut 2.54-cm x 2.54-cm in dimensions and 3 such alanine films were secured on each plane of the cube.

The 3 cylinders were then secured together with a thin elastic band and placed on the HDPE sheets that were used for attenuation to achieve doses of 0.7 and 1.4 kGy on separate samples. Upon irradiation, the irradiation dose absorbed by the alanine films was immediately measured using the Bruker e-scan II equipment with the appropriate probe for analyzing films. The readings on both cylinders were compared to explain any differences observed due to the shape of the sample or position of the cylinder relative to others in a packet.

Attachment sites for *Salmonella* on the cantaloupe rind

Confocal scanning laser microscopy (CSLM)

Bacterial culture

S. Poona harboring a plasmid for green fluorescent protein (GFP; Clontech Laboratories, Inc.,Palo Alto, CA) was used for this experiment. The parent strain was shipped to Dr. Randy Worobo at Cornell University, Ithaca, NY. In his lab, the plasmid was introduced into the strain by using electroporation following the method described by Dramsi and others (1995). When grown in a medium containing the antibiotic

ampicillin (Sigma Chem.), the strain expresses the GFP gene and produces green fluorescence. This characteristic was used to microscopically observe possible access sites for bacteria on the cantaloupe rind.

Sample inoculation and microscopy

24-h culture of GFP expressing *S.* Poona in TSB + Ampicillin was harvested and washed in 0.1% peptone water and resuspended. 50 µl of an 8 log cfu/ml suspension of cells was inoculated and spread over a 1-cm^2 area of the cantaloupe rind. After allowing it to dry for 90 min at 25 ºC, the piece of the rind was excised and observed using Meridian InSIGHT Point Confocal Microscope with Krypton-Argon Laser using an excitation wavelength of 488 nm. Microscopy was carried out at the Image Analysis Laboratory at Texas A&M University. Fluorescent salmonellae were visible against a dark matrix of rind and fruit tissue.

Scanning electron microscopy (SEM)

Inoculation of fruit

A 24-h culture of *S.* Poona grown in TSB was used for this experiment. The broth culture was centrifuged and washed 3 times in 0.1% peptone water as in previous experiments and then resuspended in equal volume of peptone water. 50 µl of a 7-log cfu/ml suspension of cells was inoculated and spread over a 1-cm^2 area of the cantaloupe rind. The melon was allowed to stand for 90 min at 25 ºC to allow the inoculum to dry and bacteria to adhere to the sample.

Sample processing and microscopy

The protocol for sample processing followed the one used by Pao and others (2001) for imaging pathogens on oranges and was fine tuned at the Microscopy Imaging Center at Texas A&M University. Excised pieces of cantaloupe rind inoculated with salmonellae were placed in glass vials and fixed overnight in 3% glutaraldehyde (Avocado Research Chemicals Ltd, Heysham, Lancashire) and 2.5% paraformaldehyde (Alfa Aesar, Wardhill, MA) in 0.1 M potassium phosphate buffer, pH 6.8 to maintain the structural integrity and secure bacteria in the tissue. Sample processing was done using Pelco® Biowave™ (Ted Pella, Inc., Redding, CA) variable power microwave oven to reduce time involve in sample preparation. The microwave oven is equipped with a Coldspot™ water recirculating device on the oven floor designed to prevent specimen heating and to ensure even distribution of the microwave radiation. All microwave-assisted steps in sample processing were performed with the sample under vacuum and at a power of 250 watts. Fixed samples were washed 3 times with 0.1 M potassium phosphate buffer, pH 6.8 for 1 min at 250 watts. Samples were post fixed with 1% osmium tetroxide (Electron Microscopy Sciences, Hatfield, PA) in 0.1 M potassium phosphate buffer, pH 6.8 and microwaved at 250 watts with a 2 min “on”, 2 min “off” and 2 min “on” cycle. Osmium was removed and specimens were rinsed for three 1 min washes in 0.1 M phosphate buffer, pH 6.8 in the microwave. Dehydration of specimens was carried out in ascending concentrations of methanol in 5 % increments starting at 5

%, and ending with 3 washes with 100 % methanol. Dehydration was done in the microwave for 1 min at 250 watts through each step. After the last wash, the 100 % methanol was removed and replaced by HMDS and dried up. For microscopic observation, samples were placed on aluminum stubs with carbon sticky tape and Electrodag 502 (Ted Pella, Inc., Redding, CA) and then sputter coated with a gold / palladium mixture (50/50) using a Hummer I Sputter Coater (Anatech Ltd., Union City, CA) for 4 min at 10 mA. Samples were observed using a JEOL 6400 scanning electron microscope (JEOL USA, Peabody, MA). Photographs were taken using a Polaroid^{TM} system with Polaroid Polapan 400 film (Polaroid, Bedford, MA).

Analysis of data

Data recorded for each parameter tested in three trials were analyzed by analysis of variance using the ANOVA procedure of SAS (Statistical Analysis Systems Institute, Cary, N.C.). When ANOVA indicated a significant difference ($P < 0.05$), mean separation was carried out by the Duncan's multiple range test. Data for subjective sensory evaluation was analyzed by the Mixed Procedure of SAS and least square means were obtained. Least square means were separated when the effect was significant in the ANOVA table ($P < 0.05$). For sensory data, panelist and panelist by main effect interactions were tested. As panelist by main effect interactions were not significant, these were pooled into the error term. Microbiological data (APCs) was also analyzed by linear regression to establish the effect of different treatments on the bacterial counts over storage time.

For the pathogen reduction study, for each organism enumerated, average cell number (log cfu/g) was plotted against irradiation dose to display the reduction achieved in the sample due to irradiation on day 0 and the effect of storage over 21 days at 5 °C. The effect of irradiation dose on the reduction of the studied organisms was analyzed by analysis of variance using the ANOVA procedure of SAS.

For the D-value study, numbers of surviving *Salmonella* against increasing doses of irradiation were plotted and a linear regression chart was established. The D-value was determined from reciprocal of the regression line as the dose in kGy required to reduce the population of *S.* Poona by 1 log cycle.

RESULTS AND DISCUSSION*

Quality and shelf-life of irradiated sliced cantaloupe

Gas composition of the package

The gas composition of the package made using two different materials at the end of 8 days is shown in Figure 1. The material of Ziploc bags had a higher oxygen permeation rate which resulted in higher oxygen levels in these packages (Figure 1). Bags containing fruit = 300 g did not show anaerobic fermentation. On the other hand, Whirl-Pak™ bags were less permeable to oxygen (Figure 1). Thus lower oxygen levels could be achieved depending on the amount of fruit in the bag. Bags containing = 200 g of fruit did not show anaerobic fermentation. The oxygen level in Whirl-Pak™ bags containing 100 g cantaloupe was 9 % and the carbon dioxide level was 3 %. Bags containing = 250 g melon pieces showed a sharp rise in carbon dioxide level below 1 % oxygen level, indicating anaerobic fermentation of fruit. Whirl-Pak™ bags containing 100 g of cantaloupe pieces were chosen for the irradiation study.

Microbiological analysis

Electron beam irradiation reduced the total aerobic microbial counts of water washed and chlorine washed fruit (Day 0, Figure 2). For the water washed melons, APCs for the non-irradiated fruit was 4.0 log cfu/g with a reduction in count by 2.0 log cfu/g at 0.7 kGy and 3.8 log cfu/g at 1.4 kGy. For the chlorine washed melons, APCs for the

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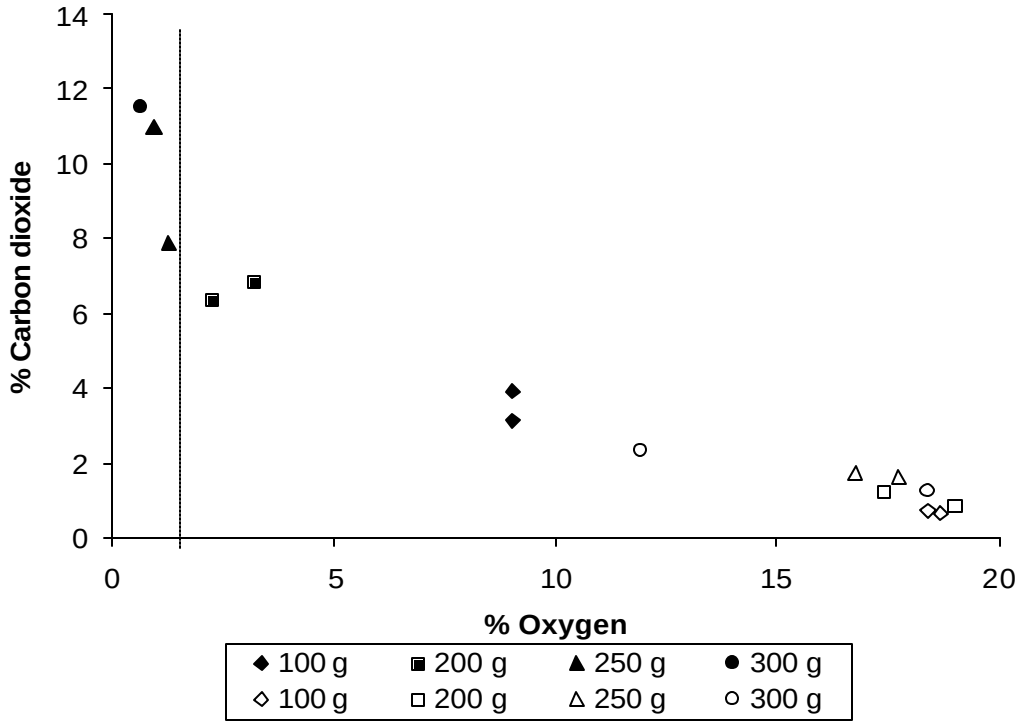

Figure 1 - Gas composition of Whirl-Pak™ and Ziploc bags containing different amounts of sliced cantaloupe after storage at 5 °C for 8 days. Solid data points denote Whirl-Pak™ bags and hollow data points denote Ziploc bags. The dotted line marks the beginning of anaerobic fermentation of fruit with increasing sample weight in the packet.

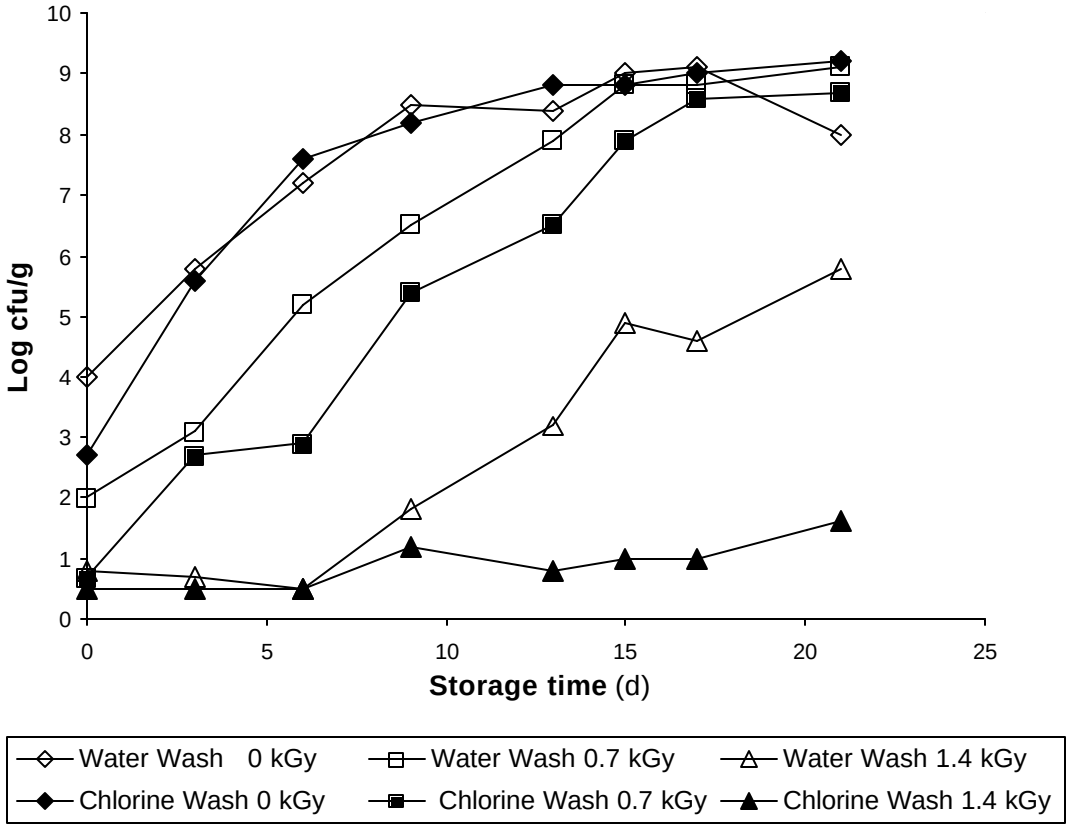

Figure 2 - Aerobic plate count of sliced cantaloupe over 21 days of storage at 5 °C after electron beam irradiation.

