EFFECT OF ELECTRON BEAM IRRADIATION ON QUALITY AND SHELF-LIFE OF TOMMY ATKINS MANGO (*Mangifera indica* L.) AND BLUEBERRY (*Vaccinium corymbosum* L.)

A Thesis

by

MARIA ALEXANDRA MORENO TINJACA

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

MASTER OF SCIENCE

December 2005

Major Subject: Biological and Agricultural Engineering

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Approved by:

Chair of Committee, Elena Castell-Perez
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ABSTRACT

Effect of Electron Beam Irradiation on Quality and Shelf-life of Tommy Atkins Mango (*Mangifera indica* L.) and Blueberry (*Vaccinium corymbosum* L.).

(December 2005)

Maria Alexandra Moreno Tinjaca, B.S., Universidad de La Salle

Chair of Advisory Committee: Dr. Elena Castell-Perez

The main goal of this research was to determine the feasibility of using electron beam irradiation as an alternative disinfestation technology while preserving the overall quality of mangoes, and to verify its suitability for the preservation shelf life of blueberries.

Physicochemical and sensory characteristics of the fruits were evaluated. Mangoes were irradiated at 1.0, 1.5 and 3.1 kGy using a 10MeV (10 kW) linear accelerator (LINAC) with double beam fixture. Samples were stored at 12ºC and 62.7% RH for 21 days. Blueberries packed in plastic clamshell containers were irradiated at 1.1, 1.6 and 3.2 kGy doses using the same linear accelerator with a single beam. The shelf life of the berries stored at 5˚C and 70.4% RH was evaluated for 14 days. The firmness of mangoes irradiated at 1.5 and 3.1 kGy significantly ($p > 0.05$) decreased during storage. There was a reduction of total sugars (8.1% and 14.1%) in samples irradiated at 1.0 kGy and 1.5 kGy, respectively. All irradiated mangoes had significantly lower (50-

70 %) ascorbic acid content throughout storage. The phenolic compounds increased in samples irradiated at 1.5 kGy (27.4%) and 3.1 kGy) (18.3%). Sensory evaluation of the fruits irradiated with 3.1 kGy showed significantly less acceptability for overall quality, color, texture and aroma. Irradiation of blueberries at 1.1 kGy had no significant ($p > 0.05$) effect on the fruits' physicochemical characteristics with the exception of ascorbic acid which decreased by 17% after 14 days. A significant decrease in texture (firmness) of irradiated berries was observed during storage time. Total sugars decreased in all irradiated fruits while total phenolics and tannins increased (10 -20%). Sensory attributes of samples irradiated with 1.1 kGy and 1.6 kGy were found acceptable by the panelists. The high dose-treated fruits were considered unacceptable.

The results from this research suggest that a 1.5 kGy is the best treatment to maintain the quality attributes of mangoes and increase the shelf life by three days. The electron beam irradiation of packed blueberries at doses of 1.1 and 1.6 kGy ensures and enhances the quality and the shelf life of blueberries up to 14 days.

DEDICATION

To my mother for all her support, effort, and dedication, that have helped me to accomplish this goal. Thanks for always being there.

To my brothers for their support, optimism, and trust in me.

To Ann Marsh for her support and her constant words of encouragement.

To God for giving me all the above and always being my guiding light.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my advisor, Dr. Elena Castell-Perez, for her invaluable support, guidance, patience, encouragement and motivation throughout my graduate studies. I also express my gratitude to Dr. Rosana Moreira for her time, support, and advice. I greatly value the knowledge that you all have passed on to me. I would also want to acknowledge Dr. Luis Cisneros-Zevallos for being part of my committee, for his advice, and for allowing me to do part of my research in his lab.

A special thanks to my friends Paulo and Carmen, for all their assistance during the development of the experiment. I also want to thank them for their unconditional friendship.

I would like to thank all the students in the Food Engineering Lab. They always had kind words and warm smiles for me. Thanks to Kim for all his support.

A special thanks to Sergio Capareda, Mr. Larry Nielsen, and Brian Jones. I appreciate all their unconditional help during the development of the volatiles analyses.

Thanks to all my friends for being supportive throughout my educational journey at Texas A&M University.

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CHAPTER I

INTRODUCTION

Fruits are important constituents of the human diet and they are rich sources of nutrients such as vitamin C, provitamin A carotenoids, and minerals. However, their high moisture content and the presence of macronutrients such as sugars make them vulnerable to spoilage by microorganisms and insects, therefore limiting their shelf-life and marketability.

Food irradiation has been identified as a new technology that can eliminate pathogenic microorganisms such as *Salmonella, E.coli* O157:H7 and *Campylobacter* from raw foods in both the fresh and frozen state (ICGFI, 1999). The application of electron beam irradiation to fresh fruits and vegetables is a way to extend shelf life and to improve disinfestation and pasteurization treatments. Dose levels of 0.5 to1.0 kGy, depending on the type of fruit, are sufficient to kill large numbers of most molds, yeasts and bacteria naturally present on the product. Lethality of irradiation is influenced by the target (insect or microorganism), the condition of the treated product, and environmental factors such as temperature, nutrients, pH, and presence/absence of oxygen.

Compared with other disinfestation methods such as the use of pesticides (which are chemicals like ethylene dibromide, methyl bromide, phosphine, etc.), electron beam irradiation may be an efficient technique that assures complete disinfestation and there

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This thesis follows the style and format of the *Journal of Food Engineering.*

would be no fumigant residues which require post-treatment. The practicability of applying ionizing radiation as a disinfestation technique depends on the fruit tolerance to the irradiation dose. Irradiation at doses below 1.0 kGy is an effective insect disinfestation treatment against various species of fruit flies, orange worm, spider mites, scale insects, and other insect species of quarantine significance in marketing fresh fruits. Decay produced by original microflora and post-handling contaminants can be eliminated or delayed by dose levels that do not affect the sensory qualities such as color, texture and flavor. However, physiological and compositional changes have been reported as a response to gamma irradiation (Lacroix et al., 1992; Beyers et al., 1983). Some alterations in quality induced by irradiation doses are concerned with softening, texture loss, and flavor changes. Citrus fruits, for example, are particularly susceptible to skin pitting and additional treatments are required to reduce the radiation doses, but in the case of fruits like strawberries it has been demonstrated that the application of an irradiation dose of up to 2.0 kGy by itself delays the development of spoilage microorganisms responsible for softening of the tissue (ICGFI, 1999).

Most of the studies of irradiated foods have been carried out with foods irradiated with gamma rays. Therefore, there is a gap in the knowledge and understanding of the effects of electron beam irradiation in the physical and organoleptic quality attributes of fruits. The new knowledge is critical because it is important to maintain a balance between the optimum doses required to achieve safety and the minimum change in the quality of the fruit.

This research focused on the application of new advances targeted to the preservation of physicochemical properties of selected fruits and consequently in the reduction of the risk of postharvest decay. The benefits of this study are not only to the food industry but also to the retail markets and the consumer. To date, there is a lack of information about the effect of this irradiation treatment on popular fruits such as mangoes and berries. Thus, the aim of this study was to evaluate the effect of electron beam irradiation on the physical, sensory and chemical properties of the two fruits.

The main goal of this study was achieved by addressing the following specific objectives:

- To identify radiation levels at which loss of quality of the selected fruits is minimized.
- To quantify the changes in the physical, sensory and chemical properties of the irradiated fruits using kinetics principles.

CHAPTER II

LITERATURE REVIEW

From 1982 to 1997, per capita consumption in the United States of fresh fruits and vegetables increased from 91.6 to 121.1 kg, an increase of 32% (CDC, 2000; FDA, 2003). This fact is associated in part with the effect of the significant role of these commodities as a part of a healthy diet, and it also involves economic benefits to the industry and the grower. However, it has been established that pathogenic microorganisms related to the consumption of these products can cause disease outbreaks (Beuchat, 1998). For instance, raw raspberries and blackberries imported from Guatemala have been associated with several *Cyclospora cayetanesis* outbreaks (CFSAN.FDA, 2001); thus there is a need for new or improved technologies that reduce risks associated with these foodstuffs.

Pathogens related to foodborne diseases are sensitive to low levels of ionizing radiation. As irradiation doses increase, more microorganisms are affected (Radomski et al., 1994). However, a higher dose, while not creating any harmful products, can induce changes in sensory qualities and chemical properties. For example, in the irradiation of mangoes with gamma rays, the concentration of phenolic compounds was higher in fruits irradiated at l.5 kGy compared with the controls; also, irradiation at 0.5-1.5 kGy caused a minor loss of vitamin C (El-Samahy et al., 2000). In studies of oranges, minor differences were found in the evaluation of the sensory quality of navel oranges treated with low doses (60-80 Krad) of gamma radiation (O'Mahony et al., 1985). It is,

therefore, important to understand how irradiation can be used as an efficient treatment strategy that will show the way for producing more high quality and safer food.

2.1. Fruits in this study

2.1.1. Mango

2.1.1.1. Botany background

The mango (*Mangifera indica* L.) is a dicotyledonous fruit of the family Arcardiaceae. It is produced mainly in the developing countries in the tropics. Two groups of mango cultivars are found: Indian and Indo-Chinese, based mainly on peel pigments and sensory characteristics of the fruit. Most of the Indian varieties posses stronger aroma and more intense peel coloration and are monoembryonic, while the cultivars grown in the Southeast Asian region are polyembrionic (Lizada, 1993). It is the largest subtropical fruit crop in India which occupies an area of 1.17 million ha with a production of 9.6 million tons (66% of world's production) (Mitra, 1997).

Mangoes are classified as climacteric fruits and ripen rapidly after post harvest. The final quality depends on the physiological processes during ripening and also on the process of development and maturation. As the fruit matures, bloom develops as wax is deposited on the peel (Lizada, 1993). Usually the development of mango fruits is divided into four stages: (a) the juvenile stage, up to 21 days from fertilization (rapid cellular growth; (b) stage of maximum growth, 21-49 days from fertilization; (c) maturation

(respiration climacteric and ripening), 49-77 days from fertilization; and (d) senescence.

Different biochemical changes occur according to the stage (Hulme, 1971).

Mango fruits are tropical in origin and therefore chilling sensitive. Temperatures low enough to delay ripening injures these fruits. Storage temperature varies depending on the fruit variety. Between 12ºC and 13ºC is considered the optimum temperature for mango storage and will give a shelf-life of 14 to 18 days for mature green fruit depending of the variety (Mitra, 1997).

2.1.1.2. Chemical composition

Mangoes are a rich source of vitamin C, provitamin A carotenoids, mineral salts and carbohydrates and they are liked for their flavor and taste (Thomas, 1986). Table 2-1 presents the average chemical composition of mango. Mango is one of the most popular tropical fruits consumed in the United States. Its per capita consumption from 1980 to date has increased by approximately 88% (Figure 2-1).

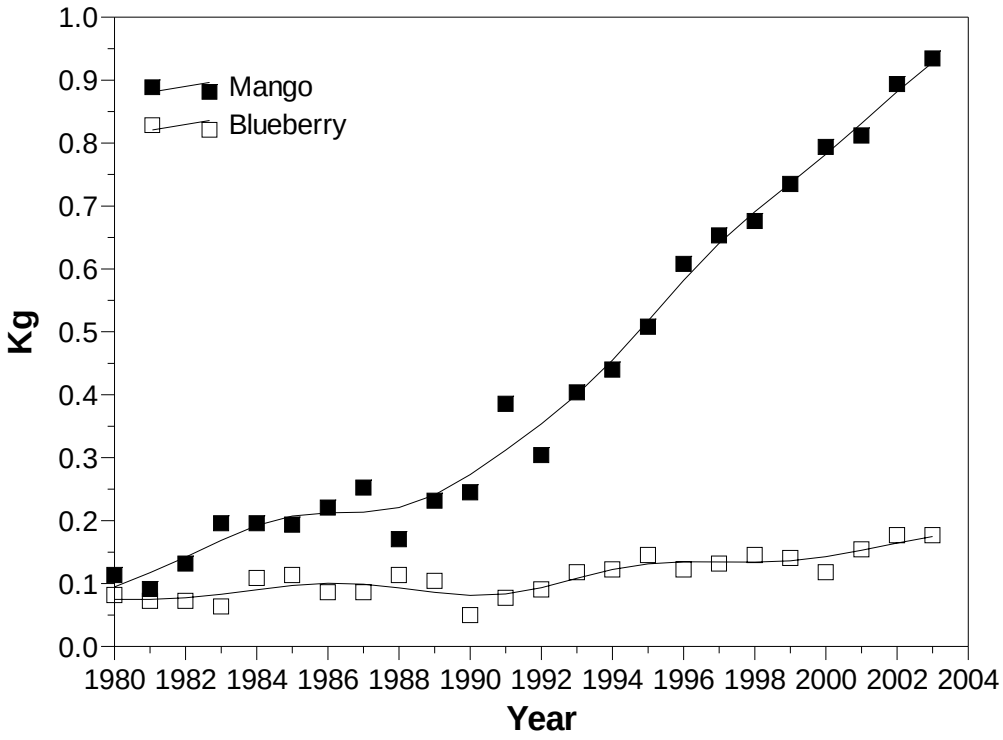

Figure 2-1. Per capita consumption of fresh fruits: mangoes and blueberries in the U.S. (adapted from USDA, 2004).

Table 2-1
Raw mangoes (*Mangifera indica*) nutritional composition data (amount in 100 grams of edible portion)

Nutrient	Units	Amount in 100 gr
Water	g	81.71-82.40
Energy	Kcal	57.00-65.00
Protein	g	0.51-0.70
Total Lipid	g	0.20-0.27
Ash	g	0.50
Carbohydrate	g	14.1-17.0
Dietary Fiber	g	1.8-2.6
Calcium, Ca	mg	10-12
Magnesium, Mg	mg	9-13
Phosphorus, P	mg	11-16
Potassium, K	mg	156-180
Vitamin C, total ascorbic acid	mg	27.7-37
Folates	µg_DFE	14
Vitamin A, IU	IU	765
Total Sugars	g	13.8-14.8
Fructose	g	3.0
Glucose	g	0.7
Sucrose	g	10.1
Alpha-carotenes	µg	16-27
Beta-carotenes	µg	445-696

Adapted from: USDA Nutrient Database for Standard Reference, Release 16-1 (2004)

2.1.1.3. Use of ionizing irradiation for fruit preservation

Fruit flies and stone weevil infestation have been a problem associated with quarantine and mango decay (Wolfenbarger, 1995). Stone weevil has been reported to occur in countries like Bangladesh, Cambodia, India, Indonesia, Japan, Malaysia, Mauritius, Mozambique, Philippines, South Africa, Vietnam, Thailand, USA, and Zanzibar. The mango weevil, *Cryptorynchus mangiferae*, is a pest that is found in

mangoes where eggs are laid on immature fruits and the emerging larvae develop, pupate and emerge as adults in the seed. The adult weevil then leaves the seed and tunnels through the edible flesh which provides access for secondary fungal infections and consequently affects the fruit's marketability and acceptability (Thomas, 1986). Therefore, it is of economic importance to have quarantine restrictions on mangoes that reduce the risk of introducing these pests in the US (Singh, 1989).

Fumigation with organic chemicals has been used for years for postharvest control of fruit flies but there is a concern about residue and human health effects (Wolfenbarger, 1995). Irradiation has been used as an effective technique for quarantine purposes and decay control. The percentage of decayed mangoes fell when the fruits had been irradiated. Von Windeguth (1986) determined that 75 Gy of gamma irradiation killed 99.9968% of *A. suspensa* on mangoes and 175 Gy provided quarantine security of adults of this insect. Milne et al. (1977) also reported that irradiation at doses above 50krad resulted in 100% mortality of mango weevil larvae and pupae and adults surviving treatments were inactivated at doses above 0.75 kGy and they were unable to emerge from the seed. Note: 1 kGy = 100 krad.

Several studies have shown that gamma irradiation at low levels extend the shelf life of mango fruits by slowing down the rates of ripening and senescence (Thomas, 1986). For instance, a delay in ripening of fruits was observed by Spalding and Reeder (1986) with irradiation treatment doses of 150, 250 and 750 Gy. Earlier studies also showed a delay in ripening of irradiated mangoes at doses up to 250 Gy of gamma rays, manifested by degreening of the peel (Dhakar et al., 1966).

In the USA between 1986 and 1987, gamma irradiated mangoes, Hawaiian papayas and apples were marketed and sold well in different states. Later, other tropical fruits and irradiated poultry products showed an increase in their consumption and acceptance by consumers (ICGFI, 1999).

2.1.2. Blueberries

2.1.2.1. Botany background

Blueberries belong to the genus *Vaccinium* in the heath family (Ericaceae). They are solely North American in origin. Four species are cultivated: 1) highbush (*V. corymbosum L*), 2) lowbush (*V. myrtilloides* Michx. and *V. angustifolium* Aiton), and 3) rabbiteye (V. *ashei* Reade). The commercial production of rabbiteye blueberries is largely confined to the southeastern United States, centering on Georgia and extending to North Carolina and to Texas. Lowbush blueberries are cultivated primarily above latitude 44ºC in the northeastern United States and Maritime Provinces of Canada. Most highbush blueberries are grown in the Midwestern, eastern, and central United States and along the Pacific coast of the northern United States and southern Canada (Caruso & Ramsdell, 1995).

The highbush blueberry is the most commercially important blueberry with annual production of about 55,000 t of fruit on about 14,000 hectares in North America. Its production is correlated with the altitude. The blueberry production season begins in Florida in mid-late April and follows in North Carolina, Arkansas, Georgia, New Jersey, Michigan, and Maine, in that order. In the Southern Hemisphere, blueberry production begins in December and extends for as long as four months (Caruso & Ramsdell, 1995).

Blueberries have very fine fibrous root systems that do not develop root hairs. Roots begin growth before bloom and can continue to grow well into the fall. Peak root growth occurs in the spring and early fall. The fruit contains many seeds and is held on corymbs or racemes. Their size depends largely on the number of developed seeds. Anthocyanis pigments give the fruits their characteristic color. Blueberry fruit enlarges after pollination according to a double sigmoid growth curve and goes through several phases of color development from immature green to translucent greenish white, greenish pink, blue-red, and finally a complete blue. Up to 50% of the increase in blueberry volume occurs during the shift from greenish pink to blue. Fruit are ripe 40-80 days after blooming, depending on cultivar and environmental conditions; the rabbiteye specie develops most slowly (Caruso &Ramsdell, 1995).