non-irradiated fruit was 2.7 log cfu/g with a reduction in count by 2.0 log cfu/g at 0.7 kGy with APCs below the detection limit at 1.4 kGy. This indicates that the electron beam exposure resulted in significant reduction of microbial populations, dependent on the dose intensity and wash treatment. On day 0, melons washed with chlorine were 1.3, 1.3 and 0.3 log cfu/g lower in APC than those washed only with water at doses of 0, 0.7 and 1.4 kGy respectively. These results were consistent across the control and irradiation doses as well as throughout the 21-day storage period. The antimicrobial action of chlorine on the surface of the melons may have resulted in fewer bacteria contaminating the flesh. A similar observation has been reported by Ukuku and others (2002) with their work using *L. monocytogenes* inoculated on the surface of whole cantaloupe. They found that sanitization of whole cantaloupes with chlorine or hydrogen peroxide has the potential to reduce or eliminate the transfer of *L. monocytogenes* on melon surfaces to fresh-cut pieces during cutting. Our results show the effectiveness of combining decontamination wash with subsequent exposure to low doses of irradiation to achieve microbial reduction. In this study, the duration of storage of cantaloupe was also observed to have an effect on microbial counts. However, after 15 days of storage, there was no significant increase in bacterial counts for 0 and 0.7 kGy treated fruit. Storage at 5 °C is not conducive for growth of most organisms typically pathogens like *Salmonella* (Golden and others 1993); however, many mesophilic bacteria can grow at that temperature. Lamikanra and others (2000) reported an increase in total aerobic bacterial counts after an induction period of 5 days on minimally processed, cut cantaloupe stored at 4 °C. O'Connor-Shaw and others (1994) observed an increase in lactobacilli

population from 3.3 x 10^4 to 3.6 x 10^6 cfu/g in fresh-cut cantaloupe stored at 4 °C. This highlights the need for appreciable reduction of bacterial load on cantaloupe during decontamination steps to prolong their proliferation to levels that would affect the fruit quality. These findings suggest that electron beam irradiation is a potentially viable decontamination tool and needs to be validated for its ability to eliminate pathogens associated with fresh-cut produce.

Objective texture and color analysis

Texture

The firmness of cantaloupe irradiated at 1.4 kGy was 4.94 Newton and was significantly lower than the control as well as 0.7 kGy. The firmness value for control was 5.86 N and was not significantly different from 5.61 N for cantaloupe irradiated at 0.7 kGy (Table 1). This observation is consistent with the study by Hagenmaier and Baker (1998) of gamma-irradiated shredded carrots. There was no difference between texture of carrots treated at a low dose of 0.5 kGy and the non-irradiated control. However, other studies have reported softening of fruits after irradiation. (Johnson and others 1965; Thomas 1986; Miller and others 1995). Yu and others (1995) reported that firmness of strawberries irradiated with electron beam at 0.5, 1.0 and 2.0 kGy were lower than control fruit. Johnson and others (1965) reported that gamma irradiated strawberries became softer as irradiation dose increased from 1 kGy to 4 kGy. In our study, the type of wash treatment used for whole cantaloupes did not influence the firmness of the cut pieces (Table 1). There was a gradual and significant reduction in

Table 1 - Mean firmness values expressed in Newtons (N) and L* Hunter color means, obtained from sliced cantaloupe as affected by wash treatment of whole melons and by dosage of electron beam irradiation of cut pieces

Effect	Firmness (N)	L*
Wash		
P-value[x]	0.22	0.003
Water[y]	5.55[a]	54.07[a]
Chlorine[z]	5.39[a]	55.58[b]
Dose		
P-value[x]	<0.0001	0.0007
0.0	5.86[a]	56.13[a]
0.7	5.61[a]	54.60[b]
1.4	4.94[b]	53.74[b]
Days		
P-value[x]	<0.001	0.54
0	6.01[ab]	54.63[a]
3	6.05[a]	55.00[a]
6	5.53[bc]	55.07[a]
9	5.48[c]	ND[e]
13	5.21[cd]	54.32[a]
15	5.09[cd]	55.22[a]
17	ND[e]	53.86[a]
21	4.93[d]	55.65[a]
Root Mean Square Error	0.73	2.77

[a-d]Means for factors within the column with the same letter are not significantly different ($P \geq 0.05$)
[e]ND-Not Determined.
[x]P values from Analysis of Variance table
[y]Manual wash consisting of dipping in distilled water, gently brushing with soft brush to remove soil. Each melon was brushed for 2 min
[z]Manual wash followed by dipping and rotating for 3 min in distilled water added with 200 mg/L sodium hypochlorite then rinsing with distilled water to remove excess chlorine

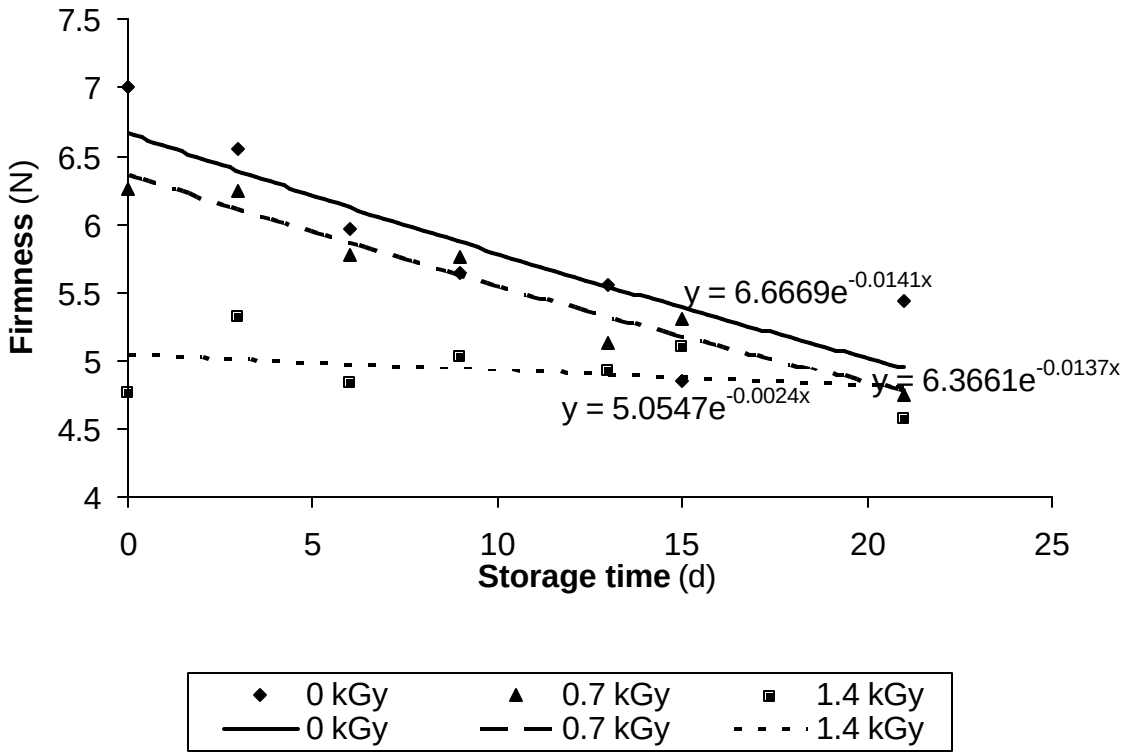

Figure 3 - Mean cantaloupe firmness values (N) as affected by interaction between storage days and irradiation dose as obtained using a texture analyzer.

firmness values with storage time which may be associated with senescence (Table 1). This tissue softening may be caused by partial depolymerization of cell wall polysaccharides, mainly cellulose and pectins (D'Amour and others 1993) and by damage to cell membranes (Voisine and others 1993) which leads to loss of intracellular water and cell turgescence. Additionally, radiation induced texture change has been associated with changes in pectic substances (Kertesz and others 1964; Somogyi and Romani 1964; Howard and Buescher 1989). There was an interaction between irradiation dose and storage time for firmness (Figure 3). Firmness values for the control and 0.7 kGy treated fruits decreased following first order kinetics at a similar rate with time whereas firmness of 1.4 kGy irradiated fruit decreased at a lower rate (Figure 3). The data indicates that irradiation at doses as low as 0.7 kGy showed no difference on texture of cantaloupe in comparison with the non-irradiated control.

Color

Wash treatment had a significant effect on the lightness of cantaloupe pieces (Table 1), with chlorine treated fruit having higher L* values than fruit washed only with water. There was an effect of irradiation dose on L* values with non-irradiated fruit having higher L* values than 0.7 and 1.4 kGy irradiated fruit (Table 1). This observation was different from that observed by Yu and others (1995) for electron beam irradiated strawberries. They observed that Hunter L* values increased with irradiation dose. Miller and others (1995) reported no change in color of 'Sharpblue" blueberries upon electron beam irradiation upto 1 kGy. Hue, 68.53, and Chroma, 27.67, were not affected

by either washing or irradiation treatment, indicating that there was no visible color change in fruit after treatment (Data not shown). In general, L*, Hue or chroma values of the fruit did not change through time.

Sensory evaluation

The least squares mean for each organoleptic parameter analyzed by the five member trained sensory panel are included in (Tables 2, 3 and 4). Irradiation dose and wash treatment did not affect sensory attributes, except sour basic taste. As irradiation dose increased, sour basic taste slightly decreased and was barely detectable for all treatments. The type of wash treatment had no influence on sensory attributes of cantaloupe. However, some sensory attributes were affected by storage time. This may have been due to variation in maturity levels of certain melons and also the senescence of fruit with time. There was an interaction (P= 0.01) effect of irradiation dose and storage days on sour aroma (Figure 4). With increased storage in control cantaloupes, sour basic taste increased; however with storage, sour basic taste increased at a slower rate in irradiated cantaloupes. There was also an interaction (P= 0.04) between dose and storage time on fermented flavor aromatic (Figure 5). On day 0, cantaloupe treated with 1.4 kGy had higher fermented flavor aromatics than non-irradiated control or 0.7 kGy treatment. With increased storage, fermented aromatic would expectantly increase. This increase was observed for control samples. Interestingly, fruit treated with 0.7 kGy did not increase in fermented aromatic as rapidly with storage as the control. This indicates that low doses of irradiation may have prevented fermentation of fruit during storage.

Table 2 - Least squares means for different subjective sensorial odor attributes[a] of sliced cantaloupe as affected by wash treatment of whole melons and by dosage of electron beam irradiation of cut pieces

Effect	Odor				
	Cantaloupe	**Fermented**	**Earthy**	**Soured**	**Fruity**
Wash	0.63[d]	0.49	0.90	0.26	0.05
Water[b]	6.1	0.7	0.4	0.1	1.8
Chlorine[c]	6.0	0.7	0.4	0.1	1.7
Dose	0.93[d]	0.61	0.98	0.07	0.78
0.0	6.0	0.8	0.4	0.1	1.7
0.7	6.0	0.7	0.4	0.1	1.8
1.4	6.1	0.7	0.4	0.1	1.7
Days	0.0001[d]		0.0035	0.0001	0.0001
0	5.3	0.5	0.4	0.0	1.9
3	5.4	0.1	0.1	0.0	2.3
6	6.6	0.3	0.6		2.1
9	6.9	0.8	0.5	0.0	1.8
13	5.8	1.0	0.5	0.1	1.4
15	6.4	0.9	0.3	0.1	1.7
17	6.4	0.9	0.5	0.4	1.7
21	5.6	1.2	0.5	0.4	1.2
Root Mean Square Error	1.83	0.94	0.70	0.47	1.03

[a]Attributes are based upon universal scales from 0 (no detection of the attribute) to 15 (highest intensity for the attribute)
[b]Manual wash consisting of dipping in distilled water, gently brushing with soft brush to remove soil. Each melon was brushed for 2 min
[c]Manual wash followed by dipping and rotating for 3 min in distilled water added with 200 mg/L sodium hypochlorite then rinsing with distilled water to remove excess chlorine
[d]P values from analysis of variance table ($P<0.05$).