Berries should be cooled as soon as possible after harvested, ideally to 0˚C within 2 hours of harvest (but no later than 12 hours), to retain their optimum quality. The optimum storage temperature is between -0.5˚ and 0˚C, and the optimum humidity is 90 to 95%. Berries that are free of physical damage, have low sugar to acid ratio, are precooled to 0˚C and are stored to optimum humidity and temperature can have an acceptable storage life of 2-3 weeks (Eck, 1988).

2.1.2.2. Chemical composition

Blueberries are low in fat, sodium free and a good source of both fiber and vitamin C (Hulme, 1970). They have high antioxidant capacity. Table 2-2 shows the chemical composition of blueberry.

Table 2-2
Raw blueberries (*Vaccinium spp*.) nutritional composition data (amount in 100 grams of edible portion)

Nutrient	**Units**	**Amount in 100 gr**
Water	g	84.21
Energy	Kcal	57
Protein	g	0.74
Total Lipid	g	0.33
Ash	g	0.24
Carbohydrate	g	14.4
Dietary Fiber	g	2.4
Calcium, Ca	mg	6
Magnesium, Mg	mg	6
Phosphorus, P	mg	12
Potassium, K	mg	77
Vitamin C, total ascorbic acid	mg	9.7
Folates	µg_DFE	6
Vitamin A, IU	IU	54
Total Sugars	g	9.96
Fructose	g	4.97
Glucose	g	4.88
Sucrose	g	0.11
Beta-carotenes	µg	32-35
Luthein-Zeaxanthin	µg	80

Adapted from: USDA Nutrient Database for Standard Reference, Release 17 (2004)

2.1.2.3. Irradiation of blueberries

Fruits such as citrus and berries are the main suppliers of vitamin C intake for men and women (Hagg et al., 1995). It is well established that citrus and berry fruits are a rich source of vitamins, minerals and dietary fiber that are essential components for normal growth and development. Additionally, berries contain phenolic substances like flavonols, tannins, and anthocyanins that are of special significance because of their contribution to the color, taste and flavor of the fresh fruit (Taylor & Tucker, 1993). On the other hand, as antioxidants and anticarcinogens they have a protective effect against chronic diseases, including cancer and heart disease (Philip & Chen, 1988). The content of micronutrients, sugars and acids in these fruits influences markedly their sensory quality. For example, softening of the tissue is associated with changes in nutritional composition. In addition, it has been reported that phenolic acids contribute to the dark color, bitter taste, and objectionable flavour of some fruits and leaves (Ayaz et al., 2000).

Besides all their nutritional properties, these fruits have economic benefits for the food industry and its consumers. North America (83%) is the primary producer of blueberries (USA: 55% and Canada: 28%). Between 2000 and 2002 the U.S. fresh blueberry exports averaged 38 million pounds, increasing in share of domestic production (44%). For instance, the U.S. production sent to Japan accounts for 7 %, up from only a fraction in the 90's (USDA, 2003). Their per capita consumption in the US has increased from 1987 to date about 54% (Figure 2-1).

Because many decay organisms such as anthracnose (*Gleosporium spp*), gray mold rot (*Botrytis cinerea*), and alternaria (*Alternaria spp*.) result in spoilage,

blueberries lose considerable market (Miller et al., 1994b). The principal insects that inhibit distribution of blueberries are the apple maggot (Ragoletis *pomonella*), blueberry maggot (*R. mendax* Curran) and plum curculio (*Conotrachelus nenuphar*) (Miller & McDonal, 1996). Lower dosages of gamma irradiatiation have been used successfully to increase the shelf life and control decay in some fruits without significant effects on quality. For example, Miller et al. (1994) reported that Climax and Sharpblue blueberry cultivars tolerated gamma irradiation up to 0.75 kGy without a loss of fruit quality. Eaton et al. (1970) found significant cultivar variation in the physiological response of high-bush blueberries to gamma irradiation at doses from 1.0 to 5.0 kGy. Hallman and Thomas (1999) reported that the prevention of phanerocephalic pupae of blueberry maggot at the 99% level was accomplished with the application of gamma irradiation at levels of 58 and 24 Gy. Miller et al. (1995) observed that Sharpblue blueberries appear to tolerate electron beam irradiation at 0.75 kGy as an acceptable nonchemical quarantine treatment.

Little information is available on how electron beam irradiation affects physiological and physical characteristics of the fruits or on how effective this treatment may be for the control of blueberry decay. Therefore, it is important to consider an approach that maintains food quality, extends shelf-life, and offers economic incentives for production and commercialization of this commodity.

2.2. Food irradiation

2.2.1. Overview

Finding the ways to prevent the deterioration of food and to control infection by microorganisms has been a big concern over the years. Controls such as refrigeration, canning, and pasteurization are usual but food irradiation techniques are being used more often and as a result are being more closely related to public health issues.

Food irradiation is a non-thermal energy method used to "pasteurize" foods. Irradiating food basically means using non-thermal energy that results in the lethal-destruction of microorganisms responsible for foodborne illness (Josephson & Peterson, 1982).

Ionizing radiations which can be used for the treatment of foods are gamma rays from Cobalt-60 (^{60}Co) and Cesium- 137 (^{137}Cs), accelerated electrons from a machine at energy of 10 MeV or lower and X-rays from a device at of 5 MeV or lower energy (Rosenthal, 1992).

Research in food irradiation began as early as 1905. Table 2-3 presents a chronological summary of the major events in the development of food irradiation.

At low doses (0.05-0.15 KGy), gamma irradiation has been used efficiently on different foods such as potatoes, onions, and garlic to eliminate insect pests, inhibit the growth of molds, inhibit sprouting, and prolong the shelf life. At higher doses (10-50 kGy) irradiation could be used on a variety of different foods to eliminate parasites and pathogenic bacteria. Many foods can be irradiated effectively, including meat, poultry, grains, and many seafood, fruits and vegetables (ICGFI, 1999).

Table 2-3
Chronological events in the development of food irradiation

YEAR	EVENT
1905	Scientists receive patents for a food preservative process that uses ionizing radiation to kill bacteria in food.
1921	U.S. patent is granted for a process to kill Trichinella spiralis in meat by using X-ray technology.
1953-1980	The U.S. government forms the National Food Irradiation Program. Under this program, the U.S. Army and the Atomic Energy Commission sponsor many projects in food irradiation
1958	The Food, Drug, and Cosmetic Act is amended and defines sources of radiation intended for use in processing of food as a new food additive. Act administered by FDA.
1963	FDA approves irradiation to control insects in wheat and flour.
1964	FDA approves irradiation to inhibit sprouting in white potatoes.
1964-1968	The U.S. Army and the Atomic Energy Commission petition FDA to approve the irradiation of several packaging materials.
1966	The U.S. Army and USDA petition FDA to approve the irradiation of ham.
1971	FDA approves the irradiation of several packaging materials.
1985	FDA approves irradiation at specific doses to control Trichinella spiralis in pork.
1986	FDA approves irradiation at specific doses to delay maturation, inhibit growth, and disinfect foods, including vegetables and species.
1990	FDA approves irradiation for poultry to control salmonella and other foodborne bacteria.
1992	USDA approves irradiation for poultry to control salmonella and other foodborne bacteria
1997	FDA's regulations permit ionizing radiation to treat refrigerates or frozen uncooked meat, meat byproducts, and certain food product to control foodborne pathogens and to extend shelf life.
2000	USDA's regulations allow the irradiation of refrigerated or frozen uncooked meat, meat byproducts, and certain food product to reduce the levels of foodborne pathogens and to extend shelf life. FDA's regulations permit irradiation of fresh shell eggs to control salmonella.

Adapted from: EPA (2004)

In fruits and vegetables, most contamination occurs on the surface. The effectiveness of ionizing radiation depends on the radiation source in addition to factors such as food shape, dimensions and type of packaging materials (ICGFI, 1999). All fruits and vegetables are perishable due to physiological changes, postharvest fungal diseases, other pathological breakdown, and insect infestation.

Ionizing radiation, commonly in the form of gamma radiation, has been an effective and efficient postharvest treatment of these products applied basically for disinfestation, shelf-life extension, and pasteurization (Josephson & Peterson, 1982).

A large number of fruits have been irradiated for several purposes. For example, strawberries have been irradiated at dose levels between 1.0-3.0 kGy in order to reduce the amount of decay produced by *B. cinerea* which causes the gray mold disease. Dose levels of gamma radiation between 0.50 to 4.0 kGy have been applied to various *Prunus* fruits such as peaches, cherries and nectarines as an effective treatment to control brown rot produced by fungi. In the case of tropical fruits, such as papaya and mango, gamma irradiation has been applied to control decay mainly produced by fruit fly infestation, as well as for shelf life extension (Josephson & Peterson, 1982).

Minea et al. (1996) reported that the application of electron beam irradiation in strawberries, cherries, apricots, nectarines and apples at radiation doses of 0.1-3.0 kGy resulted in a longer extension of shelf life without any significant change in the nutritive value of the fruits. Yu et al. (1995) found an extension of 2 and 4 days in the shelf-life of electron beam irradiated strawberries at doses of 1.0 and 2.0 kGy, respectively.

In other studies, Palekar et al. (2004) reported a reduction in the total aerobic microbial counts of cantaloupes irradiated at 0.7 and 1.4 kGy. Bari, et al. (2004) reported that irradiation at a dose of 1.0 kGy reduced the total aerobic plate count and *L. monocytogenes* by approximately 4.0 logs (99.99%) on precut bell pepper. In the same study, spoilage bacteria were reduced by 5 log cycles on carrot cubes by 1.0 kGy.

2.2.2. Types of irradiation

2.2.2.1. Gamma radiation

Gamma radiation is electromagnetic radiation produced by the nuclear disintegration of certain radioactive materials. A radioactive transformation is the change of an atom from one element to another by the involvement of a particulate radiation (alpha, beta, neutrons) (Rosenthal, 1992). Cobalt 60 and Cesium 137 are the radioactive elements used as sources for food preservation; they provide high-energy photons, called gamma rays. These rays can penetrate foods to a depth of several centimeters (McMurray et al., 1998).

2.2.2.2. Electron beam

The electron beam is a flow of high-energy electrons that are emitted from an electron gun. They can be emitted as cathode rays from the cathode of an evacuated tube subjected to an electrical current or are produced in linear accelerators. The electrons can penetrate food only to a depth of three centimeters, or a little over 2.54 cm (Scharf, 1986). For food treatment the energy of the electron beams is limited to a maximum of 10 MeV (Rosenthal, 1992).

2.2.2.3. X-rays

X-rays are usually produced by a machine in which a beam of fast electrons in a high vacuum bombards a metallic target (tungsten, molybdenum). X-rays of short wavelengths are of the same nature as gamma rays except for their origin. For food treatment the X-ray machines should be operated at an energy level of 5 MeV or lower (Rosenthal, 1992).

2.2.3. Units of measurement

The dose of irradiation is usually measured in a unit called the Gray (Gy), which is a measure of the amount of energy transferred to the food, microbe or other substance. Gray is therefore the unit of absorbed ionizing energy. A radiation dose of 1 Gy involves the absorption of 1 Joule (J) of energy of 1 kg of matter. Another unit that has been used is the rad that involves the liberation of 100 erg energy for each gram of material through which radiation passes (Josephson & Peterson, 1982).

Different doses of irradiation can be applied to foods to achieve diverse benefits. Table 2-4 summarizes the doses approved for food applications.

Table 2-4
Food irradiation applications

Benefit	Dose (kGy)	Products
Low dose (up to 1 kGy)		
-Inhibition of sprouting	0.05 - 0.15	Potatoes, onions, garlic, root ginger, yam, etc.
-Insect disinfestations and parasite disinfestations	0.15 - 0.5	Cereals and pulses, fresh and dried fruits, dried fish and meat, fresh pork, etc.
-Delay of physiological processes (e.g. ripening)	0.25 - 1.0	Fresh fruits and vegetables.
Medium dose (1 – 10 kGy)		
-Extension of shelf- life	1.0 – 3.0	Fresh fish, strawberries, mushrooms, etc.
-Elimination of spoilage and pathogenic microorganisms	1.0 – 7.0	Fresh and frozen seafood, raw or frozen poultry and meat, etc.
-Improving technological properties of food	2.0 – 7.0	Grapes (increasing juice yield), dehydrated vegetables (reducing cooking time), etc.
High Dose (10 -50 kGy)		
- Industrial sterilization (in combination with mild heat)	30 - 50	Meat, poultry, seafood, prepared foods, sterilized hospital diets.
- Decontamination of certain food additives and ingredients	10 – 50	Spices, enzymes preparations ,natural gum, etc.

Adapted from: IGCFI, (1999)

2.2.4. Effect of ionizing radiation on food nutrients

Food contains various types of compounds such as water, proteins, carbohydrates, lipids, and minerals. Chemical reactions produced by ionizing radiation are dependent upon the dose, and the amount of radiolytic products increases with the dose (Hayashi, 1991). It is well known that the chemical changes induced by radiation are influenced by temperature, water content and oxygen concentration because of their

influence on the diffusion of radicals. Dose rate also influences the production of chemical reactions. A high dose rate resulted in a lower number of chemical reactions (Hayashi, 1991).

2.2.4.1. Water

In high moisture foods water is the macrocomponent most vulnerable to direct radiolysis which yields several major decomposition products like hydrated electrons (e^-_{aq}), hydrogen atoms (H·), hydroxyl radicals (OH·), hydrogen (H_2), and hydrogen peroxide(H_2O_2) (Rosenthal, 1992) as,

$$H_2O \rightarrow e^-_{aq}, H\cdot, OH\cdot, H_2, H_2O_2. \quad (2\text{-}1)$$

Between these transformations, the hydroxyl radical (OH·) is the most reactive and accounts for the major chemical changes occurring in irradiated foods. With its unsaturated bonds it (the hydroxyl radical (OH·)) adds hydrogen atoms and with its saturated molecules it substrates hydrogen atoms. The other products of water radiolysis are more selective and have lower reactivity (Rosenthal, 1992). Hydroxyl radicals (OH·) are powerful oxidising agents while hydrated electrons (e^-_{aq}) are powerful reducing agents (Butler et al., 1984).

2.2.4.2. Lipids

The irradiation of lipids results in a nonoxidative (direct) and oxidative (indirect) effect. Nonoxidative changes are due to the susceptibility of some bonds to radiation, which break easier than others. As a result, free radicals are formed and terminated by either hydrogen atom abstraction from other molecules or by the loss of a hydrogen

atom, and by recombination with other radicals. Some of the products formed are H_2, CO_2, CO, alkanes, alkenes, and aldehydes (Rosenthal, 1992).

In the case of lipid oxidation, the changes are produced by the initiation of a free radical chain process by any nonspecific free radical source including ionizing radiation. After initiation the further mechanism is always the same, producing peroxides, alcohols, carbonyl compounds, hydroxy and keto acids, lactones and polymers. The immediate effect of lipid degradation in foods is reflected in a decrease of organoleptic quality, especially noticeable in rancid foods because of the formation of carbonyl compounds. These oxides may impart off-odors and tastes to foods and may contribute to lipid-related diseases (Rosenthal, 1992).

2.2.4.3. Proteins

The principal effect of irradiation on proteins is the splitting of large molecules into smaller units because of the weakness of the hydrogen ions which can be broken easily when they are in the ionization area. This is an effect of the introduction of a charge that disrupts electrical dipoles. These changes would be lethal to a living organism (foodborne bacteria) but do not affect the nutritional quality of the food (Rosenthal, 1992). However, in some cases irradiated proteins can form intermolecular covalent bonds which must contribute their precipitation from solutions as aggregates and therefore, there is a loss of protein activity (Butler et al., 1983). The reactions of electrons with proteins lead to deamination, the scission of peptide and disulphide bonds, the addition of aromatic and heterocyclic aminoacid residues, and the reduction of metmyoglobin (Josephson & Peterson, 1982).

2.2.4.4. Carbohydrates

Hydrolysis and degradation of carbohydrate molecules may happen due to irradiation. Lower saccharides (i.e. glucose, ribose) may be oxidized at the end of the molecule to form acids and as a result of the ring scission, aldehydes are formed. Large carbohydrate molecules (like cellulose and starch) are divided into smaller units by the breakdown of the glycosidic link, resulting in depolymerization (Rosenthal, 1992). These polymers are highly sensitive to radiation and the radiation induced damage causes considerable changes in the cell membranes and connective tissue (Josephson & Peterson, 1982).

2.2.4.5. Vitamins

Usually the destruction of vitamins is indirectly produced by the reaction of free radicals of the solvent or oxidizing species like peroxyl radicals or carbonyl compounds that react with them. Therefore, the percent of destruction is related to the content of water and oxygen. The fat-soluble vitamins would be exposed mainly to radicals produced by the direct action of radiation of lipids (Josephson & Peterson, 1982). Among the fat-soluble vitamins, vitamin E is the most radiosensitive and vitamin D the least. In the group B, thiamine is the most radiosensitive. Ascorbic acid is voluntarily converted to dehydroascorbic acid. This conversion is nutritionally insignificant. However, losses of vitamin C in irradiated foods have been reported (Rosenthal, 1992).

Ascorbic acid (Vitamin C) participates in many chemical reactions mainly as a redox system which plays a role as an electron donor in enzymatic reactions involving metals. This acid is not very stable in solutions, and its degradation depends on variables

such us temperature, salt and sugar concentration, pH, oxygen, enzymes, aminoacids, etc. Oxidative degradation involves the formation of the ascorbic acid radical ($\cdot A^-$) as in radiolytic degradation. Some of the intermediate products of this degradation are combined with aminoacids and are responsible for the browning of foods (Josephson & Peterson, 1982). In the radiation chemistry of ascorbic acid, the OH radical adds to the double bond in positions 3 or 4 of the acid molecule, followed by the elimination of water (OH^-). In neutral solutions ascorbic acid is ionized as:

$$AH^- + OH \rightarrow HO - \dot{A}H^- \rightarrow \cdot AH + OH^- \quad (2\text{-}2)$$

$$AH \leftrightarrow \cdot A^- + H^+ \quad (2\text{-}3)$$

The same radical can be produced by dehydroascorbic acid as well:

$$e^-_{aq} + A \rightarrow \cdot A^- \quad (2\text{-}4)$$

2.3. Quality parameters

Quality of food has been defined as the composite of those parameters that differentiate individual units of a product and have significance in determining the degree of acceptability by the buyer (Kramer & Twigg, 1970). This implies the degree of excellence of a product or its suitability for a particular use. Quality involves sensory characteristics, nutritive values, chemical constituents, mechanical properties, functional properties and defects (Abbott, 1999).