Table 3 - Least squares means for different subjective sensorial aroma attributes[a] of sliced cantaloupe as affected by wash treatment of whole melons and by dosage of electron beam irradiation of cut pieces

Effect	Aroma				
	Cantaloupe	**Fermented**	**Earthy**	**Soured**	**Fruity**
Wash	0.44[d]	0.30	0.50	0.74	0.53
Water[b]	6.9	1.0	1.0	0.3	2.5
Chlorine[c]	6.8	0.9	1.0	0.3	2.4
Dose	0.11[d]	0.16	0.07	0.11	0.15
0.0	6.6	1.0	1.1	0.4	2.3
0.7	7.0	0.9	0.9	0.2	2.5
1.4	7.0	1.0	1.0	0.3	2.5
Days	0.0001[d]	0.0001	0.0001	0.0001	0.0001
0	6.4	0.7	0.9	0.1	2.5
3	7.0	0.3	0.9	0.1	2.9
6	7.9	0.8	1.7	0.1	3.1
9	7.7	1.3	0.9	0.2	2.4
13	6.6	1.3	0.9	0.2	2.4
15	6.5	1.1	0.8	0.5	2.2
17	6.7	1.0	0.9	0.7	2.3
21	6.0	1.3	0.9	0.7	1.7
Root Mean Square Error	2.1	1.07	0.89	0.6	1.27

[a]Attributes are based upon universal scales from 0 (no detection of the attribute) to 15 (highest intensity for the attribute)
[b]Manual wash consisting of dipping in distilled water, gently brushing with soft brush to remove soil. Each melon was brushed for 2 min
[c] Manual wash followed by dipping and rotating for 3 min in distilled water added with 200 mg/L sodium hypochlorite then rinsing with distilled water to remove excess chlorine
[d]P values from analysis of variance table ($P<0.05$).

Table 4 - Least squares means for different subjective color, taste and texture attributes[a] of sliced cantaloupe as affected by wash treatment of whole melons and by dosage of electron beam irradiation of cut pieces

Effect	Color	Texture			Basic taste		
		Slippery	Hardness	Juiciness	Sweet	Sour	Bitter
Wash	0.41[d]	0.40	0.67	0.29	0.25	0.55	0.22
Water[b]	1.9	0.22	4.9	8.6	3.0	0.7	0.9
Chlorine[c]	1.8	0.2	4.8	8.7	2.8	0.7	0.8
Dose	0.98[d]	0.12	0.43	0.49	0.14	0.02	0.33
0.0	1.8	0.2	4.9	8.6	2.8	0.9[x]	0.9
0.7	1.9	0.2	4.9	8.6	3.0	0.6[y]	0.8
1.4	1.8	0.2	4.8	8.8	3.0	0.7[y]	0.8
Days	0.0001[d]	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0	1.6	0.1	6.1	6.8	4.6	0.2	1.0
3	1.6	0.0	5.4	9.2	3.2	0.7	1.3
6	1.9	0.0	5.0	9.0	3.3	0.7	1.2
9	1.9	0.0	5.1	10.1	2.7	0.5	0.4
13	1.9	0.1	4.7	9.1	2.6	0.6	0.6
15	1.8	0.3	4.3	6.1	2.5	0.7	0.7
17	2.0	0.3	4.0	9.1	2.2	1.0	0.8
21	2.0	0.4	4.4	9.8	2.0	1.0	0.7
Root Mean Square Error	0.49	0.37	1.27	1.86	1.28	0.86	1.06

[a]Attributes are based upon universal scales from 0 (no detection of the attribute) to 15 (highest intensity for the attribute). Color attribute is based on a 1 to 4 scale derived from standard chart with 4 colors with the points denoting the standard colors 1= SW 6888 Pizzaz Peach, 2= SW 6889 Stirring Orange, 3 = SW 6890 Osage Orange and 4 = SW 6891 Mandarin. The values between two whole numbers indicate an intermediate shade

[b]Manual wash consisting of dipping in distilled water, gently brushing with soft brush to remove soil. Each melon was brushed for 2 min

[c]Manual wash followed by dipping and rotating for 3 min in distilled water added with 200 mg/L sodium hypochlorite then rinsing with distilled water to remove excess chlorine

[d]P values from analysis of variance table ($P<0.05$).

[x,y]LS Means for attributes with the same letter are not significantly different ($P \geq 0.05$)

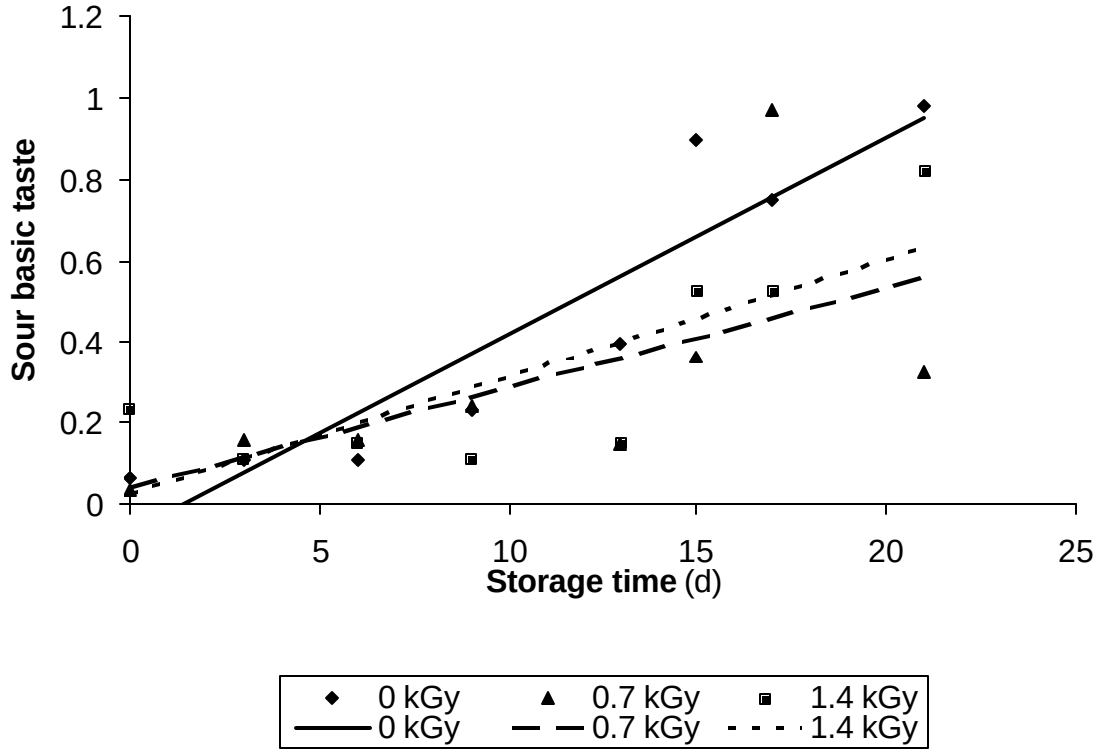

Figure 4 - Least squares means of sour basic taste due to the interaction between storage days and irradiation dose as observed by subjective sensory analysis of irradiated cantaloupe.

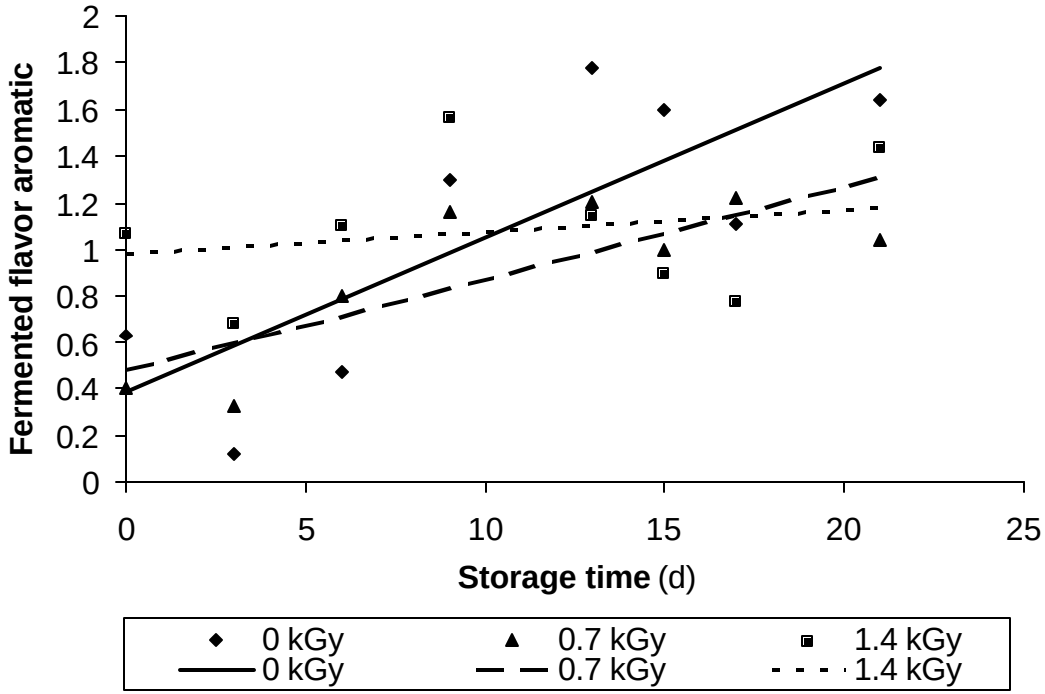

Figure 5 - Least squares means of fermented flavor aromatic due to the interaction between storage days and irradiation dose as observed by subjective sensory analysis of irradiated cantaloupe.

Cantaloupe treated with 1.4 kGy did not increase in fermented aromatic with storage even though they were initially higher at day 0 (Fig 5). This indicates that when fruit were treated with 1.4 kGy, some fermentative processes were induced but at a low level. With storage however, these fermentative processes did not continue. Sourness and fermented aroma is most significantly caused by a higher level of microbial load on the non-irradiated control than the irradiated samples resulting in greater fermentation of sugars into acid. As in objective analysis, subjective color and hardness did not appear to be significantly affected by irradiation or wash treatment. The texture analyzer used in objective texture analysis appeared to be sensitive to minute changes in firmness of cantaloupe, to an extent not perceivable to the sensory panel. As a result, although the instrument indicated difference in texture, the panel was unable to find any difference in hardness due to irradiation. Further research needs to be done to estimate the role of non-bacterial agents in the sensory quality of irradiated cantaloupe.