2.3.1. Physical properties

2.3.1.1. Color

Color is the total visual experience resulting from biological stimulus by certain intensities and wavelengths. The perception of the food color is a function of the light

absorption, reflection and scattering. The color of the food is not only determined by the chemical structure of the pigments present but also by their physical state relative to the nonpigmented constituents (Abbott, 1999).

Light reflected from the product carries information used by inspectors and consumers to judge different aspects of quality; however, human vision is limited to a small region of spectrum. Color can be described by different coordinate systems. One of the most common is the Hunter *L, a, b* system, where *L* indicates the lightness, from white to black, and *a* and *b* are XY coordinates indicating color directions: *a* is the red – green axis, and *b* is the yellow and blue axis (Abbott, 1999). Colorimeters measure light in tristimulus color space that relates to human vision. These parameters are: hue, saturation and lightness. Hue (*H)* is the perception of color resulting from differences in absorption of incident energy at several wavelengths, such us green, blue, yellow, and red. Saturation (chroma (*C*) or purity) describes the reflection of a given wavelength. The Lightness (*L*) describes the relation between total reflected and absorbed light with no regard to specific wavelength. If the light is reflected from a surface evenly at all angles, the impression of a product with a "flat" or "diffuse "appearance is created. Some quality parameters respond to wavelengths outside of visible spectrum so spectrometers and spectrophotometers are used for measuring wavelengths in the UV, visible and NIR spectral regions (Abbott, 1999).

It has been known that irradiation can destroy pigments in fruits and fruit juices and its effect depends on the dose and conditions during irradiation. (Hulme, 1971). Therefore, changes in color could be noticeable. Several authors have investigated the

effect of irradiation on fruit color. Mitchell et al. (1992) reported no significant effect on *L* and *b* values of lemons at 75 and 300 Gy gamma irradiation but *a* values were lower and fruits were greener. In the same study, it was shown that when the irradiation dose was increased, the redness (*a* value) of mangoes was reduced. In the application of gamma irradiation on apple slices treated with and without calcium ascorbate at doses of 0.5 kGy and 1.0 kGy, Fan et al. (2005) found a slight decrease in *L* values at both doses. Castell-Perez et al. (2004) reported a significant decrease in *a*, *b* and chroma values of cantaloupe, both fresh-cut and whole, when irradiated with an electron beam at 3.1 kGy.

2.3.1.2. Texture

Texture is a critical quality attribute for the acceptability of fruits and vegetables and it involves the structural and mechanical properties of a food and their sensory perception in the hand or mouth. Some of these mechanical properties include the resistance to mechanical damage. Compression is one of the types of mechanical loading and the force required to attain a specified deformation or to rupture the fruit is measured (Abbot & Harker, 2000) (Figure 2-2).

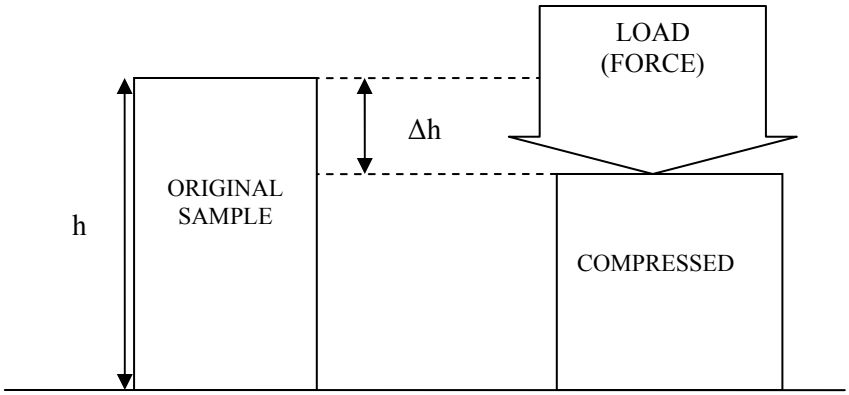

Figure 2-2. Schematic of uniaxial deformation of a solid under constant force (adapted from Bourne, 1982).

Textural attributes refer to the structural, physiological and biochemical characteristics of the living cell; their change over time, and their alteration by processes like freezing, cooking, etc. Because of the continuous physiological changes in the living cells and the variability between the individual units of the commodity, the evaluation of texture is difficult (Abbot & Harker, 2000).

2.3.1.2.1. Physiological basis for texture

To evaluate and develop an adequate method for testing the texture of a product, it is important to understand many of the anatomical and physiological aspects that are the main elements in the strength or softness of tissues and are responsible for the textural parameters (Abbot & Harker, 2000).

Figure 2-3 represents a diagram of the cell structure of a plant cell. In the parenchyma cells, the mechanical properties are determined by a mixture of pectin and hemicelluloses and by the fibrous polysaccharides present in the cell wall. The wall's plasticity (flexibility) and rigidity (strength) are conferred by the polysaccharides. The arrangement and packing of the parenchyma cells in the tissue is another factor that influences the mechanical strength of the produce (Abbot & Harker, 2000). In apples, for example, the cortical tissue cells are large and elongated along the fruit radius and organized into distinct columns which results in higher tissue stiffness and the strain at fracture is lower when tissue plugs are compressed in a radial rather than a vertical or tangential orientation (Khan & Vincent, 1993).

The primary cell wall of the parenchyma cell is composed of a mixture of cellulose, hemicellulose and pectin. Changes that occur in the cell wall during the ripening of fruit, the storage of product and cooking are critical to the texture of the final product. During maturation some cells become lignified which results in toughening of the product. Also, in the period of ripening, cell changes include solubilization and degradation of pectin and a net loss of the noncellulosic neutral sugars galactose and aracbinose, and there may be a decrease in the molecular weight distribution of hemicellusose. The cell wall also influences the perception of juiciness through its ability to hold and release fluid. In some fruits the cell wall swells greatly during ripening (Abbot & Harker, 2000). In mangoes, the ripening is characterized by softening

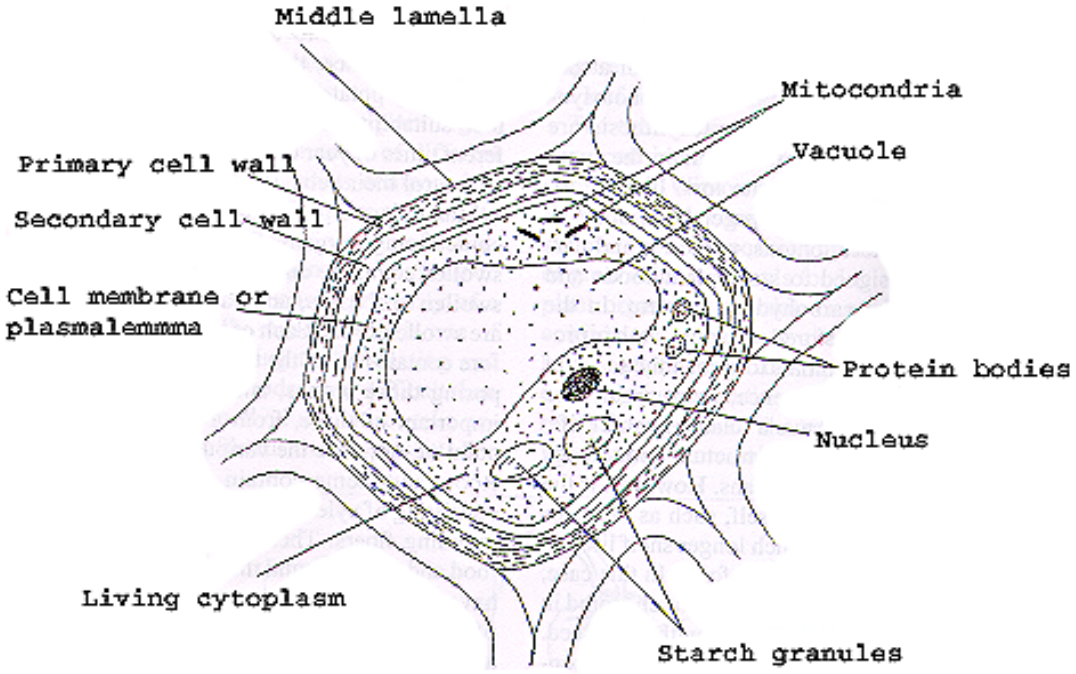

Figure 2-3. Diagram of the structural appearance of a plant cell (adapted from Rosenthal, 1999).

of the flesh (Mitra, 1997). Mitcham & McDonal (1992) observed that neutral sugars and cell wall hemicellulose of 'Keitt 'and 'Tommy Atkins' mangoes decreased with ripening. Woodruff et al. (1960) reported a decrease in the soluble pectin in blueberry fruit after red coloration was reached during the ripening.

2.3.1.2.2. Effect of gamma irradiation on plant cells

The microstructure of food materials has been used to explain and predict physical and chemical behaviors of foods. Texture, which is an important quality indicator, is dependent on the microstructure of the fruit. In scanning electron microscopy (SEM), the image of the sample is obtained by scanning electrons on the sample surface and recoiled electrons are detected by a detector in the SEM, which is operated under high vacuum to provide the ideal conditions for electron movements (Kim, 1996).

One of the effects of ionizing radiation is the interaction with atoms and molecules in the cell, especially with water, to produce free radicals which can diffuse far enough to reach and produce damage to different important compounds in the plant cell. This indirect effect of irradiation is especially significant in vegetative cells, the cytoplasm of which contains about 80% water. Softening and loss of cell cohesion of fruit tissues is based on an increase in water soluble pectin and is accomplished with loss of protopectin (Kovacs & Keresztes, 2002). Exo- and endo- polygaracturonases are bound to the cell walls and carry out autolysis of cell wall pectin. Irradiation, by increasing the activity of polygalacturonase and methyl esterase, results in a significant decrease of pectin. Kovacs & Keresztes (2002) found that the structure of apple changed as a function of the irradiation dose (1-2.5 kGy) and storage time. The cotex cells in their radiated apples shrunk and collapsed. Foa et al. (1980) reported that the total polysaccharide of the cell wall of soft fruits irradiated with gamma radiation at doses of

10 and 100 Krad reacted as a quadratic function of the dose, and the most affected are primarily pectincs followed by hemicellulose.

2.3.1.2.3. Objective measurement of texture

Compression test

Firmness of a fruit can be measured by compression or puncture with various probes at different forces or deformation levels, depending on the purpose of the measurement and how quality is defined. Deformation is the change in height and diameter of a food under the application of a force (Bourne, 1982). The deformation test usually measures the force under a constant deformation. The test involves the contact of the product (usually small cylinders) with small flat or curved indentors or with parallel plates significantly larger than the area of contact (Abbott & Harker, 2000). Figure 2-4 shows a linear force-deformation relationship for a firm, medium and a soft product. The application of a force F to three ideal commodities gives the deformations *f* for a firm product, *m* for a medium and *s* for a soft product (Bourne, 1982).

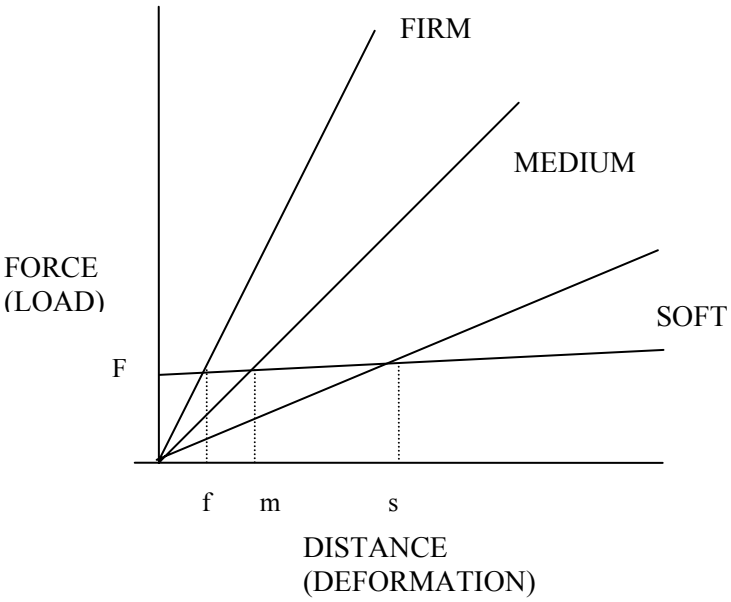

Figure 2-4. Deformation of ideal firm, medium, and soft solids (adapted from Bourne, 1982).

Kramer shear test

The maximum force is defined as firmness in the Kramer shear test. The Kramer shear device is used extensively in the food processing industry and is used by some fresh-cut processors for quality control. The key component of the original Kramer shear device is a multiblade cell with ten blades 2.9 mm (around 7/34 in) thick attached to the press ram that engage with holes in the bottom of the box (67x67x63 mm). The cell is usually filled with randomly oriented pieces of the product. The force measured by the test involves compression, shear, extrusion and friction between the tissue and the blades. In operation, the food is placed in the test cell; the lid is put on, and the test cell

is placed in the machine such that the slits formed by the bars in the lid are aligned with the blades on the ram. When the ram is activated, the set of blades is forced down through the box, first compressing and then extruding the material. Some of the material extrudes upward between the moving blades, and the remainder extrudes downward through the bars in the bottom of the test cell. The moving blades are pushed down until they pass between the bars in the bottom of the test cell. When the ram is reversed, the moving blades return to their original position. As they ascend, the bars of the stationary test cell lid scrape off into the cell the food lodged between the moving blades (Bourne, 1982).

Plant materials have a high tendency to soften after irradiation. The effect of irradiation on texture would probably be less in soft fruits than in firm fruits. It is probable that most of the fruits soften at doses below 2.0 kGy. The correlation between the degree of polymerization of the cell wall and pectin substances of the fruit, plays an important role in the texture of irradiated of fruits (Hulme, 1971).

Different studies have been done to evaluate the texture of irradiated fruits. Fan et al. (2005) reported that firmness of fresh-cut apples was decreased (22%) as radiation dose increased (0.5 and 1.0 kGy, gamma rays). Yu et al. (1995) found that firmness of strawberries was less after electron beam irradiation at 0.5, 1.0 and 2.0 kGy. Similar findings were reported by Castell-Perez et al. (2004) when whole cantaloupes were irradiated with an electron beam at 1.5 and 3.1 kGy. Samples were less firm than the control and those irradiated at 1.0 kGy. Eaton et al. (1970) showed that the effect of gamma irradiation on the texture of blueberries at doses of 1.0, 1.71, 2.92, and 5.0 kGy

changed according with the cultivar but in general all irradiates samples were softer that the controls. El-Samahy et al. (2000) found that the application of gamma irradiation on mangoes at doses of 0.5, 0.75, 1.0 and 1.5 kGy caused a significant decrease in firmness, and this decrease was proportional to the applied dose. Ladaniya et al. (2003) reported a significant decrease in firmness of oranges when they were treated with gamma irradiation at 1.0 and 1.5 kGy. Palekar et al. (2004) reported a lower force of compression when cantaloupe was irradiated with electron beam at 1.4 kGy than when it was treated with 0.7 kGy.

2.3.1.3. Respiration rates

The process of respiration involves combining oxygen in the air with organic molecules in the tissue (usually sugars) to form various intermediate compounds and eventually, carbon dioxide and water. The energy and organic molecules produced during respiration are used by other metabolic processes to maintain the health of the commodity (Saltveit, 1997). The overall equation for respiration can be written as follows:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6\ H_2O + 686\ \text{kcal/mol} \qquad (2\text{-}5)$$

The water produced remains in the tissue but the CO_2 escapes and accounts for part of the weight loss of harvested fruits (3-5% weight loss).

From measurements of CO_2 and O_2 it is possible to evaluate the nature of the respiratory process. The ratio of the volume CO_2 release to the volume of O_2 absorbed in respiration is termed the respiratory quotient (RQ). For aerobic respiration, values of RQ range from 0.7 to 1.3, depending on the substrate being oxidized (Robertson, 1993).

Respiration is affected by different environmental factors such as light, chemical stress, radiation stress, water stress, growth regulators, and pathogen attack. The most important postharvest factors are temperature, atmospheric composition, and physical stress (Saltveit, 1997).

The storage life of fruit varies according to their rate of respiration. This is because respiration supplies compounds that determine the rate of metabolic processes directly associated to quality parameters, like firmness, sugars, flavor compounds, etc. Respiration plays an important role in the postharvest life of fresh commodities because it reflects the metabolic activity of the tissue including the loss of substrate, the synthesis of new compounds, and the release of heat energy (Saltveit, 1997). The two major substrates found in fruits are sugars and organic acids; both are largely sequestered in the vacuole and form a major contribution to the overall flavor of the fruit. However, they are released and therefore available for respiration.

Glycolysis, oxidative pentose phosphate (OPP) and tricarboxylic acid (TCA) are the respiratory pathways utilized by the fruit for the oxidation of sugars. The increased respiration of sugars on climacteric fruits seems to be mediated mainly by an increased flux through glycolysis (Saltveit, 1997).

Radiation induces an immediate increase in the respiration rate of most fruits (Hulme, 1971). Radiation appears to induce some modification in the respiratory apparatus or mechanisms which results in an enhanced rate of gaseous exchange, because oxygen uptake may also be stimulated as well as carbon dioxide production. The increase in the rate of carbon dioxide is a characteristic of the maturation process in

climacteric fruits. This rise is usually related to some metabolic changes leading to ripening of the fruit (Thomas, 1986). Akamine and Goo (1971) reported that the respiration of green preclimateric mangoes exposed to gamma irradiation doses of 0.25, 0.50, 0.75 and 1.0 kGy showed an initial increase in respiration rate, reaching the peak one day after the treatment and then a decrease was observed. They also reported a slight increase in the respiration of papaya immediately after irradiation at 1.0 kGy. Similar findings have been reported for bananas and tomatoes (Thomas, 1986). Paul (1996) found higher levels in the respiration rate of papaya irradiated with gamma rays at 750 Gy. Ladaniya et al. (2003) reported an increase in the respiration rate of mandarin and oranges when the fruits were exposed to gamma irradiation at 0.25, 0.5, 1.0 and 1.5 kGy.

2.3.1.4. Density

Density is important in both liquid and solid foods. Density may be used in determining the composition of liquid mixtures, evaluating seed purity, and determining fruit and vegetable maturity. Loss of density could be an indicator of quality that is characterized by changes in the tissue like the lysis of the cells due to different effects such as stresses produced by deficiencies of water, oxygen and minerals (Saltveit & Mangrich, 1996).