Reduction of *S*. Poona and native flora on irradiated sliced cantaloupe

Microbiological analysis

S. *Poona*

Electron beam irradiation reduced *Salmonella* by 1.1 log cfu/g at 0.7 kGy and 3.6 log cfu/g at 1.5 kGy, in comparison with the non-irradiated control, on day 0 (Fig. 6). The day 0 counts indicate the immediate effect of irradiation treatment on reduction of salmonellae. Storage of the irradiated cantaloupe for 21 days at 5 °C resulted in gradual reduction in *Salmonella* counts with time. However, complete elimination was not

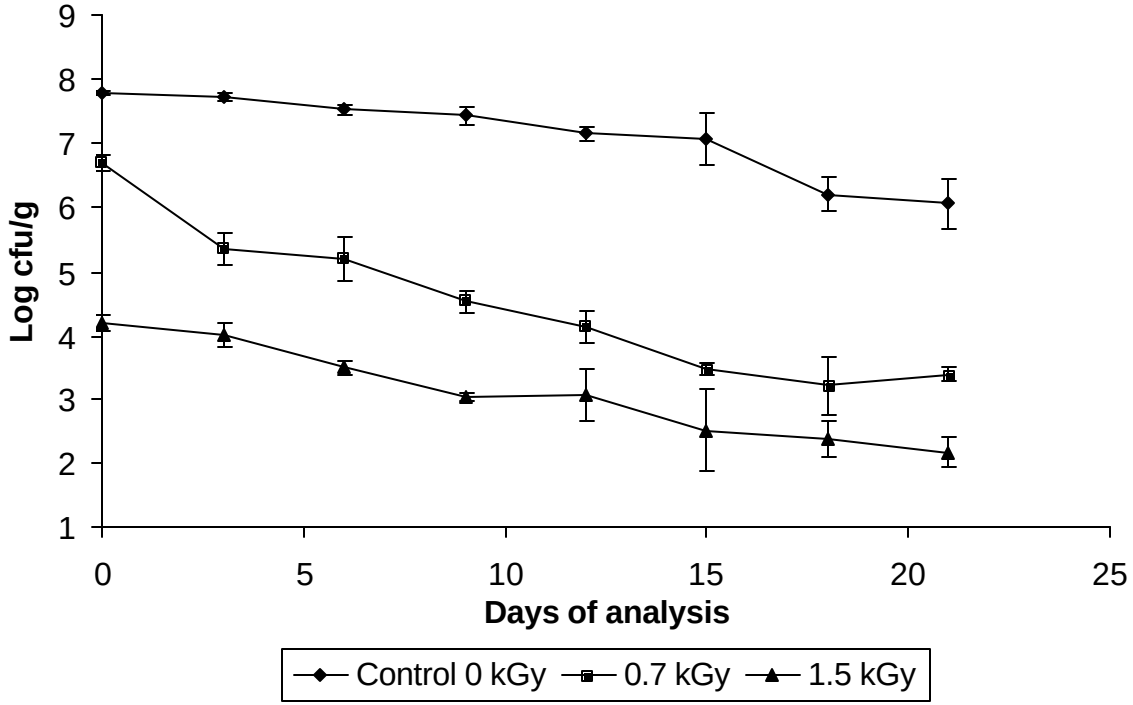

Figure 6 - Survival of *S.* Poona on irradiated sliced cantaloupe stored at 5 °C over 21 days.

observed up to the end of the study (21 days). Cantaloupe, being a nutritionally rich medium, has been shown to be a supportive medium for survival and proliferation of *Salmonella* (Golden and others 1993). Up to day 15 of storage, the decline in number was greater for 0.7 and 1.5 kGy irradiated samples in comparison to the control. After day 15, salmonellae in the control were reduced at a faster rate than the 0.7 and 1.5 kGy samples. At the end of 21 days, the non-irradiated samples had 6.0 log cfu/g while those irradiated at 0.7 kGy and 1.5 kGy had 3.4 log cfu/g and 2.2 log cfu/g respectively. This implies that irradiation of sliced cantaloupe is effective in reducing *Salmonella* and that subsequent storage of irradiated fruit at refrigeration temperature prevents proliferation of survivors. It stresses the importance of effective interventions followed by optimum storage and handling post-intervention to minimize the risk of pathogen regrowth. According to Prakash and Foley (2004), the level of contaminating pathogens in produce is usually low (= 3 log cfu/g) and hence low doses of irradiation can be effective in eliminating the threat posed by them. Taken this into consideration, the reduction that was achieved in our experiment looks encouraging. The effect of temperature on proliferation of salmonellae on sliced melons was studied by Golden and others (1993). They found that at 23 °C over 24 h of storage, *Salmonella* grew rapidly in sliced cantaloupes, honeydew and watermelons. However, at 5 °C the population did not increase in spite of having viable survivors in the samples. Our study was carried out over 21 days at 5 °C and our data shows a slow reduction in salmonellae over time. Thus, there seem to be more factors in addition to temperature of storage that contributed towards the reduction in salmonellae on cantaloupes after irradiation. One likely factor

would be the proliferation of surviving native flora such as lactic acid bacteria and yeasts that would compete with the pathogen for survival. Lactic acid bacteria, yeasts and molds can grow at refrigeration temperatures and compete with weaker survivors for space and nutrients. Thus, gradually over time, *Salmonella* may have been edged out by the native flora as seen in the dipping survival curves (Fig. 6). There has not been much work done to study the interaction of pathogens with native microorganisms on irradiated fresh produce. Lactic acid bacteria have been known to produce bacteriocins such as nisin, lacticin, pediocin, sakacin, enterocin, plantaricin and are effective biocontrol agents against a variety of gram-negative as well as gram-positive pathogens (Leverentz and others 2003). Studies report that lactic acid bacteria, specifically a strain of *Lactobacillus casei* and its culture permeate was effective in controlling pathogens like *S.* Typhimurium, *S. aureus* and *L. monocytogenes* in ready-to-use fruits and vegetables (Vescovo and others 1996; Torriani and others 1997). It is likely in our study, that the native lactic acid bacteria in the sliced cantaloupe released bacteriocins against *S.* Poona as they proliferated over 21 days of storage. Also, lactic acid bacteria may have fermented the abundant sugar present in cantaloupe to produce enough acid to lower the pH that can directly inhibit salmonellae or reduce their ability to withstand storage at low temperature.

An interesting observation in our study was the comparative reduction of *Salmonella* at different doses of irradiation. Irradiation reduced *Salmonella* on cantaloupe by 1.1 log cfu/g at 0.7 kGy and 3.6 log cfu/g at 1.5 kGy. It would be logical to expect a proportionate reduction at double the dose which is not seen in this case.

Matic and others (1990) determined the radiation resistance of two inoculum levels of *S. enteritidis*, *S. typhimurium* and *S. lille* in whole egg powder as a function of dose and storage time. They reported a similar trend in reduction as observed in our study. At 1 kGy, they reported a reduction in salmonellae of less than 1 log and at 2 kGy it was 3 logs with the non-irradiated control having 2.6 log cfu/g. When the inoculum level of the control was 4.7 log cfu/g, they reported a reduction of around 0.5 log at 1 kGy and nearly 2 logs at 2 kGy. Such disproportionate reduction was not seen at doses higher than 2 kGy in their study. This hints at the possibility that irradiation results in varying extent of bacterial killing at different dose ranges. At low doses (<1 kGy), killing seems to be at a lesser extent than that observed at higher doses (1-2 kGy). Further research needs to be done to investigate the effect of varying inoculum levels and irradiation doses on the efficiency of pathogen reduction on various commodities.

Lactic acid bacteria

Electron beam irradiation reduced lactic acid bacterial counts by 0.2 log cfu/g with 0.7 kGy and 2.9 log cfu/g with 1.5 kGy on day 0 (Fig. 7). There was no significant difference in counts between the control and samples irradiated at 0.7 kGy through the entire duration of the study. Yeasts, molds, and gram-positive spoilage organisms, such as lactic acid bacteria, are more resistant to irradiation than gram-negative organisms, such as *Salmonella* (Monk and others 1995). However at high dose (1.5 kGy), the decline in counts was far greater than at 0.7 kGy. This difference in bacterial reduction at different dose ranges follows the same trend that was observed in our results with *S.*

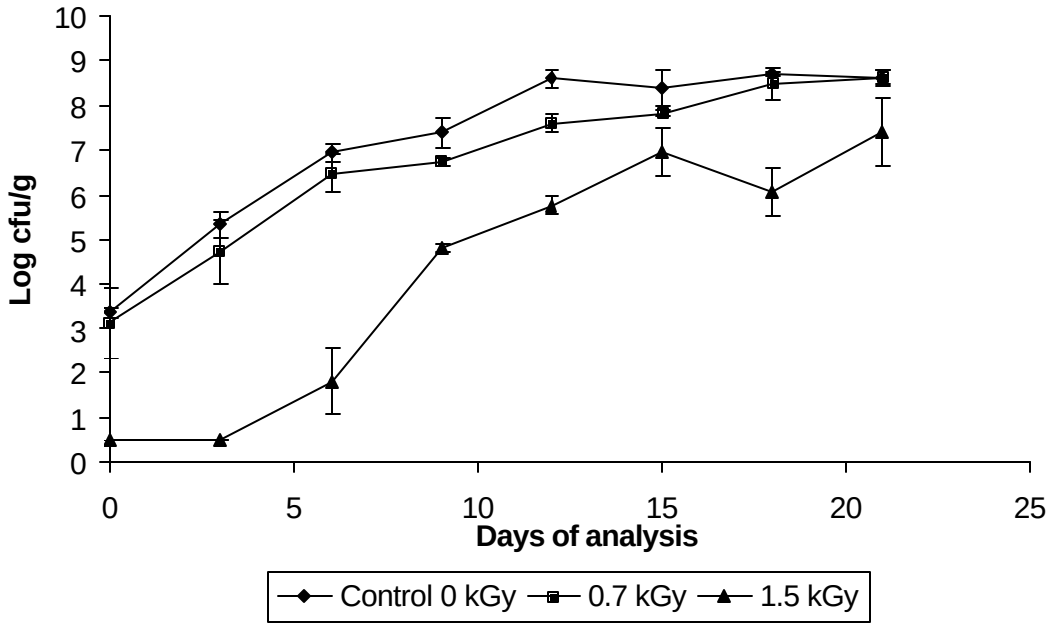

Figure 7 - Growth of lactic acid bacteria on irradiated sliced cantaloupe stored at 5 °C over 21 days.

Poona. At 5 °C, the number of lactic acid bacteria increased steadily over 21 days of storage for all irradiation doses. Studies have shown that lactic acid bacteria grow well at refrigeration temperatures especially in a sugar-rich medium like cantaloupe. They have been shown to increase by 2.3 log cfu/g on fresh-cut cantaloupe stored at 4 °C for 11 days and can also reach counts as high as 7.0 log cfu/g after storage for 12 days at 5 °C (Lamikanra and others 2000). We observed that in samples irradiated at 1.5 kGy, the lactic acid bacterial counts were below detection limit until day 4, after which they rose sharply to reach 7.4 log cfu/g by day 21. Their ability to grow rapidly at low temperatures gives them a significant advantage to proliferate and cause spoilage due to fermentation of sugars in the fruit. Thus it is important to reduce their initial numbers significantly to increase the shelf-life of fresh-cut fruit. At low temperature of storage, they have an edge over gram-negative bacteria and can effectively compete with them for nutrients. Bacteriocin production and reduction of pH by fermentation of sugars are two factors that provide them a competitive advantage over other bacterial populations. Lactic acid bacteria have been known to inhibit *L. monocytogenes*, *S. aureus* and other pathogens on a variety of commodities (Leverentz 2003). In our experiment, lactic acid bacteria proliferated over 21 days with a corresponding decline in *Salmonella* counts at a slower rate. Further research needs to be done to conclusively establish the lethal effect exerted by lactic acid bacteria on *Salmonella* in irradiated cantaloupe.

Yeasts and mold

Electron beam irradiation did not reduce yeasts to a significant extent at either

0.7 or 1.5 kGy. Yeasts have a higher resistance to radiation damage than bacteria (Farkas 2001). Over 21 days of storage the control showed a greater increase in number of yeasts than cantaloupes irradiated with 0.7 or 1.5 kGy, both of which had no significant difference in counts through the entire duration of storage (Fig. 8). At all three doses, storage over 21 days at 5 °C did not result in a big increase in number of yeasts like lactic acid bacteria. Over 21 days of storage, the increase in yeast counts in the control, 0.7 and 1.5 kGy cantaloupes were 1.5, 1.1 and 0.9 log cfu/g respectively. This may be a result of slower growth at 5 °C or competing flora.

Fungi are known to be as sensitive to irradiation as vegetative bacteria (Farkas 2001). We made a similar observation with day 0 counts where 0.7 and 1.5 kGy irradiation resulted in a 0.5 and 1.7 log cfu/g reduction in fungi compared to the control (Fig. 9). Unlike the growth patterns of *Salmonella* and other native flora, the fungal counts in the control as well as irradiated melons declined steadily with storage and dropped below detection limit by day 21. It is interesting to note that the fungal counts in the control dropped rapidly with time. This may be due to competition for sugars and other nutrients that depleted with time due to activity of other competing organisms. Fungi grow best under aerobic conditions and hence low levels of oxygen in the package may have contributed towards the reduction in fungal counts over time. Our observation, in addition to reports from other researchers, indicates that fungi do not grow well at low temperatures.

Prakash and Foley (2004) reported a 3.7-log reduction of yeasts and mold on cilantro irradiated at 0.5 kGy. This stark contrast between the reduction reported on

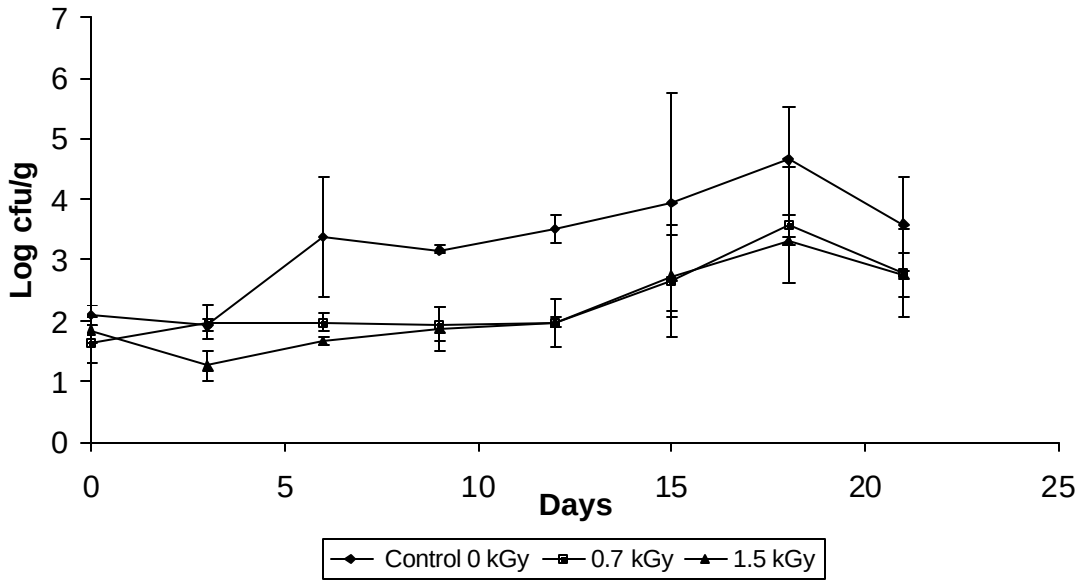

Figure 8 - Growth of yeasts on irradiated sliced cantaloupe stored at 5 °C over 21 days.

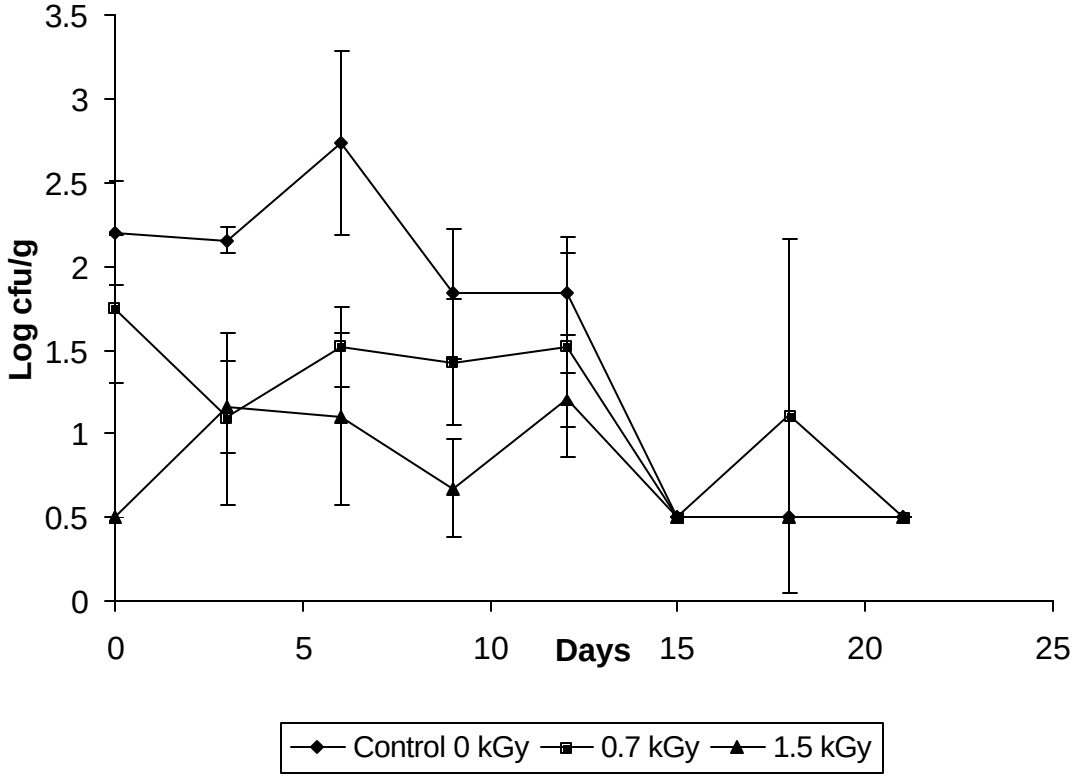

Figure 9 - Growth of mold on irradiated sliced cantaloupe stored at 5 °C over 21 days.

cilantro and our findings with cantaloupe underlines the role played by the food matrix in determining the efficacy of irradiation. In a study conducted by Aziz and Moussa (2002), they irradiated a variety of fruits at doses of 1.5 and 3.5 kGy and enumerated surviving fungi over time under refrigeration. They found that on day 0, fruits irradiated with 1.5 kGy and 3.5 kGy had an average reduction in fungal counts of around 2 log cfu/g and 3 log cfu/g respectively and even after 14 days of storage under refrigeration, they reported no increase in fungal counts. In spite of the downward trend in our observations, there was a large fluctuation in counts on certain days on analysis. No visual mold was observed in the control as well as irradiated melons after 21 days. O'Connor and Mitchell (1991) irradiated strawberries using gamma rays at 1.2 kGy and enumerated surviving yeasts and mold on day 1 and day 5 after storing the fruit at 8 °C. They experienced a large variation in counts and concluded that yeasts and mold counts cannot be used as standards for determining efficiency of irradiation. Our findings suggest that electron beam irradiation of cantaloupe is effective in reducing mold but not yeasts, and that storage at low temperature can keep surviving flora from growing to undesirable levels.

pH measurement

pH is a broad estimate of the microbiological activity in foods. A high sugar commodity like cantaloupe has a pH close to neutral when fresh. However, abundance of microflora such as lactic acid bacteria, pseudomonads and yeasts utilize the sugars to produce acidic compounds leading to off-flavor and reduced shelf-life. In cantaloupe,

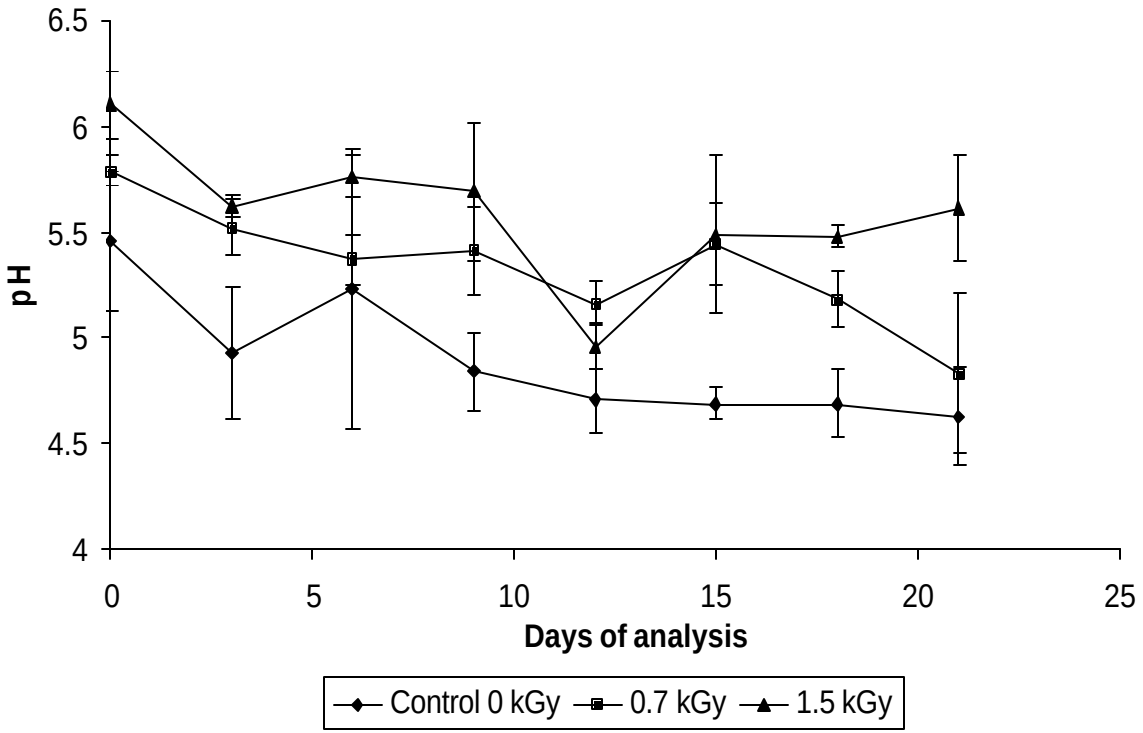

Figure 10 – Surface pH of irradiated sliced cantaloupe stored for 21 days at 5 °C.

we could associate differences in pH of the control, 0.7 kGy and 1.5 kGy doses with the activity of lactic acid bacteria (Fig. 10). The control had the lowest pH (5.46) indicating maximum fermentative activity whereas 0.7 and 1.5 kGy samples had higher pH values of 5.79 and 6.1 respectively. Thus, electron beam irradiation was capable of reducing the fermentation associated with bacterial activity at varying levels depending on the irradiation dose. This difference in surface pH with dose levels was observed throughout the 21- day storage period by the end of which there was no significant difference between pH of the control (4.63) and cantaloupe irradiated at 0.7 kGy (4.83). The cantaloupe irradiated with 1.5 kGy ended up with a pH of 5.61 and maintained its significant difference over the other two doses through 21 days. Temperature of storage may also have played a role in controlling the decrease in pH with storage. Over 21 days at 5 °C, cantaloupe irradiated with 0.7 kGy showed the highest pH reduction of 0.96 while the control dropped by 0.83 and 1.5 kGy samples dropped by 0.49.

D-value for *S*. Poona on irradiated sliced cantaloupe

Irradiation of cantaloupe discs at increasing dose levels between 0 and 1.06 kGy yielded a linear (R^2 = 0.99) dose-survival curve for *S*. Poona (Fig. 11). Each of the 10 data points is an average of *Salmonella* counts from six cantaloupe discs. There was a 4.8-log cfu/g reduction from the non-irradiated control (7.64 log cfu/g) and discs irradiated at 1.06 kGy (2.8 log cfu/g). The D-value for *Salmonella* was calculated as a reciprocal of the slope of the linear regression line (Farkas 2001). The slope of the line was - 4.371 as obtained from the linear equation y = mx + c where m stands for the slope

of the line (Fig. 11). The downward trend of the line produced a negative slope in the plot. A D-value of 0.211 kGy for *S.* Poona in cantaloupe is proposed. Numerous studies have been conducted in the past to determine the D-value for pathogens in various foods using gamma irradiation however, not much has been reported for D-value using electron beam irradiation. Numerous factors play a role in determining the radiation sensitivity of organisms and hence their D-value is specific to certain parameters under which it is determined. Composition of the medium is the most significant factor. According to Farkas (2001), cells irradiated in phosphate buffer are much more sensitive than those irradiated in foodstuffs. More the complexity of the medium, greater is the interaction of medium components with the free radicals formed from water, thus protecting the molecules. According to Pillai (2004), presence of shielding or "quenching" molecules in foods can prevent achievement of the desired level of nucleic acid damage in the matrix. This is significant in low-dose irradiation of foodstuffs where such scavenger molecules reduce the desired dose and render the previously established D-values ineffective. Other parameters that would significantly influence D-values using irradiation are temperature of the product and moisture content. Thus, it is important to mention the environmental conditions while suggesting D-values for pathogens.