The density of a substance at a determined temperature is defined as the mass divided by the volume (Saltveit & Mangrich, 1996). The density of some fruits is: 1.02 g/cc for pears, 0.906 g/cc for oranges, mangos are between 0.99 and 1.065 and blueberry 1.030 – 1.050 specific gravity (Seymour, 1993).

The bulk density is considered when transporting, string and packaging particular matter. It is known as the density of the bulk material including the volume of the air spaces when the material is poured into a container. The values of the bulk density of some fruits are: 0.54-0.60 g/cc for apples, 0.768 g/cc for lemon, 0.608 g/cc for peaches, 0.3-0.6 g/cc for blueberries, and 0.58g/cc for mangoes (Lewis, 1987).

2.3.2. Chemical properties

2.3.2.1. Moisture

Water is the major component in every food and is found bounded in different ways as free, absorbed and bound water. Free water occurs in the intergranular spaces and pores of a food and is the least tightly bound. Absorbed water is water taken from the surface of the macromolecular colloids. The most tightly held water is bound within the food, which is also known as water of hydration. The moisture content is determined by measuring the mass of a food before and after the water is removed by evaporation (Fennema, 1996). The moisture content is relatively high in the flesh of most fruits at maturity and it is an important quality factor in the preservation and packaging of some products (Fennema, 1996).

The selection of the method to measure moisture is influenced by the difficulty of breaking up these bonds with water. Moisture methods can be classified as loss from drying, distillation, chemical assays and physical procedures. One of the most common and widely used is oven drying. This method consists of tare-weighing the drying dish, filling the dish with a specific amount of sample, drying the sample, cooling the dried sample and dish, and, finally calculating the moisture content. Other types of drying use

vacuum ovens, infrared heating, and microwave ovens. Vacuum-oven drying allows the removal of the last small amount of water left in the food material. By drying under reduced pressure (25-100 mm Hg), it is able to obtain the complete removal of water and volatiles without decomposition within a 3-6 hr time (Multon, 1997).

Fruits average water content is between 80-90%. For mangoes, water content is about 81.71% and for blueberries it is about 84.21% (USDA, 2004).

2.3.2.2. pH

Most foods are complex materials that contain many substances such as proteins, organic acids, and weak inorganic acid- phosphate salts, which participate in the pH control and buffering systems. In plants, buffering systems containing citric acid (lemons, tomatoes, rhubarb), malic acid (apples, tomatoes, lettuce), and tartaric acid (grapes, pineapple) are common, and they usually work in combination with phosphate salts maintaining the pH control (Fennema, 1996). The pH of an extract is a way to express the acidity of a fruit. The buffer ratio ΔpH/ΔNaOH changes during fruit growth. pH changes are associated with the ripening process of the fruits; the ratio of H^+/tritable acidity is used as an index of maturity (Hulme, 1971).

The pH value changes according to the acid content in the fruit, for instance, for citrus fruits like lemons and nectarines the pH is around 2.2. and 3.4, respectively. For mangoes it is approximately 4.9 (Mitchell et al., 1992). The pH of blueberries ranges between 2.85-3.49 (Hulme, 1971).

Gamma irradiation treatment had no significant effect on the pH of fresh-cut fruits and tropical fruits (Mitchell et al.1992; Fan et al. 2005). Similar findings were

reported by Yu et al. (1995) who found no differences in the pH values of electron beam irradiated fruits (up to 2.0 kGy) and non-irradiated strawberries. Also, Miller and McDonal (1996) reported no differences in the pH values of blueberries when irradiated with gamma rays at 0.5 and 1.0 kGy.

2.3.2.3. Acidity

Most of the fruits contain several organic acids that play an important role in fruit metabolism which involves process like photosynthesis, respiration, synthesis of lipids, phenolics compounds and volatile aromas, etc. During the fruit development stages the acid content varies according to the maturity level. However, there is a big significance between the acid content and the flavor of the fruits; sometimes sweetness is preferred but in other cases acidity is best (Hulme, 1971). The ratio of sugar/acid is often used as an index of consumer acceptability and quality in fruits. It is an important parameter to predict the maturity of the fruit and is also decisive for their basic taste and palatability. For example, in citrus fruits like mandarins and novel oranges this value is 8, for blueberries it ranges from 11.2 to 14.3, for mangoes from 5.26 to 100 according to the maturity stage (Dantas de Morais & Simanao de Asis, 2004).

Total acidity represents the sum of all the acids present, free or combined with cations, while tritratable acidity is the free acidity which is measured by neutralizing the fruit extract with a strong base. The aim is to measure the total neutralization of all acidic groups including phenols, amino acids and other constituents which would combine with the alkali (Hulme, 1971).

The acidity in mango declines as the fruit ripens. The predominant acid is citric, with malic and succinic also found in some quantities. In blueberry, the non-volatile acids are the most important in determining fruit acidity. The major organic acids are citric and malic acid (Mitra, 1997).

Different findings have been reported about the effect of irradiation on the acidity of fruits. Mitchell et al. (1992) and Fan et al. (2005) found no significant effect on tritratable acidity of irradiated apple slices or tropical fruits (like lemons, mangoes, mandarins and nectarines) respectively. The acidity of gamma irradiated mango samples was higher than the unirradiated samples (Youssef et al., 2002).

2.3.2.4. Total soluble solids

Soluble solids content is a chemical factor used as an indicator to define the optimum stage of fruit maturity. This parameter is also associated with the sweet flavor of the product (Seymour et al, 1993).

The total solids content in soft fruits such as blackberry, blueberry and strawberry ranges between 1.02 and 15.2%. In mangoes according to the variety and maturity, this parameter ranges between 9.1 and 18.27% (Hulme, 1971).

Ladaniya et al. (2003) reported an increase in the total soluble solids of irradiated mandarins and oranges at doses up to 1.5 kGy. Eaton et al. (1970) found an increase in the soluble solids of blueberries and cherries irradiated with gamma doses of 100, 171, 292 and 500 Krad. Lacroix et al. (1992) showed that the level of soluble solids of mangoes irradiated with doses between 0.3 and 0.6 kGy was significantly higher that the control samples. Mitchell et al. (1992) reported no effect of irradiation on the soluble

solids of mango irradiated at doses of 75, 300 and 600 Gy. However, in the same study the author found a reduction in the soluble solids of peaches at 75 Gy.

2.3.2.5. Water activity

The water activity is related to the intensity with which water associates with nonaqueus constituents. This is a parameter used to predict food stability, safety and perishability. Water tightly associated with other food constituents is less available for microbial growth and chemical reactions to cause decomposition. Water activity between 0.6 and 0.7 is a critical value below which no microorganism can grow (Fennema, 1996).

Relative humidity plays an important role in food product development, storage and packaging. A hygroscopic product equilibrates with a humid environment after prolonged exposure and reaches the equilibrium relative humidity. The equilibrium humidity between the moist food and the surrounding is termed the water activity (a_w) (Fennema, 1996).

2.3.2.6. Sugars

Sugars constitute around 91% of the dry matter in foods. They are very common components of foods in natural form and also as added ingredients. They have different molecular structures, shapes, and sizes and exhibit a variety of chemical and physical properties. Fruits are attractive for their flavor, appearance and texture; in all these properties sugars play an important role. Flavor is the balance between sugar and acid. Specific flavor constituents are glycosides. The color of many fruits is determined by sugar derivatives of anthocyanidins and texture is governed by structural

polysaccharides. Usually their molecules contain carbon atoms along with hydrogen and oxygen atoms. D-Glucose is the most abundant carbohydrate that belongs to the group of monosaccharides whose molecules cannot be broken down. It has six carbons atoms and therefore, is called hexose (Hulme, 1971).

Glucose, sucrose and fructose are the most common sugars found in fruits. Their content varies considerably with the variety of the fruit, and the soil and climatic conditions during their life on the plant. In addition, climacteric fruits show considerable changes in sugar content between harvesting and eating ripeness (Seymour et al., 1993).

Sugars form rings that involve the aldehyde or ketone group. The ring forming and opening again is reversible unless the hemiacetal or ketal hydroxyl group has become involved in another link. Rings that are locked have no aldehyde or ketone group to react (unless there are several rings, and one can open) and are *non-reducing* sugars. Sucrose is an oligosaccharide composed of α-D-glucopyranosyl unit and β-D-fructofuranosyl unit linked head to head (reducing end to reducing end) and therefore, is a non reducing sugar (Fennema, 1996). Glucose and fructose are the main reducing sugars. However, in most of the fruits glucose concentration exceeds that of fructose. Table 2-5 presents the sugar content of several fruits.

Changes in the sugar content of irradiated fruits have been investigated. Mitchell et al. (1992) reported no effect of gamma irradiation at 75 and 300 Gy on the sucrose and fructose content of custard apples. However, for glucose levels a significant increase was observed in irradiated samples. In the same experiment, they found no effect in the fructose and glucose content of lemons but an increase in sucrose at 75 Gy; for mangoes

they reported higher levels of glucose in irradiated samples and no effect on the fructose content.

In other studies, El-Samahy et al. (2000) found no effect of gamma radiation (0.5, 0.75, 1.0 and 1.5 kGy) on total sugars of mangoes but a slight increase in reducing sugars was observed. Castell-Perez et al. (2004) reported no effect of electron beam irradiation (1.0, 1.5 and 3.1 kGy) on the total sugars in cantaloupe (whole) however, for fresh-cut samples a reduction (8%) of fructose content was observed.

Table 2-5
Glucose, fructose and sucrose contents of selected fruits

Fruit	Sugar content (g/100g edible portion)		
	Glucose	Fructose	Sucrose
Apple	1.82	5.01	2.4
Banana	5.82	3.78	6.58
Blackberry	3.24	2.88	0.24
Blueberry	4.88	4.97	0.11
Lemon	0.52	0.92	0.18
Cantaloupe	1.16	1.8	5.4
Mango	0.7	2.9	9.9
Pineapple	2.9	2.1	3.1
Strawberry	2.2	2.5	1
Watermelon	1.6	3.3	3.6

Adapted from: Hulme (1971); USDA (2004)

2.3.2.7. Total phenolics

Phenolic compounds are important because of their contribution to the nutritional and sensory quality of fruits (color, astringency, bitterness, and flavor). Some of these compounds, especially flavonoids, have health benefits because of their antioxidant capacity. It has been reported that free radicals cause oxidative damage to lipids, proteins and nucleic acids (Prior et al., 1998). Free radicals may be related to the etiology of different diseases such as cancer, heart, vascular and neurodegenerative diseases. Chemically, phenolics are defined as substances possessing an aromatic ring supporting one or more hydroxyl substituents, including their functional derivatives (Shahidi & Naczk, 1995).

The accumulation of phenolics in fruits may be higher or lower than in other parts of the plant, such as bark, leaves or heart wood. The concentration of phenolics decreases as fruit matures, but usually the amount per fruit increases (Seymour et al., 1993). It also varies commonly from species to species, variety to variety, season to season, and location to location. The common phenolics in fruits are cinnamic acids and derivatives, flavans, anthocyanidins and anthocyanins, flavonol and flavonol glucosides and other condensed polyphenols. The predominant phenolic acids present in foods are derivates of hydrobenzoic and hydroxycinnamic acids. These derivates differ in the patterns of hydroxylations and methoxylations of their aromatic rings. High concentrations (75%) of these compounds have been reported in blueberries (Macheix et al., 1990). Among the flavonoids, (+) catechin and (-) epicatecin are the most common forms in fruits (Hulme, 1971).

There have been several reports of the accumulation of phenolic compounds following irradiation. El-Samahy et al. (2000) and Youssef et al. (2002) showed an increase in total phenolics content in gamma irradiated mango. Tan & Lam (1985) also found a rise in the total phenolics of papaya and mango fruits which were irradiated with gamma rays at 1.0 kGy and 1.50 kGy, and 0.25, 0.50, 075 and 1.0 kGy, respectively. Gamma irradiation of citrus clementina fruits at 0.3 kGy stimulated the synthesis of phenolics compounds (Oufedjikh et al., 2000). Breitfellner et al. (2003) showed that gamma irradiation of strawberries at doses of 1 up to 6 kGy increased flavonoid compounds.

2.3.2.8. Antioxidant activity index

Fruits and vegetables contain many phytonutrients that have antioxidant properties. Besides the Vitamin C and carotenoids, phenolics like flavonoids, antocyanins and tannins are important components in fruits that have strong antioxidant activity (Prior et al., 1998). Enzymes like phenylalanine ammonia-lyase (PAL) are significant in the metabolism of phenolics (Frylinck et al., 1986).

Lipid auto-oxidation is a radical process that involves a chain reaction including induction, propagation and termination steps. Different compounds are formed during these stages which are responsible for organoleptic and nutritional alterations due to the formation of off-flavor volatile compounds from degradation of hydroperoxides and the disappearance of essential fatty acids (Bondet et al., 1997). Also, the radicals formed are involved in the ageing process of tissues and pathologies such as cancer or cardiovascular diseases. Therefore, it is important to protect food lipids and human

tissues against free radicals by endogenous or exogenous antioxidants from natural or synthetic origin (Bondet et al., 1997).

Antiradical antioxidants act by donating hydrogen atoms to lipid radicals. Radicals obtained from antioxidants with molecular structures such as phenols are stable species and will then stop the oxidation chain (Bondet et al., 1997).

The DPPH$^{\bullet}$ free radical method is based on the reduction of two radicals 2,2-difenil-1-picrilhidrazil (DPPH$^{\bullet}$) by the donation of one hydrogen atom for the phenolic molecule. This reduction is accompanied by a decrease in its absorbance in the methanolic solution while the radicals are being reduced by the phenolics (Bondet et al., 1997).

Prior et at. (1998) reported a linear relationship between the phenolics content and the total antioxidant activity of blueberries. Oufedjikh et al., 2000 showed that the application of gamma irradiation at 0.3kGy of citrus clementina increased the PAL (Phenylalanine ammonia-lyase) activity. Lorinda et al. (1986) also reported an increase in the PAL activity of mangoes after gamma radiation at 0.75, 1.25, 1.75 kGy.

2.3.2.9. Ascorbic acid

Fruits like mango and blueberries are high sources of vitamin C (ascorbic acid), an essential nutrient in the human diet. Additionally because of its reducing and antixiodative properties, vitamin C is effective in the treatment and prevention of atherosclerosis and other diseases (Prior et al., 1998).

Different factors affect the content of ascorbic acid in fruits, for example sunlight, rain, fertilization, ripeness and variety. It is known that vitamin C decreases

during storage time (Hagg et al., 1995). The changes in concentrations of acid depend on the type of fruit (Seymour et al, 1993). Table 2-6 presents the average value of ascorbic acid in several fruits.

Several studies have been conducted to illustrate the effect of irradiation on vitamin C, ascorbic acid. Michell et al. (1992) reported that the application of gamma irradiation doses of 75 and 300 Gy on mangoes had no effect on total vitamin C, however at 600 Gy a significant reduction was observed.

In addition, Youssef et al. (2002) reported a significant decrease (15.0-18.0%) of ascorbic acid in gamma irradiated (0.0 - 2.0 kGy) mango pulp. Beyers & Thomas (1979) found that gamma irradiation at 0.75, 1.50 and 2.0 kGy caused a 17% loss of ascorbic acid content in mangoes. Contrary results were reported by Fan et al. (2005) who observed that gamma irradiation at doses of 0.5 and 1.0 kGy had no significant effect on ascorbic acid levels of cut apple slices treated and nontreated with calcium ascorbate. Ladaniya et al. (2003) concluded that the vitamin C content of orange, mandarin and acid lime was reduced by 15.84%, 26.80% and 29.20% respectively, when the fruits were irradiated at 1.5 kGy using gamma rays.

Table 2-6
Ascorbic acid content in selected fruits

Fruit	Ascorbic acid (mg/100g)
Apple	2-10
Banana	10-30
Blackberry	15
Blueberry	10
Grapefruit	40
Guava	300
Lemon	50
Mango	27
Orange	50
Pineapple	25
Strawberry	60

Source: Hulme (1971)

2.3.2.10. Volatiles

Aroma is one of the important quality attributes of fruits. Volatile compounds provide the characteristic flavor and aroma for different fruits; they involve diverse compounds like alcohols, acids, ketones, aldehydes, esters and many other chemical groups that are distributed differently in each fruit (Hulme, 1971).

In mangoes, more than 270 volatile compounds have been identified as free forms (Bartley & Schwede, 1987; Olle et al., 1998) and about 70 compounds as glycosidically bound aromas (Olle et al., 1998). MacLeod & Gonzalez De Troconis (1982) reported that monoterpenes (such as α- and β-pinene, myrcene and limonene) and sesquiterpene hydrocarbons were the most abundant compounds of the mango aroma.

The volatiles responsible for the aroma of blueberries are esters and alcohols, and their distribution varies among the cultivars. For instance, in highbush blueberries these compounds account for a third of the volatile compounds, while terpenoids compromise an additional 20 to 30%. In wild lowbush blueberries, esters accounted for 10 to 50% of the volatiles, alcohols for 25 to 40%, and terpenoids for 2 to 15% (Forney, 2001; Balonga et al. 1995). Some of the main compounds in this fruit included ethanol, 1-ethyl-1-hexanol, phenol, methyl acetate, bezyl alchol, and linalool. (Forney, 2001).

Information concerning the impact of irradiation on volatile compounds is limited. Fan and Mattheis (2001) reported that gamma irradiation of apple at 0.44, 0.88 and 1.32 kGy inhibited the production of some volatile compounds; however, a stimulation of aldehyde production at 1.32 kGy was noticeable after 8 weeks of storage. Lee et al. (2004) presented a difference in the volatile profiles of red pepper powder irradiated at 3.0, 5.0 and 7.0 kGy (gamma rays).

2.3.2.11. Pigment

Peel color is an important criterion of fruit acceptability. Pigments are natural indicators of fruit ripeness and some of them, including carotenoids and flavonoids, have vitamin activity (Seymour et al., 1993).

2.3.2.11.1. Carotenoids

Mango is rich in carotene and other carotenoid pigments. Approximately 60% of the total carotenoids in mango are β carotenes. In addition to their contribution to the fruit color as natural pigments, they also have an important role as a food nutrient since they are vitamin A precursors (Wilberg &Rodriguez, 1995).

Peel color development is usually accompanied by ultrastructural changes associated with chloroplast-to-chromoplast transition. The thylakoid membrane systems in the peel of 'Alphonso' and 'Tommy Atkins' mangoes gradually break down, while osmiophilic globules enlarge and increase in number. The loss of membrane integrity is related to chlorophyll degradation, while the appearance of osmiophilic globules involves an increase in carotenoid levels (Medlicott et al., 1986).