D-values for *Salmonella* have been specific to species as well as strains within the same species in some cases. Research initiatives in the past have been focused on irradiation of meats with little work done on produce. Hence most of the literature cited pertains to pathogen reduction in meats. In a study conducted by Mulder (1984), D

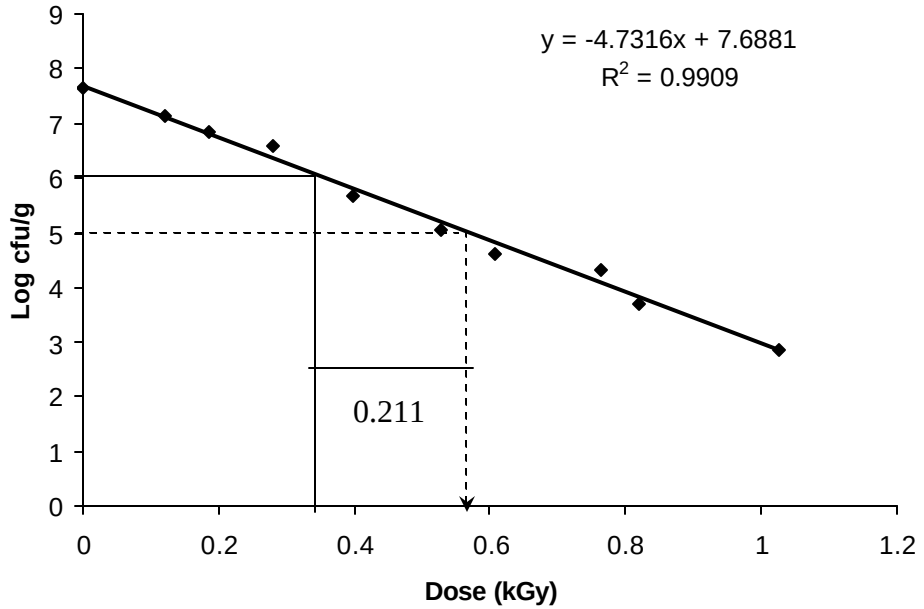

Figure 11 – Death curve of *S.* Poona with increasing doses of irradiation. The lines drawn serve the purpose of illustrating the D-value but not for calculation.

values were different for different species of *Salmonella* ranging from 0.77 kGy in whole egg for *S. give* to 0.33 kGy within the same product for *S. enteritidis. S. paratyphi* B had a D-value of 0.3 kGy in crab meat and as high as 1.07 kGy in horse meat. In ready-to-eat meats, *L. monocytogenes* displayed significant variability in radiation resistance (Sommers and Thayer 2000). D-values for *L. monocytogenes* inoculated onto commercially available beef, mixed meat and poultry frankfurters ranged from 0.49 to 0.71 kGy. Olson (1998) compiled D-values of the most significant foodborne pathogens on various meats. In chicken irradiated at 2 °C, he reported D-value for *Salmonella* spp ranging from 0.38 – 0.77 kGy. The significantly high water content (greater than 90%) of cantaloupe may be one of the reasons for achieving greater killing, resulting in lower D-value than meats.

The previous section presented the reduction of *S.* Poona on irradiated cantaloupe pieces. In that experiment it was observed that a 1.1-log reduction of *S.* Poona occurred at 0.7 kGy and 3.6 log reduction at 1.5 kGy (Fig. 6). Comparing these results with the D-value of 0.211 kGy proposed, the reductions achieved on cut cantaloupe are far less than expected. Going by the D-value, a 3-log reduction should have occurred at 0.7 kGy and a 7-log reduction at 1.5 kGy. However, due to several differences in the way the two experiments were designed, this may amount to an invalid comparison.

The effect of difference in the shape and size of the two samples on the irradiation efficiency was studied. When the cylindrical pieces of cantaloupe were laid down and irradiated, the dual electron beam was incident on a curved surface from the top and bottom of the 1 inch cylinder. When the 4-mm flat discs of cantaloupe were

used, the electron beam was incident on a flat surface. It was hypothesized that the curved surface of the cylindrical pieces achieved an inconsistent dose over the entire surface as opposed to the flat surface of the disc.

A difference existed in the method of inoculation in the 2 experiments. Cylindrical pieces were inoculated by dipping in a suspension of cells of known density and allowed to stand to let the excess suspension drain. However, the discs were placed in a dish and inoculated with a fixed volume of suspension of known density. The effect of inoculation methods on efficiency of irradiation needs to be looked at more closely to explain this hypothesis. The difference in packaging environment may also be a factor to consider. According to Farkas (2001), the lethal effect of irradiation on microbial cells increases in the presence of oxygen. In an oxygen-free environment and in the presence of moisture, radiation resistance usually increases by a factor of 2 to 4 and in dry conditions without oxygen, resistance can increase by a factor of 8 to 17 (Farkas 2001). The cylinders were irradiated in packages containing 9 % oxygen, whereas the discs were irradiated in air (20.9 % oxygen). The low oxygen levels in the packet may have contributed to the increase in resistance of *S.* Poona to irradiation treatment. D-values for any foodstuff should be established by taking into consideration all possible physical and environmental parameters beyond the characteristics of the target pathogen.

Dose-mapping of cantaloupe cylinders and cubes

Dose-mapping of cylindrical and square cantaloupe pieces using alanine films

provided substantial information on the dose distribution over a curved and flat surface. Each cylinder was covered entirely with 20 films of alanine, yielding 20 data points spanning the entire circumference of the cylinder. The distribution of dose over samples when irradiated at 0.7 kGy is shown in Figure 12. The distribution can be explained using clock positions to indicate position of dosimeters over the cylindrical surface. On the cantaloupe located at the edge of the packet, the dosimeters placed at 12:00 and 6:00 position on the cylinder absorbed identical doses of energy (0.70 and 0.72 kGy respectively). However, the dose absorbed at other points gradually decreased outward from both of those reference points and dropped to as low as 0.44 and 0.35 kGy at the 3:00 and 9:00 position on the cylinder at the edge. In the cylindrical piece located at the center, the trend was similar with doses of 0.8 and 0.88 kGy absorbed at the 6:00 and 12:00 position and gradually reducing along the circumference with the lowest doses being absorbed at the sides of the cylinder. This may have been a factor contributing to lower reductions of *S*. Poona observed on the cylindrical cantaloupe pieces in previous experiments. When a cubic piece was tested, the square cantaloupe piece had a flat plane exposed to the incident beam of electrons and, hence, there was no significant variation in dose over the sample (Fig 12). The flat plane of the cube was representative of the flat plane of the disc of cantaloupe that was used for the D-value study. This points out the difference in consistency of dose absorbance as a function of the shape of the plane exposed to the electron beam. As a result, it may not

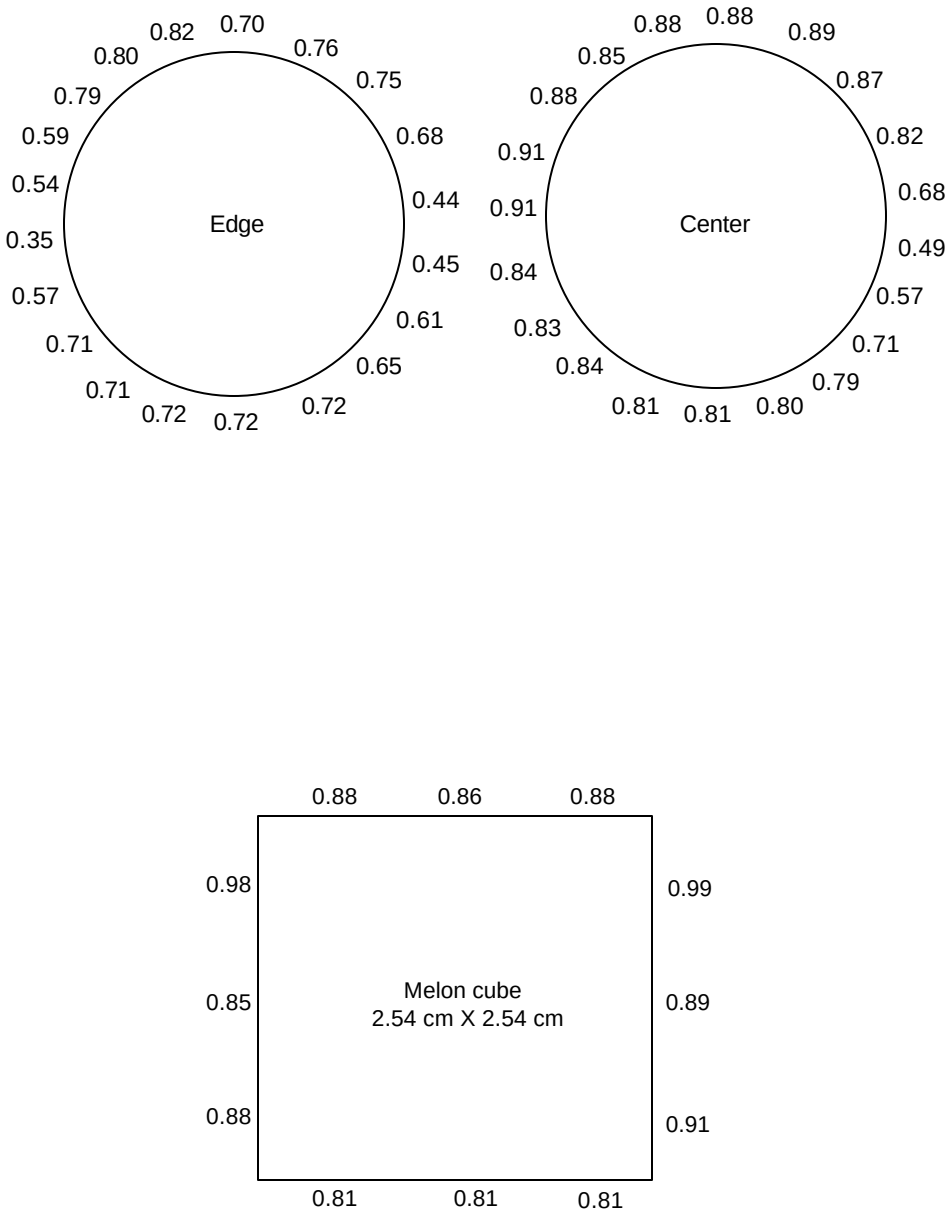

Figure 12 – Dose-maps of cylindrical and square-shaped cantaloupe pieces irradiated at 0.7 kGy with dual beam of electrons. “Edge” and “Center” denote the location of the cantaloupe piece inside the packet.