Some of the carotenoids found in fruits are β-carotene, α-carotene, lycoxanthin, lutein, lycopene, and zeaxanthin (Hulme, 1971). Table 2-7 presents the β-carotenoid content of several fruits.

Several authors have investigated the effects of radiation on pigment stability of fruit juices. Lacroix et al. (1993) reported an increase in Vitamin A content of irradiated (gamma rays) mangoes at 0.63 and 0.56 kGy. EL- Samahy et al. (2000) also found an increase in the total carotenoids content of mangoes as a result of gamma irradiation (0.5-1.5 kGy). Similar findings were found by Youssef et al. (2002) with gamma irradiation of mangoes at doses between 0.5 and 2.0 kGy. Castell-Perez et al. (2004) reported an increase of approximately 25% in the carotenoid content of whole cantaloupes exposed at 3.1 kGy with electron beam irradiation. The same trend was observed in that study for fresh-cut cantaloupe.

Table 2-7
β-carotene levels in selected fruits

Fruit	B-carotene (µg/100g edible portion)
Blueberries	35
Papaya	276
Melon	450
Mangoes	445
Peaches	334
Persimmons	374

Source: Holden et al. (1999)

2.3.2.11.2. Tannins

The phenolic compounds contributing to the color of soft fruits are precursors of polymeric proanthocyanidins. They are more prominent in the skin and peel of the fruits, although they are also distributed throughout the flesh (Hulme, 1971).

Tannis are water soluble poliphenolic compounds that have the ability to precipitate alkaloids, gelatin and other proteins. They range in color from yellowish-white to light brown (Fennema, 1996).

Tannins are responsible for the astringency of many edible fruits. The process of fruit ripening brings about changes in the composition and quality of tannins, thus lowering astringency and improving palatability of the fruit (Shahidi & Naczk, 1995). Table 2-8 presents the content of tannin in several fruits.

Table 2-8
Proanthocyanidin tannins content in several fruits

Fruit	Proanthocyanidin content (mg/100g fresh weight)
Blackcurrant	1.12
Apricot	1.32
Cranberry	7.26
Apple	5.94
Blueberry	3.46
Grapes	1.36

Source: Shahidi & Naczk (1995); USDA (2004)

In early studies, Markakis et al. (1959) found 50% destruction of anthocyanin content in some strawberry juices irradiated (gamma) at 0.5 Mrad. Later, Lees & Francis (1972) showed that gamma radiation at levels of 150 and 300 krad stimulates the synthesis of anthocyanins and flavonoids of cranberries when stored at 31° and 40°F. Similar findings were reported by Lu et al. (1993) who reported that the phenolic and anthocyanin content in peaches treated with gamma irradiation at 0.1 kGy was higher than that in non-irradiated fruits.

2.3.3. Sensory quality of fruits

Sensory evaluation focuses on evaluating the goodness and badness of food that can be used and consumed. The objective is the measurement of the sensory attributes. Sensory testing can establish the value of a commodity or even its very acceptability. The principal uses of sensory testing are in quality control, product development and research (Meilgaard et al., 2000).

Sensory analysis studies the relationship between a given physical stimulus and the subject's response. The sensory attributes perceived in a food product are: appearance, odor/aroma, consistency and texture, and flavors (aromatics, chemical feelings, taste) (Meilgaard et al., 2000).

2.3.3.1. Appearance

Appearance is usually the parameter that determines the decision to purchase or consume. General appearance characteristics include color, size and shape, surface texture, and clarity (Meilgaard et al., 2000).

2.3.3.2. Odor/aroma

The odor of a product is detected when the volatiles enter the nasal passage and are perceived by the olfactory system. Aroma is the odor of a food product. The amount of volatiles that escape from a product is affected by the temperature and by the nature of the compounds (Meilgaard et al., 2000). For example, esters are characterized by their fruity aromas and some terpene alcohols by their floral odor (Hulme, 1971).

2.3.3.3. Texture and firmness

Texture and firmness are perceived by sensors in the mouth. They refer to the viscosity (for homogeneous Newtonian liquids), consistency (for non-Newtonian or heterogeneous liquids and semisolids) and texture (for solids and semisolids) (Meilgaard et al., 2000).

2.3.3.4. Flavor

Flavor is an attribute that results from the sum of impressions perceived via chemical senses from a product in the mouth. It includes the aromatics that are the

olfactory perceptions caused by volatile compounds, the tastes or gustatory perceptions and the chemical feeling factors such as astringency, spice heat, and cooling (Meilgaard et al., 2000).

Ahmed et al. (1972) showed that the texture of strawberries irradiated with gamma rays at 1.5 and 3.0 kGy was rated softer by sensory evaluation. However, Lacroix et al., (1992) evaluated the effect of gamma irradiation on biochemical and organoleptical changes in mangoes and papayas and their results indicated that up to 0.95 kGy (gamma rays) the sensory evaluation of these commodities was not changed. In a later study, Lacroix et al. (1993) showed similar results when the (gamma) irradiation treatment of mangoes at an average of 0.75 kGy did not affect the overall quality of the fruits.

2.4. Kinetics of quality changes

Food quality involves microbiological, chemical, physical and enzymatic changes during processing and storage. In food science and in food engineering, most kinetic models that are used are limited to zero order, first order or second order (Boekel, 2000).

The undesirable changes in quality usually involve degradation of color, flavor and texture, deterioration of the functional properties of ingredients and loss of nutritional value and on development of potentially toxic compounds. Similar reactions occur post-processing at a rate that is determined by the inherent properties of the food, the type of packaging, and conditions of storage and distribution. These factors determine the shelf-life of the food (Fennema, 1996).

Foods are kinetically stable but it is important to understand the appropriate use of kinetics for prediction of quality. This research only focused on quantifying the chemical and physical changes of the tested fruits due to irradiation and storage time.

Many of the changes occurring during processing of the food may be described by the following first order equation:

$$-\frac{dC}{dt} = -kt \qquad (2\text{-}6)$$

where C = quantity of quality attributes (units of amount, such as concentration), t = time of exposure to process (eg, min), and k = reaction rate constant (min^{-1}).

Although Eq. (2-6) suggests that a first order reaction may be causing the quantitative change in a given quality attribute, most of the changes occurring in foods are more complex; therefore, the reaction order and quality function should be selected properly.

In fruits and vegetables, color and texture degradation usually follow a first order reaction kinetics (Ahmed et al., 2001; Lau et al. 2000; Dixon & Hewett, 1998). Little information is available about the kinetics of quality changes on irradiated products. However, Bourne (1995) reported that the softening of carrots exposed to gamma radiation at doses ranging from 1.3 to 50 kGy showed a first order kinetic rate process. Ibarz et al. (2005) found that the effect of UV irradiation on brightness of apple, peach and lemon juices increased following a first order kinetics. Nayak et al. (2005) evaluated the effect of gamma irradiation (3.0-9.0 kGy) and osmotic treatment on mass transfer

during rehydratation of carrots. The authors estimated the diffusion coefficient for water considering Fickian diffusion described by the equation,

$$F = -D\frac{\partial C}{\partial x} \tag{2-7}$$

where F is the mass flux of solute per unit area per unit time and D is the effective diffusion coefficient in a porous medium. They found that the increase in dose resulted in decrease of water and increase in the solute diffusion coefficient. Korkmaz & Polat (2001) reported that the radical kinetics and characterization of the free radicals in gamma (10 kGy) irradiated red pepper evaluated by electron spin resonance corresponded to a second-order kinetics. Valdivia et al. (2002) observed that the oxidative rancidity of the gamma irradiated avocado at dose levels between 0.5 and 2.5 kGy followed a first order kinetics.

In other studies, Ochoa et al. (2001) evaluated the color change in preserves of raspberries and sweet and sour cherries exposed to different lighting conditions (light and darkness) at constant temperature. The authors found that the changes followed a first order kinetics. Vikram et al. (2004) also reported that the thermal degradation of vitamin C in orange juice heated by electromagnetic and conventional methods followed a first order kinetics.

CHAPTER III

METHODOLOGY

3.1. Fruit samples

One hundred sixty-eight (168) mangoes (*Mangifera indica*) and ninety-six (96) trays of blueberries (*Vaccinium corymbosum L.)* were purchased at a local market the night before the irradiation test was performed. The fruits were labeled with the specific treatment (radiation dose) and kept at refrigeration temperature (10ºC) over night.

3.1.1. Irradiation of mangoes

Samples were subjected to four (4) treatments: (1) zero dose (control), (2) high dose (3.0 kGy), (3) medium dose (1.5 kGy), and (4) low dose (1.0 kGy). The effect of irradiation was evaluated at four different time periods up to 21 days of storage. Table 3-1 shows the experimental design of the fruit quality study. A total of forty-two (42) mangoes were used for each irradiation dose. At each dose level, eight (8) mangoes were placed in molded pulp packaging fruit trays (CMC Industries) and then placed inside open cardboard boxes (0.508 x 0.609 x 0.102m) (Figure 3-1 A). The samples were then irradiated using a double beam fixture (top and bottom) 10 MeV (10 kW) Electron Beam Linear Accelerator located at the National Center for Electron Beam Food Research facility at Texas A&M University. To determine the applied dose, four dosimeters (B3WIN Radiochromic Films, Gex Corporation Centenial, CO, USA) were placed evenly at the surface of the mangoes: two on the top and two in the back of the fruit.

Dose was varied by running the conveyor belt at three different speeds: 0.10, 0.20, and 0.30 m/s. The dose rate was 0.8 kGy /s. The measured doses were 3.1 kGy, 1.5 kGy and 1.0 kGy, respectively. Table 3-2 presents the dosimeter readings. Control samples were non-irradiated.

Table 3-1.
Experimental design for irradiation of mangoes using a 10MeV electron beam accelerator (double beam fixture)

Storage time (Days) At 12˚C	**Dose**				
	Control[a] (0 kGy)	**Low (1.0 kGy)**	**Medium (1.5 kGy)**	**High (3.1 kGy)**	**Subtotal (units)**
0	5	5	5	5	20
5	5	5	5	5	20
10	5	5	5	5	20
21	5	5	5	5	20
Subtotal	20	20	20	20	80
Respiration rate	6	6	6	6	24
Other quality tests	16	16	16	16	64
Total (units)*	42	42	42	42	**168**

*One unit represents one fruit
[a]Non-irradiated fruits.

Figure 3-1. Experimental setup for irradiation of mango fruits. (A) placement of fruit on the trays, (B) location of film dosimeters on the product.

The blank dosimeter was used to estimate the dose absorbed by the dosimeter alone (no produce). The difference in the values of the dosimeter readings was small and the dose absorbed was also the same by the sides of the fruit (T1 and T2 in Table 3-2, for example). Figure 3-2 represents the simulation of the dose distribution in the mangoes when irradiated by running the conveyor at 0.30 m/s (higher speed). Since the fruits were exposed to the double beam (upper and lower), a high-dose region was located in the middle of the mango.

At the side edges of the fruit, the absorbed doses were significantly high (>2.5 kGy, orange to red color on the color scale) due to the overlapping high dose area of the single beam. However, this was a very small portion (2.4%) of the fruit.

In order to minimize the effects of high dose on the later measurements, samples taken from the mangoes after irradiation were mixed thoroughly. The uniformity ratio (Dmax/Dmin) was 2.4, which is an acceptable commercial situation.

Table 3-2
Dosimeter reading after irradiation of mangoes using a 10 MeV electron beam accelerator (double beam fixture)

Conveyor speed / **Dosimeter location**	**Dose [kGy]**		
	0.30m/s	**0.20m/s**	**0.10m/s**
Blank	0.9±0.1	1.3±0.1	2.9±0.1
T1*-Top left	0.9±0.1	1.3±0.1	2.9±0.1
T2- Top right	0.9±0.1	1.3±0.1	2.9±0.1
T3- Back left	1.0±0.1	1.5±0.1	3.0±0.1
T4- Back right	0.8±0.1	1.5±0.1	3.0±0.1

*indicates position of radiochromic film on fruit (see Fig 3.1B)

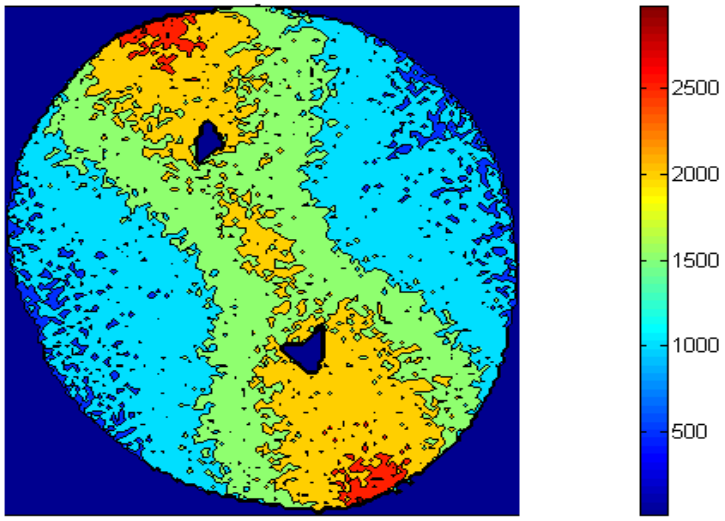

Figure 3-2. Monte Carlo simulation of dose distribution in a mango irradiated using a 10 MeV electron beam in double fixture. Conveyor speed 0.30 m/s (adapted from: Kim, 2005).

3.1.2. Irradiation of blueberries

Seventy two (72) blueberry trays (0.09 x 0.09 x 0.04m, and ~190.0 g) were irradiated using the same 10 MeV Electron Beam Linear Accelerator located at the National Center for Electron Beam Food Research facility at Texas A&M University. The conditions for the experiment were the same as for the mangoes but in this case a single beam fixture was used (due to the small thickness of the trays). Eight (8) blueberry trays were placed inside open cardboard boxes (Figure 3-3 A) with the same dimensions as those used for mangoes and then irradiated using a top single beam. Three

replications for treatment were performed. Untreated (non-irradiated) samples were used as controls.

The effect of irradiation on the fruit quality was evaluated up to 14 days of storage. Twenty-four (24) trays of blueberries were irradiated at each dosage (zero (control), high, medium and low dose) using the same conveyor speeds of 0.10, 0.20, and 0.30 m/s, respectively. Table 3-3 presents the experimental design. In the same manner as for mangoes, radiochromic films were used to determine the absorbed dose. For the blueberries, two dosimeters were placed at the top (T) and two at the bottom (B) of the sample trays (Figure 3-3 B).

Table 3-3
Experimental design for the irradiation of blueberries using a 10 MeV electron beam accelerator (single beam (top) fixture)

Storage time (Days) At 5˚C	**Dose**				
	Control[a] (0 kGy)	**Low (1.1 kGy)**	**Medium (1.6 kGy)**	**High (3.2 kGy)**	**Subtotal (trays)**
0	5	5	5	5	20
3	5	5	5	5	20
7	5	5	5	5	20
14	5	5	5	5	20
Subtotal	20	20	20	20	80
Respiration rate	1	1	1	1	4
Other quality tests	3	3	3	3	12
Total (trays)	24	24	24	24	**96**

[a]Non-irradiated fruits

Figure 3-3. Experimental setup for irradiation of blueberry fruit. (A) placement of fruit on the trays, (B) location of film dosimeters on the product.

The dosimeter readings for all speeds are shown in Table 3-4. The measured doses were 1.1 kGy, 1.6 kGy and 3.2 kGy. In this case, variation in dose among the locations was observed. Doses at the bottom (B1 and B2) were higher than at the top (T1 and T2, Table 3-4) due to dose buildup; secondary electrons are more effectively absorbed in the medium as an electron beam penetrates. In the simulation of the absorbed dose in blueberries when irradiated by running the conveyor at 0.30 m/s (Figure 3-4) it was observed that the dose went up to 1.85 kGy (red color), which was

relatively high. However, only 2.5% of the absorbed dose was higher than 1.5 kGy.

Even though no clear pattern was observed in the dose distribution of blueberries, most of the dose at this speed (0.30 m/s) ranged between 1.0 kGy and 1.5 kGy (green to yellow color).

Table 3-4
Dosimeter reading after the irradiation of blueberries using a 10 MeV electron beam accelerator (single beam (top) fixture)

Conveyor speed / **Dosimeter location**	**Dose [kGy]**		
	0.30m/s	**0.20m/s**	**0.10m/s**
Blank(cardboard box)	1.0±0.1	1.4±0.1	2.8±0.1
T1* (top left)	0.8±0.1	1.4±0.1	2.9±0.1
T2 (top right)	0.8±0.1	1.3±0.1	2.8±0.1
B1 (back left)	0.9±0.1	1.6±0.1	3.5±0.1
B2 (back right)	1.1±0.1	1.7±0.1	3.0±0.1

*indicates position of radiochromic film dosimeter on fruit

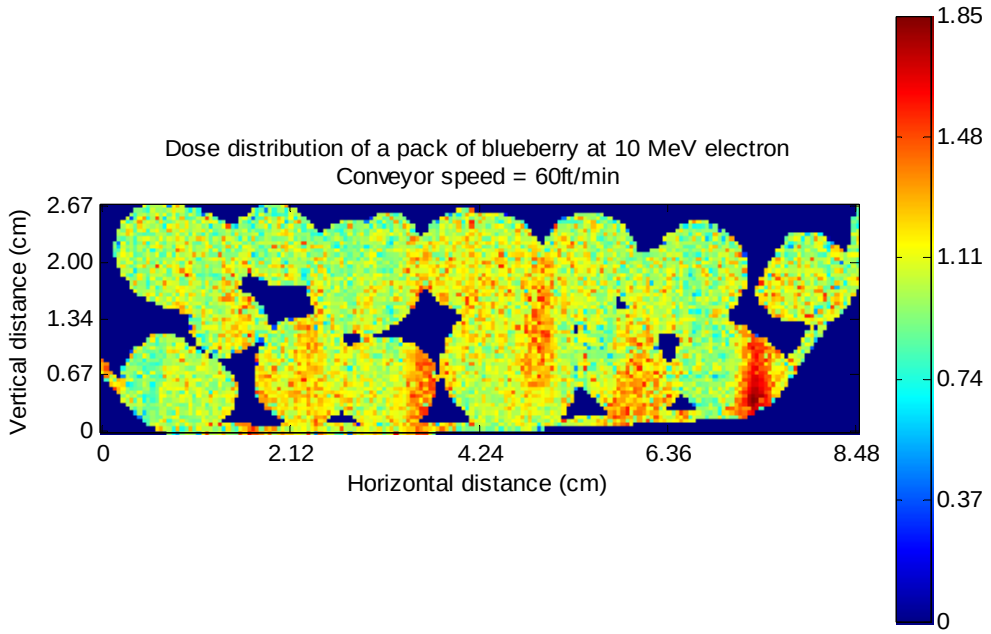

Figure 3-4. Monte Carlo simulation of dose distribution in blueberries irradiated using 10 MeV electron beam in single fixture. Conveyor speed 0.30 m/s (adapted from Kim, 2005).

3.1.3. Shelf-life study

Treated (irradiated) and control (non-irradiated) fruits were stored at refrigeration temperature to determine the effect of ionizing radiation on fruit shelf-life. Mangoes were stored at 12˚C and 62.7% RH while the blueberry trays were stored at 5˚ C and 70% RH. The physicochemical properties of the fruits (Figure 3-5) were analyzed right after irradiation treatment (day 0) and during refrigerated storage at intervals of 5 days up to 21 days for mangoes and every 3 days up to 14 days for blueberries..

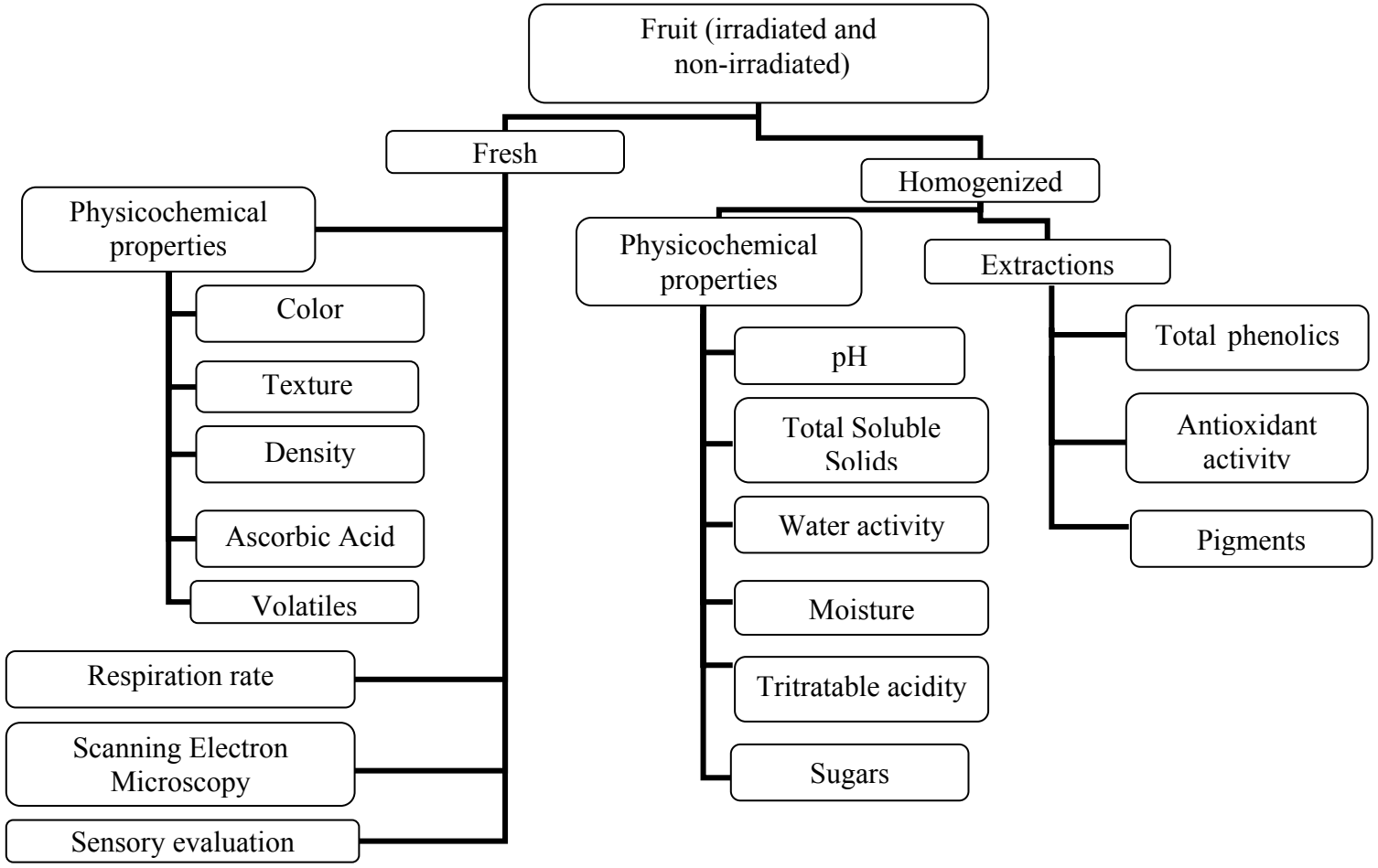

Figure 3-5. Physical and chemical properties measured in mangoes and blueberries. All the properties were the same in both fruits, except for the pigments that were determined as carotenoids in mangoes and as tannins in blueberries.

3.2. Quality parameters

3.2.1. Physical properties

3.2.1.1. Color attributes

The color of the fruit was measured with a Lab Scan XE DP-9000 colorimeter (Hunter Associates Lab II, Preston VA). Mango samples consisted of slices of approximately 5cmx7cm obtained from the interior of the fruit (without peel) while a random amount (around 70g) of blueberries were used for color measurements. The values of *L* (lightness), *a* (redness to greenness) and *b* (yellowness to blueness) were recorded for each sample. Six replications per sample were used. Chroma (*C*) values were calculated as: $(a^{*2}+b^{*2})^{1/2}$. Hue angle, (θ), was determined as $\tan^{-1}(b/a)$, and the total color difference, ΔE, as $(\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2})^{1/2}$ (McGuire, 1992).

3.2.1.2. Texture

3.2.1.2.1. Mangoes

Cylindrical samples (20 mm length x 17 mm diameter) were carved out from the whole mango using a fruit perforator with the edges flattened in a plastic mold. A Texture Analyzer (TA.XT2, Texture Technologies Corp., Scarsdale, NY) was used to compress the samples with an applied force of 0.35 N within two parallel plates (top diameter of 14 cm) (Fig 3-6). Tests were designed for a final deformation of 15% of the original sample height (15% strain), since sample permanent deformation (i.e., sample destruction) occurred prior to this limit. Force (N) versus distance (mm) values were recorded (Figure 3-7) and used to calculate the following texture parameters: (1) the

rupture or breaking force (F_R), in Newton, which is the force required to cause a permanent deformation; (2) the toughness (T) or the area under the force-distance curve up to the point of rupture of the fruits, in Joules (N.m), and (3) the modulus of elasticity (Young's Modulus) which is a measure of the stiffness based on the stress/strain ratio. A minimum of nine replications was performed for each treatment (irradiated and non-irradiated) up to 21 days. All tests were conducted at room temperature.

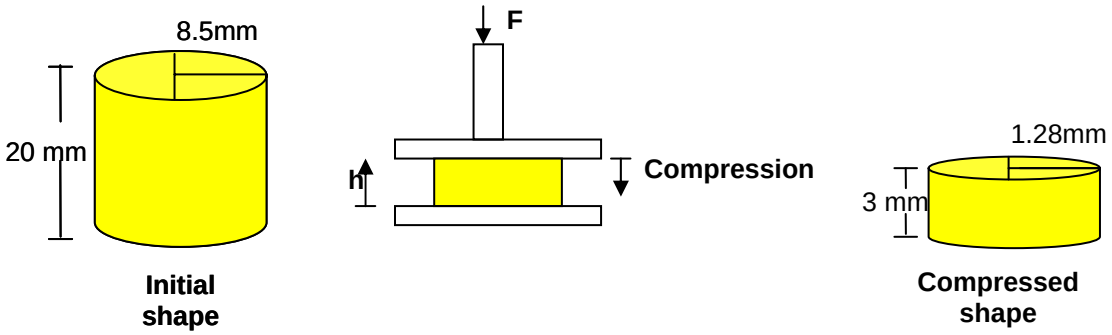

Figure 3-6. Compression test on mangoes.

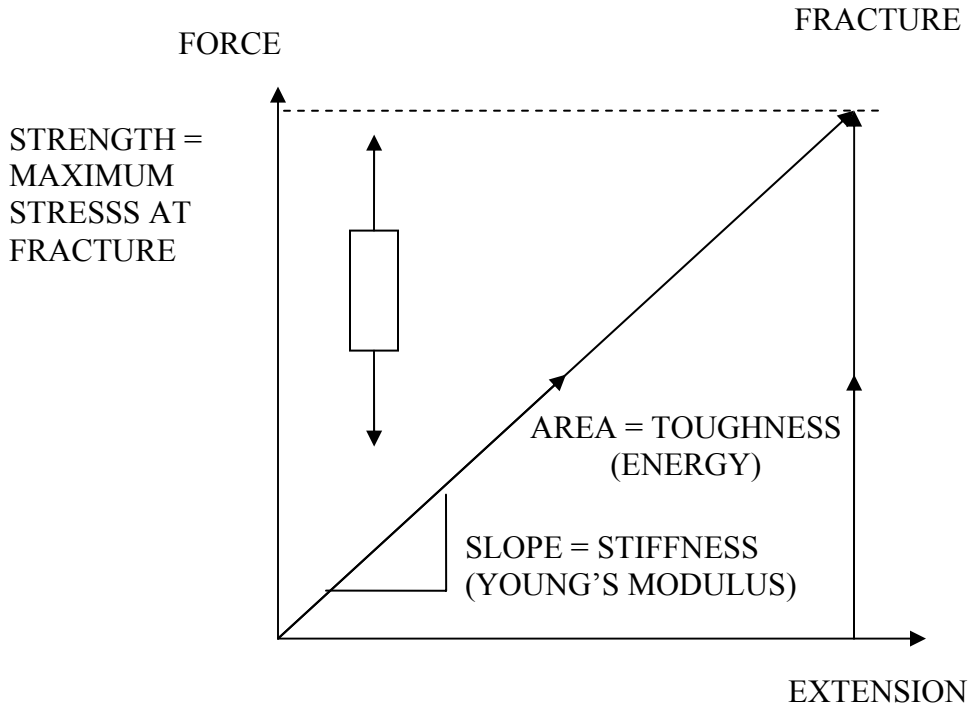

Figure 3-7 Strength, toughness, stiffness and rupture force (adapted from Rosenthal, 1999).

3.2.1.2.2. Blueberries

Firmness of blueberries was measured using a Shear Kramer Press with 5 blades (TA-91) attached to a TA-XT2 Texture Analyzer (Texture Technologies Corporation, Scarsdale, NY). Approximately eighty (80) grams of fruit were placed into the square metal container and a 5 flat-plate plunger was forced through the blueberries. The probe was set at 35 mm from the bottom of the 5 flat-plate plunger and moved downward at 5.0 mm/s (Fig. 3-8). The maximum force, F_m, (N) and area, A, (N.m) were recorded by the Texture Expert software program, v.1.16 (Texture Technology corp., Scarsdale, NY) and used to calculate the firmness, toughness and stiffness of the fruit. Three

measurements were performed for each sample (irradiated and nonirradiated) up to 14 days. All tests were conducted at room temperature.

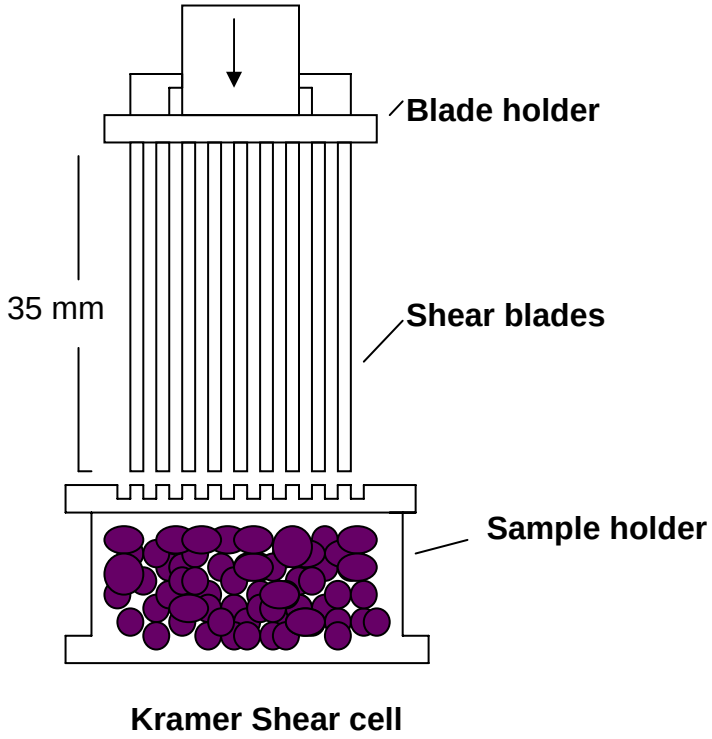

Figure 3-8. Kramer shear test on blueberries.

3.2.1.3. Scanning Electron Microscopy (SEM)

Since the objective of this test was to evaluate the effect of irradiation on cell structure and because most of the reaction effects after processing occur in the first five days, approximately ten days after irradiation treatment the samples were evaluated.

Pieces of fresh tissue of mango and blueberries were cut with a surgical blade with approximate dimensions of 7x7x8 mm and 5x5x4 mm, respectively. The samples were fixed chemically with a 2% glutaraldehyde/phosphate buffer then washed with the same phosphate buffer for 30 min and post-fixed in 1% phosphate buffered osmium solution. The following day the samples were washed with distilled water and dehydrated under vacuum through ascending concentrations of methanol(EMD Chemicals, Gibbstown, N.J.) series, starting with 5% until 100% each for one minute. 0.3 mL Hexamethyldisaline (HMDS) (Sigma Aldrich, St Louis, MO) was added and the samples were dried over night at room temperature. After dehydration, the samples were mounted on stubs and coated with gold-palladium for 90 s in a coating unit, osmium in TECHNICS sputter coater. SEM was performed on a JOEL JSM 6400 scanning electric microscope (Princeton Gamma- Tech. PGT Prism Digital Spectrometer, Japan) at 15 kV (Kim, 1996). Three samples were prepared for each treatment.

3.2.1.4. Unit density

Density of the fruit (treated and control) was determined by the weight and volume displacement method. Samples of fruit (mango: cylindrical pieces of 5cm x 7cm, blueberry: one unit) were weighed and then pierced with a long thin stainless–steel pin. A stand was constructed that held the end of the pin and could be used to lower and completely immerse the pieces in a beaker with water on top of an analytical balance. The increase in weight loading the samples in the water equals the weight of the displaced water. The tests were conducted at room temperature during the entire storage

time (21 days for mango and 14 days for blueberry) and four replications were made for each sample. Density of the fruit was calculated using the following equation:

$$\rho(g/cc) = \frac{W_{sample}}{W_{displaced\ water}} * \rho_{water} \tag{3-1}$$

where W_{sample} = sample weight in grams, $W_{displaced\ water}$ = displaced water weight in grams, ρ = density of the fruit in g/cc and ρ_{water} = density of water in g/cc.

The specific gravity (SG) corresponds to the relationship between the unit density (ρ_{unit}) and the density of water (ρ_{water}) at a reference temperature (T). Therefore it was calculates as:

$$SG = \left(\frac{\rho_{unit}}{\rho_{water}} \right)_T \tag{3-2}$$

The bulk density of the blueberries was calculated as

$$\rho(g/cc) = \frac{W_{bulk}}{V_{bulk}} \tag{3-3}$$

where W_{bulk} = bulk weight in grams, V_{bulk} = bulk volume in cc.

The porosity (ε) that is the fraction of the total volume that is occupied by the air was calculated as follows:

$$\varepsilon = 1 - \frac{\rho_{bulk}}{\rho_{product}} \tag{3-4}$$

where ρ_{bulk} = bulk density in g/cc and $\rho_{product}$ = density of the fruit in g/cc.

3.2.1.5. Respiration rates

Although ethylene production has an effect on the respiration rate, in this study the respiration rate was evaluated in terms of the CO_2 production as a direct product of the respiration process and because the equipment available only determines the concentrations of O_2 and CO_2. The respiration rate of the fruits throughout storage was monitored in a closed system (Figure 3-9). Approximately 50 grams of blueberries were placed in sealed glass jars (450 ml) and stored at 5°C for up to 14 days. Approximately 900 grams of mangoes (2 units) were placed in sealed glass jars (4L) and stored at 12°C up to 21 days. After 2 hours of blueberries being completely sealed and 40 min for mangoes, the gas concentration (CO_2) inside the jars was measured. Internal gases were collected from the headspace using a 5 mL syringe having a side hole needle. The withdrawn gas was immediately injected into a PIR-2000 infrared CO_2 analyzer (IRGA) (Horiba Instruments, Irvine, CA) to determine the levels of CO_2.

Figure 3-9. Experimental set up for measuring respiration rate of tested fruits. (A) blueberries, (B) mangoes.

A standard curve for CO_2 concentrations was obtained and the measured peak was fitted into a curve to obtain the corresponding gas concentration for each sample (treated and control). Tests were conducted in triplicate, and the respiration rate was calculated using the following equation:

$$(mlCO_2/kg.h) = \frac{\%CO_2 * V_{final}(mL)}{W_{sample}(kg) * t(h)} \quad (3\text{-}5)$$

where V_{final} = headspace volume in mL, W_{sample} = sample weight in grams, and t = time of gas collection (40 min for blueberries and 2 hours for mangoes) (Saltveit, 1997).

3.2.2. Chemical properties

To prepare the samples, approximately three (3) mangoes and two (2) blueberry trays were taken from each batch treatment (control and irradiated at low, medium and high doses), and homogenized in a blender at high speed (Oster Regency Kitchen Center) using a shredder disc to obtain a pulp sample for the chemical analyses.

3.2.2.1. Moisture content

Moisture content of the fruit was measured using a vacuum oven (Squared Lab Line Instruments, Melrose Park, IL), by taking 5 g of flesh (mesocarp) and drying at 70^{o}C under pressure ≤ 100 mm Hg (13.3 KPa) to a constant weight following the AOAC Method 920.151 (AOAC, 1980). Tests were run in triplicate and were conducted throughout the storage time. Moisture in wet basis was calculated as follows:

$$MC(\%w.b.) = \frac{(W_{wet} - W_{dry})}{(W_{wet})} x100 \quad (3\text{-}6)$$

where W_{wet}= weight of wet sample and W_{dry}= weight of dry sample in grams.