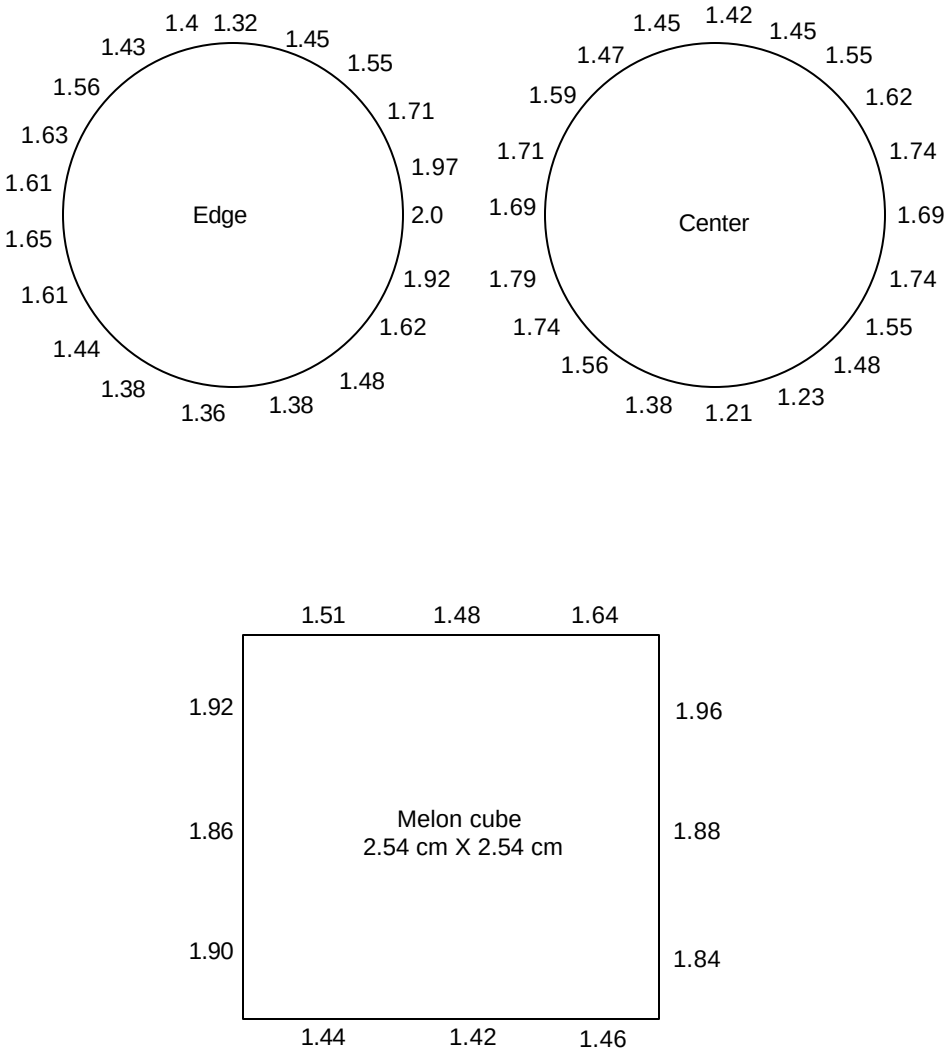

Figure 13 - Dose-maps of cylindrical and square-shaped cantaloupe pieces irradiated at 1.4 kGy with dual beam of electrons. “Edge” and “Center” denote the location of the cantaloupe piece inside the packet.

be appropriate to correlate the findings of the D-value study with the reductions in salmonellae observed on the cylinders.

The cantaloupe located at the center absorbed a slightly higher dose over the surface than the one at the edge. The reason for this is that the piece at the center was surrounded on either side by cantaloupe mass as opposed to air on one side of the piece at the edge. For the cantaloupe cylinders irradiated at 1.4 kGy, the absorbed dose was close to the target at the 12:00 and 6:00 positions of the cylinder; however, unlike the 0.7 kGy samples, the dose absorbed increased gradually towards the sides of the cylinder (Fig 13). This opposite effect at 1.4 kGy was most likely the effect of scattered electrons. At high doses of irradiation, there is an increased scatter of electrons that cause the additional absorption of dose in addition to the primary incident electrons from the beam (Maxim, personal communication 2004). At low doses like 0.7 kGy, electrons are weakened by the extra attenuation sheets and hence scatter is less for 0.7 kGy than 1.4 kGy. In case of cubes irradiated at 1.4 kGy, the absorbed dose on the flat plane was consistent as was observed at 0.7 kGy. The sides of the cube however, received slightly higher doses due to the dose accumulated in the mass along that plane. In all these estimations, it is important to take into consideration the error margin of ± 7.5 % that is inherent in the system being followed for dose delivery and measurement (Maxim, personal communication, National Center for Electron Beam Food Research, College Station, TX 2004). This experiment demonstrates that a flat surface is ideal to achieve consistency in dose distribution over the entire sample and cylindrical shapes can lead to substantial variation over the circumference.

Attachment sites for *Salmonella* on the cantaloupe rind

Confocal scanning laser microscopy

CSLM images of cantaloupe rind inoculated with GFP-expressing *S.* Poona showed bright green cells against a dark sample background. Salmonellae embedded in crevices and along the netting of the rind were observed in some images (Fig. 14). Very few images obtained were of significance, primarily because though fluorescent bacteria could be seen, the structure of the rind surface was not clearly visible. CSLM is most effective in imaging extremely thin and relatively flat samples (Mouneimne, personal communication, 2004). It has been used in imaging bacteria adhering to leafy commodities that would enable screening thin and flat specimens. Morris and others (1997) observed native flora and microbial biofilms on spinach, lettuce, parsley and broad-leaved endive. Frank and Takeuchi (2000) observed *E. coli* O157:H7 on cut edges of lettuce leaves and were able to study attachment of bacteria on leaf surfaces. Brandl and Mandrell (2002) observed the attachment of GFP-expressing *S.* Thomson in cilantro phyllosphere using CSLM and demonstrated its ability to colonize the leaf surface. In our study, the cantaloupe rind with its netting proved to be too uneven to be able to explore the entire specimen for adhering bacteria. There were some regions of the rind that were relatively flat and could be focused to provide meaningful images. Figure 14A shows fluorescent bacteria adhering along the edge of the netting and figure 14B shows bacteria colonizing crevices on the rind surface. The dark well-like regions on the rind surface appeared to be deep enough to hold bacteria. The images demonstrate the ability of bacteria to colonize crevices in the surface and possibly escape from chemical

A. **B.**

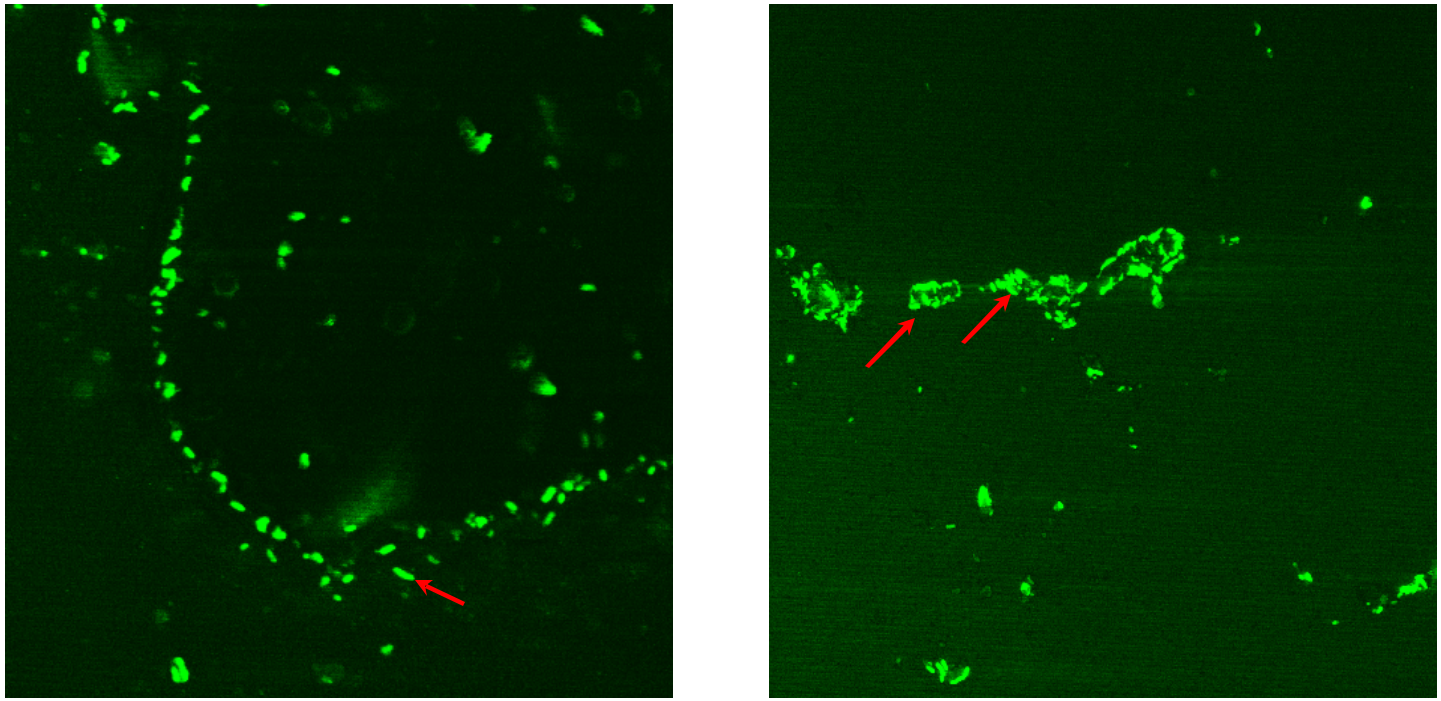

Figure 14 – CSLM photomicrographs showing the adherence of GFP-expressing *S*. Poona onto the rind surface. Red arrows point to fluorescent bacteria. (A) Bacteria lined along the edge of the netting on the rind. (B) Bacteria lodged within crevices on the rind surface.

decontaminants. There was a significant amount of green background fluorescence from the rind tissue on most of the images, making it difficult to observe bacteria. To sum it up, CSLM was found to be inadequate to explain the structure of the cantaloupe rind and likely regions of bacterial colonization on its surface. Hence, we decided to explore SEM as a possible tool to reveal bacterial adherence sites on the rind.

Scanning electron microscopy

SEM images provided fine structural details of the cantaloupe rind that were unable to be seen using CSLM. Figure 15 reveals the distinct structural difference between two regions of the cantaloupe rind; the deeply fissured and raised netting (A) and the relatively smoother skin (B). The netting appeared to be extremely rough with abundant deep crevices and compartments that harbored a much greater amount of bacteria in its complex structure than the smooth skin of the rind (Fig 15). Salmonellae were clearly visible over the entire specimen and could be imaged at very good resolution digitally as well as on Polaroid film. The bacteria colonizing the netting have the potential of forming microcolonies and escape removal by chemical decontaminants. Very few studies have imaged the surface of cantaloupes to observe attachment sites of bacteria. Annous and others (2004) observed the development of biofilms within the netting using SEM images of the rind. Most of the attention has been focused on the structural aspects of the netting and the skin but not on surface structures such as trichomes or lenticels that are abundant on the rind. Trichomes are hairy outgrowths from epidermal cells of plants and are found in large numbers on the melon surface (Fig

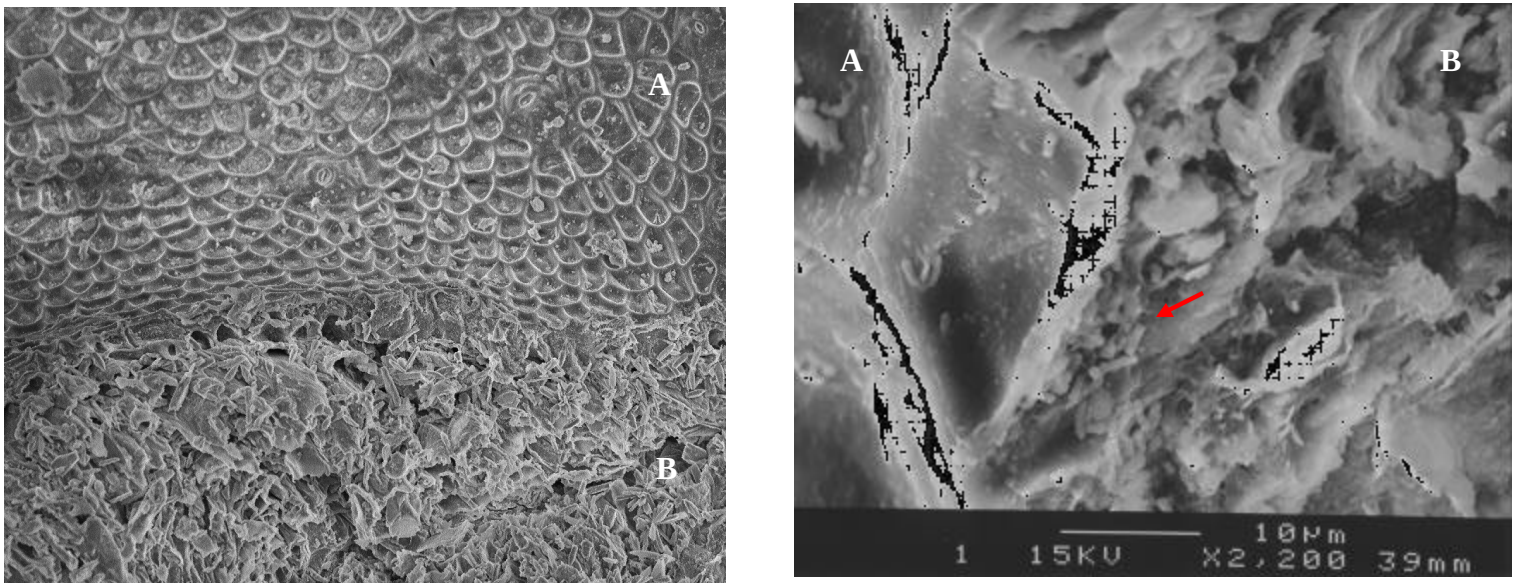

Figure 15 – Scanning electron micrographs of the cantaloupe rind. (A) The smooth, non-netted region of the rind harbors fewer bacteria than the deeply fissured, rough and complex structure of the netting (B). The red arrow points to a cluster of salmonellae colonized inside the fissures of the netting.