3.2.2.2. Water activity

Water activity was determined using a Rotronic Hydrometer (Rotronic Instrument Corp., Huntington, NY) at room temperature. Approximately 38g of pulp from each sample (treated and control) were placed in an air-tight chamber connected to a panel display where the corresponding water activity and temperature were recorded. Readings were made in triplicate and were reported throughout the storage time at the specified intervals.

3.2.2.3. pH

The pH of the fruit samples was determined using a digital pH meter (Corning model 350 pH/ion analyzer, Corning, Inc) following AOAC Method 32.025 (AOAC, 1980). After previous calibration with standard solutions (pH 4.0, 7.0 and 10.0) a glass electrode was immersed in the pulp of the fruit. The pH was recorded by direct reading. Three replications per sample were used. Measurements were conducted at room temperature and throughout the storage time.

3.2.2.4. Titratable acidity

Acidity was determined by titrating ten (10) grams of flesh with 0.1 N alkali (NaOH) following AOAC Method 22.060 (AOAC, 1980). Ten (10) grams of the pulp were diluted to 250 ml with neutralized water and approximately 0.3 ml of phenolphthalein (Fisher Scientific Company, Fair Lawn, NJ) was added. The sample was stirred and titration was made with 0.1N NaOH (Fisher Scientific, Fair Fawn, NJ) until a pink color persisted for 30 sec and the pH reading was 8.1.Three replications per

each sample were used. Determinations were made throughout the storage time. Results were expressed in terms of dominant acid as grams of citric acid per gram (AOAC, 1980).

3.2.2.5. Total soluble solids

The total soluble solids content was determined using samples of the fruit's juice with a hand refractometer (TS Meter Refractometer, American Optical, Buffalo, NY). Soluble solids by the refractometric method are defined as the concentration (by weight) of sucrose in solution that has the same refractive index (n) as the solution analyzed. The refractive index is measured in the refractometer using prisms and by total reflection. The instrument was calibrated with water at 20ºC, and a drop of the sample (juice) was placed on the prism of the instrument at 20ºC. The corresponding percentage of soluble solids was obtained by direct reading. Three replications per each sample were used. Determinations were conducted throughout the storage time.

3.2.2.6 Sugars

3.2.2.6.1 Total sugars

The total sugar content of the samples was determined using a modified version of the phenol-sulphuric acid method by Dubois et al. (1956). Approximately 5 grams of pulp were weighed and diluted in 100 mL distilled water. Diluted samples were left at refrigeration temperature (10°C) for 17 hours. After this time, the solution was filtered using a Whatman® filter No.4 and a vacuum pump (KNF Neuberger, Inc., Trenton, NJ) and the volume was increased to 200mL. A new dilution was made by taking 1 mL of the extract solution and completed to 100 mL with distilled water. Two (2) mL of sugar

solution were placed into an essay tube and 0.05 mL. of 80% phenol were added. Then, 5 mL of concentrated sulfuric acid (EMD Chemicals, Gibbstown, NJ) were added rapidly. The tubes were allowed to stand 10 minutes and then shaken using a Vortex Genie 2 (Scientific Industries, Bohamia, NY) and placed for 10 to 20 minutes in a water bath (Baxter Scientific Products, Miami, FL) at 20^{o} to 30^{o} C before readings were taken. The absorbance of the characteristic yellow-orange color was measured using an UV-1601 Spectrophotometer (Shimadzu Corp., MD). The wavelength was fixed at 490 nm. Blanks were prepared by substituting distilled water for the sugar solution.

The amount of sugar was then determined by referring to a standard curve previously developed with concentrations ranging from 40.0 to 160.-0 µg/ml (Figure 3-10) for the particular sugar under examination. From the standard curve, *x* is the sugar concentration and *y* corresponds to absorbance. Thus,

$$y = -0.02275 + 0.00842x,\ R^2 = 0.999 \qquad (3\text{-}7)$$

Tests were conducted in triplicate and throughout the storage time. The concentration of total sugars was calculated as:

$$x(g/100g) = \left(\frac{\left(\frac{y + 0.02275}{0.0084} \right) \frac{\mu g}{ml} * \frac{200*100ml}{w_{sample}(g)} * \frac{0.000001g}{1\mu g}}{2} \right) *100 \qquad (3\text{-}8)$$

where W_{sample}= sample weight in grams.

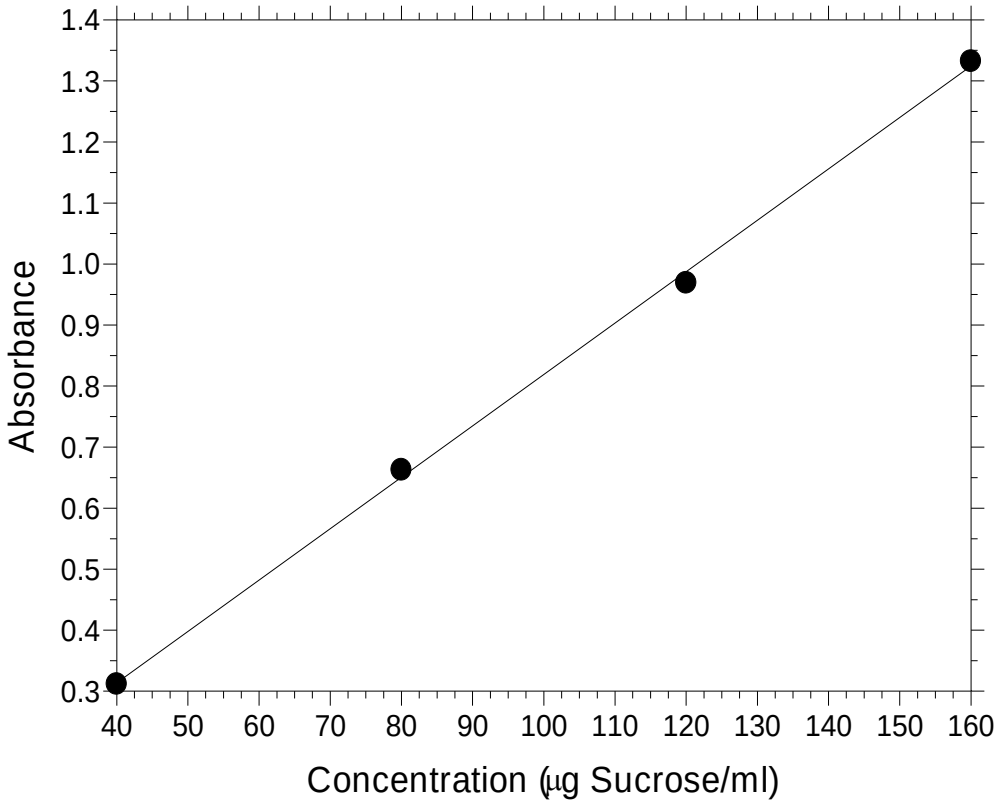

Figure 3-10. Standard curve for total sugars (µg sucrose/ml).

3.2.2.6.2 Reducing sugars

The reducing sugars (glucose) content was determined by spectrophotometer following the dinitro-salicylic method (Miller, 1959). This method tests for the presence of free carbonyl group (C=O), the so-called reducing sugar. It involves the oxidation of the aldehyde functional group present in, for example, glucose and the ketone functional group in fructose. Simultaneously, 3,5-dinitrosalicylic acid (DNS) is reduced to 3-amino, 5-nitrosalicylic acid under alkaline conditions:

oxidation

aldehyde group ----------> carboxyl group (3-9)

reduction

3, 5-dinitrosalicylic acid ----------> 3-amino, 5-nitrosalicylic acid (3-10)

Because dissolved oxygen can interfere with glucose oxidation, sulfite, which itself is not necessary for the color reaction, is added to the reagent to absorb the dissolved oxygen (Miller, 1959).

The above reaction scheme shows that one mole of sugar will react with one mole of 3,5-dinitrosalicylic acid. However, it is suspected that there are many side reactions and that the actual reaction stoichiometry is more complicated than that previously described. The type of side reaction depends on the exact nature of the reducing sugars. Different reducing sugars generally yield different color intensities;

thus, it is necessary to calibrate each sugar. In addition to the oxidation of the carbonyl groups in the sugar, other side reactions such as the decomposition of sugar also compete for the availability of 3,5-dinitrosalicylic acid. As a consequence, carboxymethyl cellulose can affect the calibration curve by enhancing the intensity of the developed color (Miller, 1959).

Three (3) mL of sugar solution were placed into an essay tube and 3 mL of dinitrosalicylic acid (Sigma Aldrich, St Louis, MO) solution were added. To avoid the loss of liquid due to evaporation, the test tubes were covered with a piece of paraffin film and heated at 90º C for 5-15 minutes to develop the red-brown color. One (1) mL of a 40% potassium sodium tartrate (Rochelle salt, Sigma Aldrich, St Louis, MO) solution was added to stabilize the color. After cooling to room temperature in a cold water bath, the absorbance was recorded with a UV-1601 Spectrophotometer (Shimadzu Corp., MD). The wavelength was set at 575 nm. A standard curve (Figure 3-11) was prepared using different glucose concentrations from 0.0 to 2.5 mg /ml. From the curve, x is the sugar concentration and y corresponds to absorbance. Thus:

$$y = -0.038395 + 0.49661x,\ R^2=0.998 \qquad (3\text{-}11)$$

Two replicates were done for each sample. Analyses were carried out throughout the entire storage time and the concentration of reducing sugars was determined as follows:

$$x(g/100g) = \left(\frac{y + 0.38395}{0.49661} \right) \frac{mg}{ml} * 200ml * \frac{0.001g}{1mg} * 100 \qquad (3\text{-}12)$$

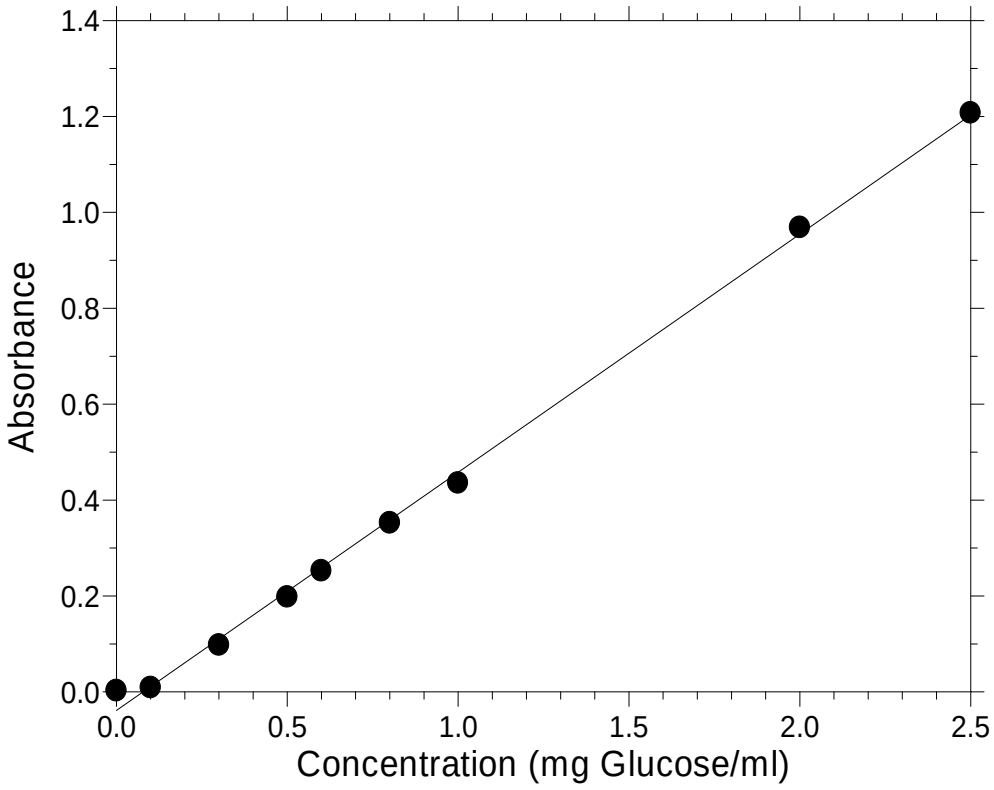

Figure 3-11. Standard curve for reducing sugars (mg glucose/ml).

3.2.2.7. Total phenolics

Total phenolics were determined by spectrophotometric analysis following the Folin-Denis assay using the AOAC method 9.098 (AOAC, 1980). Approximately 5 grams of pulp were weighed and diluted in 25 mL of methanol (EMD Chemicals, Gibbstown, NJ). Diluted samples were left standing at room temperature for 24 hours. After this time, samples were filtered and the volume was completed to 50 mL. Then, 7.5 mL distilled water was added to a 1.0 mL of sample extract and was shaken with a Vortex Genie 2 (Scientific Industries, Bohemia, NY). Half (0.5) mL of Folin-Denis reagent (Fluka, St Louis, MO) was added to this solution. After 3 min standing, 1 mL saturated sodium bicarbonate solution was added. The absorbance was measured using an UV-1601 Spectrophotometer (Shimadzu Corp; MD) at 725 nm after standing 1h at room temperature. Samples were run in triplicate. This test was conducted throughout the storage time.

The results were determined by using a standard curve previously developed with six different concentrations ranging between 0.0 and 150 μg/mL (Figure 3-12) and expressed as mg Gallic acid / 100 g of fresh fruit. From the standard curve, *x* is the Gallic acid concentration and, *y* is the absorbance. Thus:

$$y = -0.00959524 + 0.00692238\,x,\ R^2=0.99813 \qquad (3\text{-}13)$$

Total phenolics concentration was calculated as follows:

$$x(mg/100g) = \left(\frac{y + 0.00956}{0.00692}\right)\frac{\mu g}{ml} * \frac{50ml}{W_{sample}(g)} * \frac{0.001mg}{1\mu g} * 100 \qquad (3\text{-}14)$$

where W_{sample} = sample weight in grams.

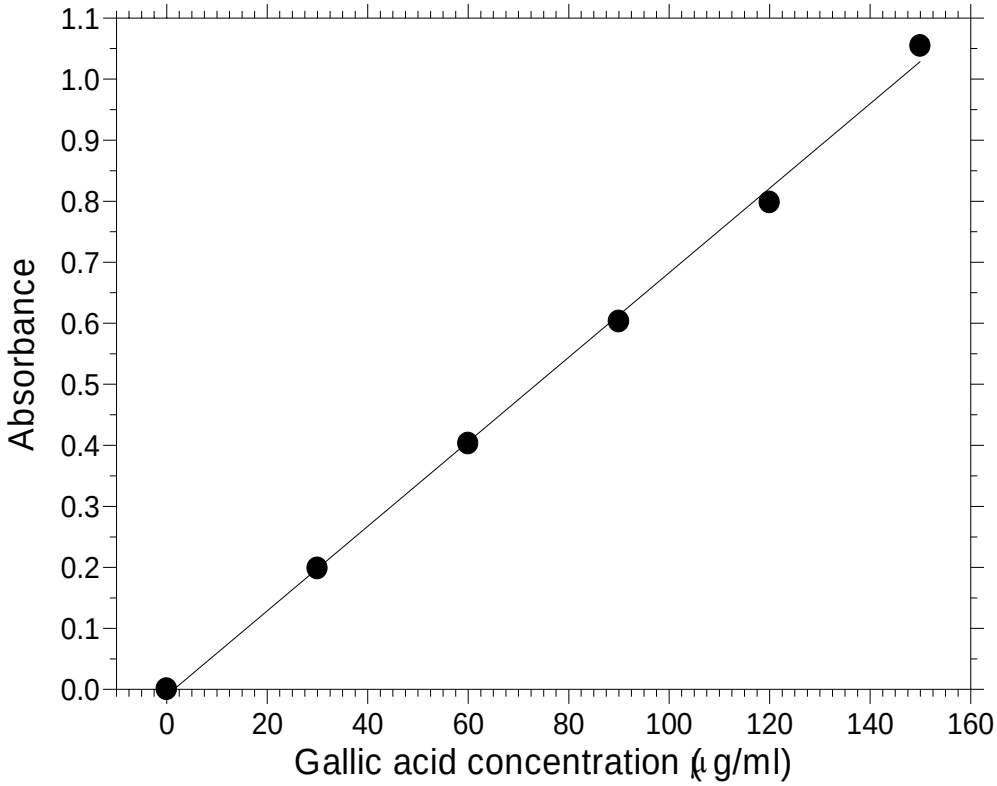

Figure 3-12. Standard curve of total phenolics (µg gallic acid/ml).

3.2.2.8. Antioxidant activity index

The determination of the antioxidant activity index was obtained following the diphenylpicrylhydrazyl (DPPH) free radical method (Bondet et al., 1997). From the same phenolic extraction obtained in section 3.2.2.7, 0.5 mL were taken and added to 3.5 ml of methanolic DPPH (Sigma Aldrich, St. Louis, MO) solution so that the initial DPPH concentration in the cuvettes (10 mm x 4mm x 45 mm) was approximately 6 x 10-5 mol/L. The absorbance was read at 515 nm by spectrophometric measurements in a kinetics modem using an UV-1601 Spectrophotometer (Shimadzu Corp., MD), until the reaction reached a plateau for 1 min (absorbance final). The percentage of antioxidant activity index (*Q*) was calculated as,

$$Q(\%/g) = \left(\frac{\frac{(Abs_{initial} - Abs_{final})}{Abs_{initial}}}{W_{sample}(g)} \right) * 100 \qquad (3\text{-}15)$$

where $Abs_{initial}$ = absorbance at time 0, Abs_{final} = absorbance at 1 min, and W_{sample} = sample weight in grams. Tests were conducted in triplicate throughout the storage time.

3.2.2.9. Ascorbic acid (Vitamin C)

Vitamin C content was determined using the 2.6-dichlorophenol-indophenol titrimetric method following the 43.059 AOAC standards (AOAC, 1980). In this method, L-ascorbic acid is oxidized to L-dehydroascorbic acid by the indicator dye. At the endpoint, excess unreduced dye is rose-pink in an acid solution. L-dehydroascorbic

acid can be determined by first converting it to L-ascorbic acid with a suitable reducing agent. The red-color endpoint should last at least 10 sec to be valid (AOAC, 1980).

About 10 grams of the fresh fruit were pulverized by grinding. Methaphosphoric acid-acetic acid solution (HPO_3-HOAc) (Sigma Aldrich, St Louis, MO) was added. The solution was filtered using a Whatman® filter No. 4 and a vacuum pump (KNF Neuberger, Inc., Trenton, NJ) and then tritrated until the sample became in suspension. Three replications for each sample were done. Tests were conducted at room temperature and throughout the storage time.