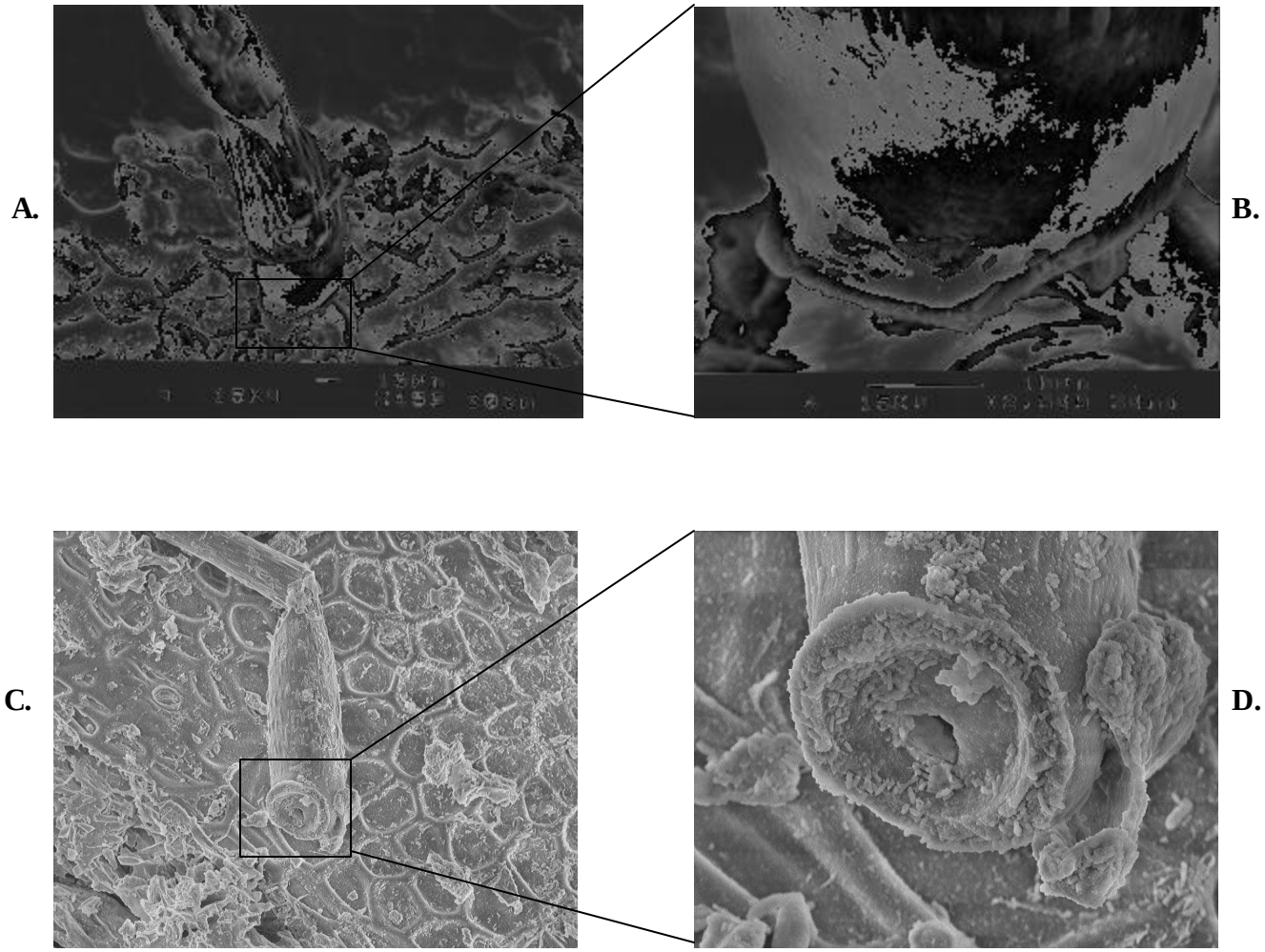

Figure 16 – Scanning electron micrographs of salmonellae associated with the trichomes on the cantaloupe rind. (A) Intact trichome with salmonellae clustered at its base (B). A broken trichome (C) with its base heavily colonized by bacteria (D).

A.

B.

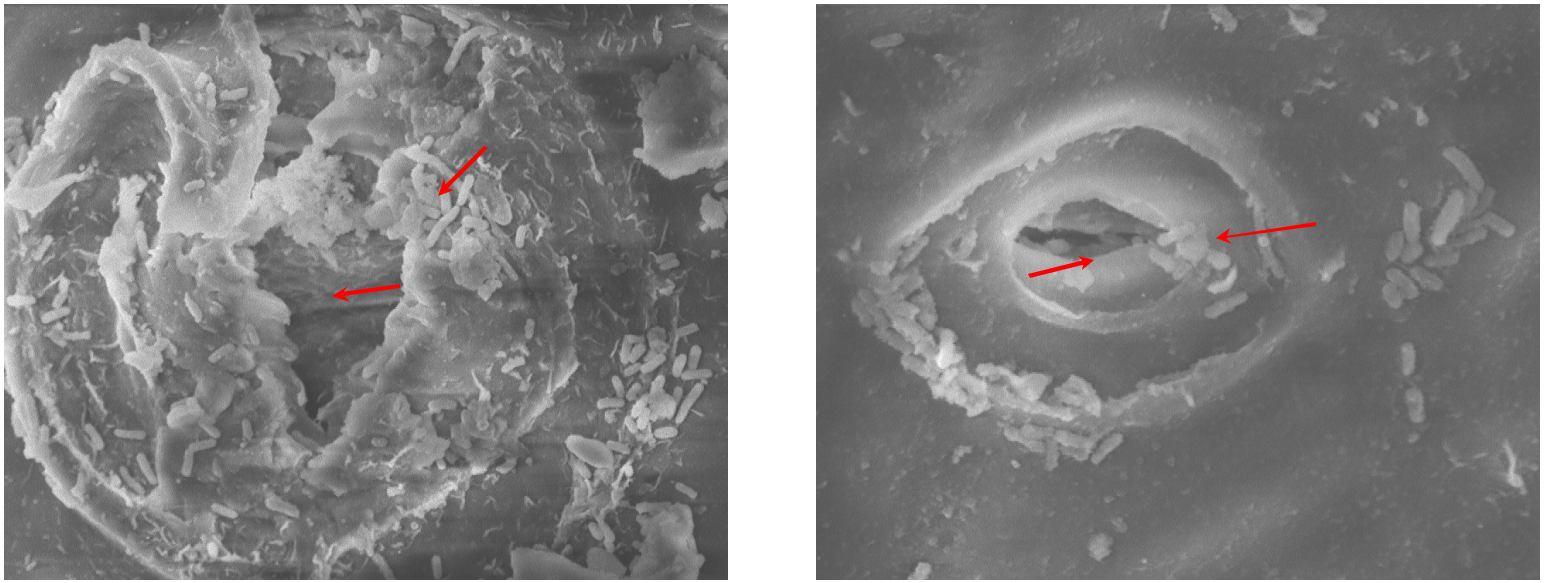

Figure 17 – Scanning electron micrographs of natural openings on the cantaloupe rind. (A) The point of attachment of a broken trichome to the cantaloupe rind. The red arrows indicate the channel going into the rind and also a cluster of salmonellae close to the opening into the rind; (B) Lenticel opening on the rind with salmonellae penetrated all the way into the opening as pointed by the red arrows.

16A). The inoculated salmonellae were found to associate with these structures and accumulate themselves within them. Figure 16B shows one such trichome with salmonellae at its base. We also observed trichomes with cracks or openings near their base which may be due to abrasion or wear and tear. Bacteria can enter these openings and colonize the trichomes from inside. Trichomes appear to be hollow and tubular as seen in Figure 16C of a broken trichome with its base exposed. The magnified image of its base exposes the colonization sites on the inner regions of the trichome (Fig. 16D) which may shield bacteria from direct contact with decontaminants. An image believed to be of the point of contact of a trichome that broke off from the rind, shows salmonellae densely colonizing the region. The region also shows a channel going into the rind which may act as an access point for internalization of pathogens into the melon rind (Fig. 17A). Lenticels are widespread and numerous over the rind non-netted region of the rind surface. Figure 17B shows a lenticel opening on the rind with salmonellae present at the opening and also deep into the cavity. The lenticels open and close for exchange of gases through the rind and can be another likely point for internalization of pathogens into the rind.

SEM was far better than CSLM in determining the sites of attachment and colonization of *S.* Poona on cantaloupe rind. SEM images of *S.* Rubislaw on oranges presented by Pao and others (2001) were better understandable than their stereomicroscopy images which did not provide clear structural details of the orange stem scar region. From our findings it is evident that pathogens like *Salmonella* can lodge themselves in sites inaccessible to chemical sanitizers and can penetrate the

surface through openings or with the knife during cutting. Thus, we propose that physical treatment like irradiation can be an effective intervention for elimination of these deep-rooted or sub-surface internalized pathogens in cantaloupe.

CONCLUSIONS

Electron beam irradiation at low doses was found to be an efficient tool for decontamination as well as extension of shelf-life of sliced cantaloupe. However, it is best applied in combination with the existing methods of chemical decontamination. Our study shows that a combination of chlorine wash of pre-cut cantaloupes and low dose electron beam irradiation can be an excellent tool for ensuring the reduction of spoilage organisms and extension of shelf life of sliced cantaloupe. Irradiation was effective in reducing the aerobic microbial load of sliced cantaloupe. It was also found to be effective in reducing levels of *S.* Poona increasingly at 0.7 and 1.5 kGy. The D-value for *S.* Poona on electron beam irradiated sliced cantaloupe was found to be 0.211 kGy. Irradiation followed by storage at 5 °C was efficient at pathogen reduction and prevention of proliferation of survivors. Spoilage organisms varied in their response to irradiation. Lactic acid bacteria were reduced appreciably at 1.5 kGy but not as much at 0.7 kGy. Yeasts seemed to be unaffected by irradiation as the reductions obtained at low doses were minimal. Mold was reduced by irradiation as effectively as vegetative bacteria and was in agreement with previous reports. The sensory data indicated that the color and texture of melons was not affected appreciably at 0.7 kGy. Also, no major off-flavors were detected other than the low level of sour taste and fermented aroma due to microbial growth in the control and to a small extent in the low dose sample. Thus, with the use of chlorine decontamination, irradiation and modified atmosphere packaging, the shelf life of fresh cut cantaloupe was successfully extended to more than 10 days.

Factors such as shape and size of the foodstuff being irradiated affect the

uniformity and hence effectiveness of absorbed dose. Thus, it is important to suggest D-values for pathogens after taking into consideration several physical, chemical and processing parameters of the commodity of interest. Scanning electron microscopy images of salmonellae on the rind of cantaloupe demonstrate the ability of pathogens to lodge themselves in areas possibly inaccessible to chemical washes. In such a scenario, irradiation of whole cantaloupe may be an excellent option if uniform dose can be delivered over its entire surface.

From this research, it is proposed that electron beam irradiation can be effective at the last step in the processing of packaged fresh-cut produce after all the existing steps in chemical decontamination. The proposed combined technology for shelf life extension may have the potential to be applied to various other produce commodities to address quality and shelf life issues faced by the fresh-cut produce industry.

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