3.2.2.10. Volatiles

Volatile compounds were identified by headspace solid phase microextraction (HS-SPME) analysis using a mass spectrophotometer gas chromatograph (MS-GC). Nine grams of fruit sample were placed in 40 ml headspace sampling vials and equilibrated at 25°C. A Carboxen™/PDMS Stable Flex™ 85 µm SPME fiber (Supelco Co., Bellefonte, PA) was used to absorb the head space volatiles. The fiber was inserted into the sample vial through the septum and exposed to headspace for 60 min. Before sampling, the PDMS fiber was reconditioned for 30 min in the GC injection port at 250°C. Volatile compounds absorbed by partition on the SPME fiber were thermally desorbed at 250°C for 3 min in the injector port of an HP6890/5973 GC-MS (Hewlett Packard, Palo Alto, CA) with a CP-Wax BP20 capillary column (25m x 0.53mm i.d., 1.0µm film thickness). Helium was used as a carrier gas. A splitless mode was used. The oven temperature was maintained at 40ºC for 3 min, followed by an increase to 220ºC at the rate of 7ºC /min and held for 25 min. The HP 5973 mass spectrometer was operated

in the electron ionization mode at 70 eV. The tests were conducted by duplicate for each sample and evaluated on the first and the last day of the storage time.

Data were collected with HP CHEMSTATION software (Agilent Technologies) and searched against the Wiley registry of mass spectral data compounds (6^{th} edition, Palisade Corp., Newfield, NY). Compounds were identified by matching their spectra with the library database, and total ions counts were presented. Because, there was a dominant compound recovered in most samples of mangoes and blueberries, respectively, the data was evaluated from the total ion chromatograms on a relative percentage basis. The ion count of δ-3-carene in mango samples, was divided by the total ion count of all integrated compounds, and then expressed as a relative percentage. Thus, reported volatile data are semi-quantitative (Beaulieu & Lea, 2003). The same approach was used for (E)-2-hexenal in blueberry samples.

3.2.2.11. Pigments

3.2.2.11.1. Carotenes

In this study, the content of β-carotenes in mango samples was determined by spectrophotometric measurements using an UV-1601 Spectrophotometer (Shimadzu Corp; MD) calibrated at 453 nm following the methodology cited by Rodrigues-Amaya (1989). All tests were carried out without the presence of light in order to protect the compounds from diffused light and oxygen. Fifty (50) mL of acetone were added to 5 grams of the fruit's pulp. The extraction was made after 24 hours. The solution was filtered using a Whatman® filter No. 4 and a vacuum pump (KNF Neuberger, Inc., Trenton, N.J.). The acetonic solution was placed in decantation balloons and 50 mL of

petroleum ether was added. The residue was washed with approximately 5-7 washes of 100 mL distilled water. The carotene extract was concentrated in a Rota-vapor-R110 (Brinkman Instruments, Westbury, N.Y.) at 32˚C for 10 min to evaporate the petroleum ether. The spectrophotometric determination was made by diluting the concentrate with 5 mL of hexane (EM Science, Gibbstown, N.J). Tests were conducted in triplicate at room temperature and throughtout the storage time.

The concentration of β-carotenes was determined with a previous standard curve developed at concentrations between 1.0 and 2.5 μg/ml (Figure 3-13) in which *x* and *y* correspond to concentration and absorbance, respectively,

$$y = -0.0125 + 0.2442x \text{ , } R^2=1.000 \tag{3-16}$$

The concentration of carotenes was calculated as:

$$x(\mu(\mu 100g) = \left(\frac{y + 0.0125}{0.2442}\right)\frac{\mu g}{ml} * \frac{60ml}{W_{sample}(g)} * 100 \tag{3-17}$$

where W_{sample} = sample weight in grams.

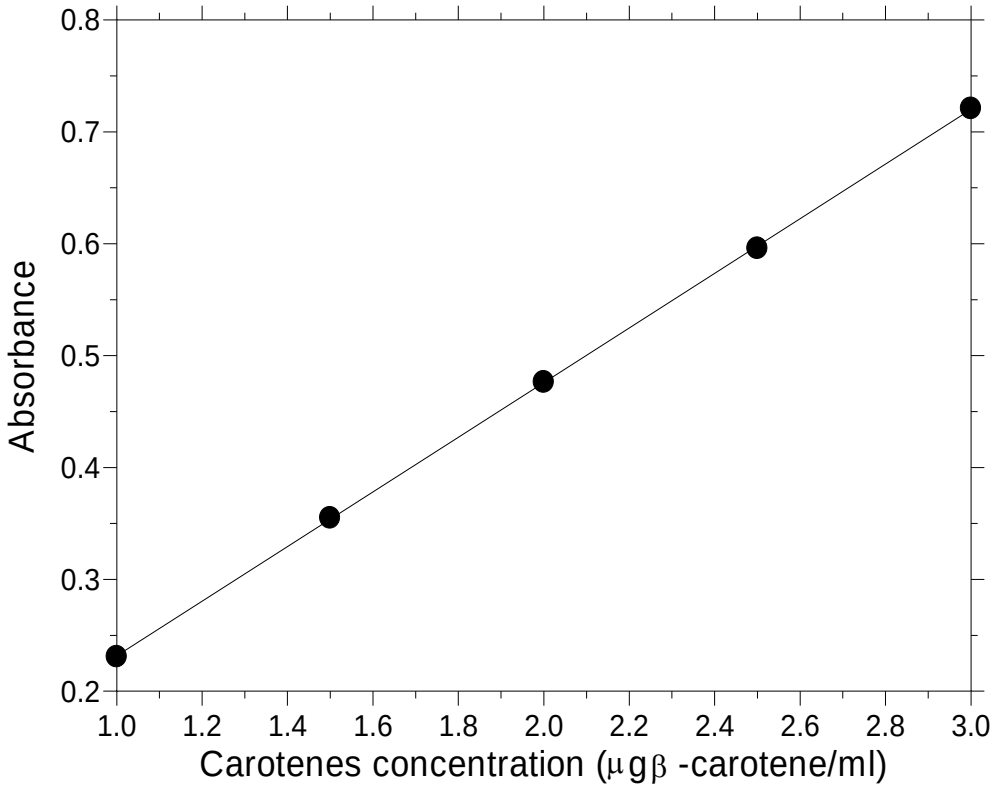

Figure 3-13. Standard curve for carotenes (μg β-carotenes/ml).

3.2.2.11.2 Tannins

The tannin content in blueberries was determined colorimetrically using the vanillin assay for their quantification (Shahidi & Naczk, 1995). Approximately 5 grams of pulp were weighed and extraction was made with 25 mL of methanol (EMD Chemicals, Gibbstown, N.J.). Diluted samples were left standing at room temperature for 24 hours. Samples were then filtered and the volume increased with methanol to 50 mL. Five (5) mL of freshly prepared vanillin solution in methanol containing 4% of concentrated HCl was added to a 1 mL solution of condensed tannins and mixed thoroughly with a Vortex Genie 2 (Scientific Industries, Bohemia, NY). Blanks were prepared by substituting the vanillin solution in methanol for a 4% concentrated HCl solution in methanol. The absorbance (Abs) was measured at 500nm after standing for 20 min at 30ºC. The results were determined by using a standard curve previously developed with different concentrations between 0.0 and 200 μg/ml (Figure 3-14) in which *x* and *y* correspond to concentration and absorbance, respectively,

$$y = 0.00857143 + 0.00495679x,\ R^2 = 1.000 \quad (3\text{-}18)$$

Tests were conducted by triplicate at room temperature and throughout the storage time. The concentration of condensed tannins was expressed in mg of catechin/100g of sample calculated as:

$$x(mg/100g) = \left(\frac{(Abs_{sample} - Abs_{blank}) - 0.00857143}{0.00495976} \right) \frac{\mu g}{ml} * \frac{50ml}{W_{sample}(g)} * \frac{0.001mg}{1\mu g} * 100 \quad (3\text{-}19)$$

where Abs_{sample} = absorbance of the sample, Abs_{blank} = absorbance of the blank and Wsample = sample weight in grams.

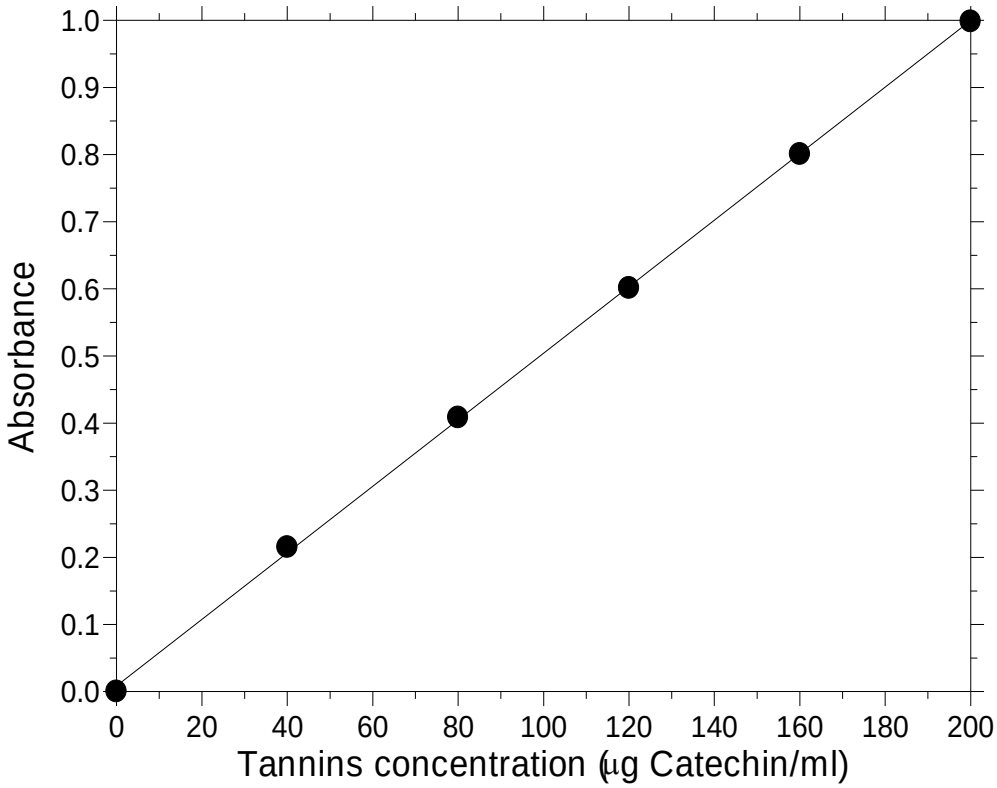

Figure 3-14. Standard curve for tannins (µg catechin/ml).

3.2.3. Sensory evaluation

The sensory test helps to understand the attributes of a product from a consumer point of view that is critical to its acceptance.

The combined effect of irradiation dose and storage time on the sensory quality of the mangoes and blueberries was evaluated (overall quality, color, texture and aroma) throughout the specified storage time by 50 untrained and randomly chosen panelists. The samples (control and irradiated) were presented to the panelist for a total of four samples of the respective fruit every week. For overall quality and color, the scale was 1-5 (hedonic scale) as described by Meilgaard et al. (2000). On this hedonic scale, a score of 1 represented the attributes most liked and a score of 5 represented the attributes most disliked. For texture, samples were rated as 1-5 with 1(firm) and 5 (soft). The aroma was rated as 1 (strong) to 5 (none) (see also appendix A-A). The samples were placed on top of white paper plates identified by three digits and randomly placed in the trays. Samples were only evaluated by visual inspection.

3.3. Statistical analysis

A completely randomized design with a 3*3*4 factorial experiment was conducted for each measurement: Three irradiation dose levels, three replications, and four days of analysis during the storage time. All experiments were conducted in triplicate with untreated samples used as controls. Analysis of Variance (ANOVA) using statistical analysis package (SPSS 11.0, 1999) with mean separation by Student-Neuman-Keuls (SNK) method at $P < 0.05$ was utilized to analyze the data.

CHAPTER IV

RESULTS

4.1. Effect of irradiation on physical and chemical properties of mango

4.1.1. Physical properties

4.1.1.1. Color attributes

4.1.1.1.1. Visual changes

Visual changes in the color of the fruits were noticeable throughout the entire storage time (Figures 4-1 to 4-3). Changes in the color of fruit are usually related to different stages of maturity. However, irradiation can induce some changes in metabolism that cause a delay in the ripening process. The outer skin of the mango fruits irradiated at a higher dose (3.1 kGy) was greener than the skin of the control (non-irradiated) samples by the end of storage (Figure 4-3); however, pitting (scars and holes) and browning (dark contour) of the internal flesh tissue was also observed (Figure 4-3, Appendix A-B). This observation suggests that the absorbed dose was higher in these areas (as discussed in section 3.1.1) which could have increased the activity of enzymes such as polyphenoloxidase and phenylalanine ammonia lyase. The browning and discoloration of the mango skin have been attributed to the ozone formation during the irradiation process and also to changes in enzyme (polyphenoloxidase) activity (Thomas, 1986). Injury and discoloration or browning of skin and fresh tissues have been reported when irradiating mangoes with gamma rays at doses as low as 0.5 kGy and 0.75 kGy (Spalding & Von Windeguth, 1988).

Figure 4-1. Irradiated and non-irradiated mangoes right after irradiation treatment (day 0). (Control =non-irradiated, Low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.1 kGy).

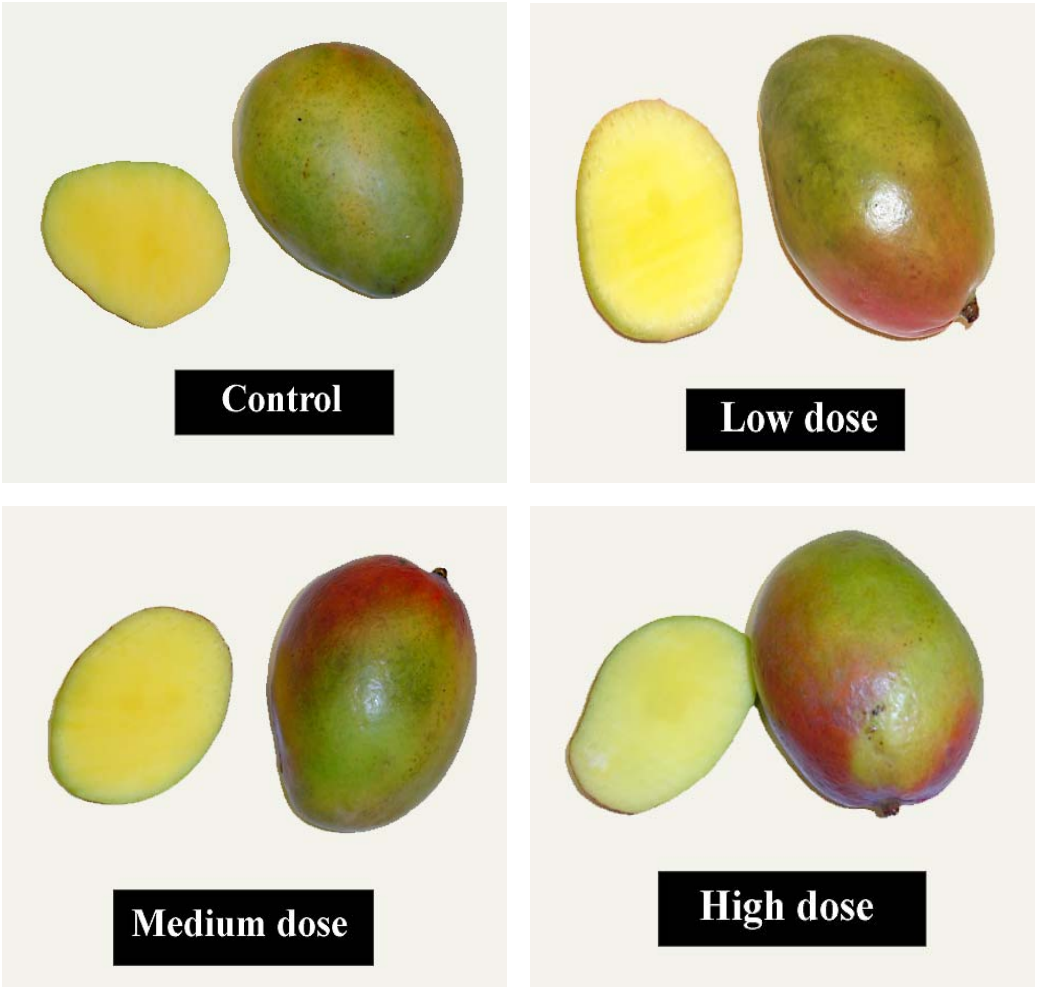

Figure 4-2. Irradiated and non-irradiated mangoes after 5 days of storage at 12ºC. (Control =non-irradiated, Low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.1 kGy).

Figure 4.3. Irradiated and non-irradiated mangoes after 21 days of storage at 12°C. (Control =non-irradiated, Low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.1 kGy).

It is important to consider that the color of a fruit is also influenced by the harvesting conditions which may also affect the appearance of the fruit. In some control (non-irradiated) fruits black spots in the skin were observed at the beginning of the experiment (Figure 4-1). In addition, the non uniformity in maturity levels among the fruits used in this study may have caused differences in color.

4.1.1.1.2. Objective measurement

The effect of ionizing radiation on the color (Hunter parameters) of the mangoes is shown in Tables 4-1 and 4-2. Compared with the control, no differences in the lightness (*L*) values were observed after irradiation (day 0). However, all the irradiated fruits became significantly (P> 0.05) lighter (higher values of *L*) by the last day of storage. The increases in *L* value were 6.43%, 8.30% and 4.0% for samples treated with low (1.0 kGy), medium (1.5 kGy) and high (3.1 kGy) doses, respectively (Table 4-1). The increase in *L* values may be associated with higher chlorophyll content in these fruits, in addition to the decrease in *a* values for all irradiated samples on this day (21). During the storage time, the samples exposed to medium (1.5 kGy) and high (3.1 kGy) doses showed significant (P> 0.05) darkening (decrease in *L* value) at days 5 and 10 (Table 4-1). The reduction was of about 7.87% and 4.62% for samples treated with medium dose, and 12.24% and 4.0% for samples exposed to high dose on days 5 and 10, respectively. For control fruits and samples treated with low dose (1.0 kGy) no significant differences (P> 0.05) in *L* values were found. These results could be

Table 4-1
Effect of irradiation dose on the color attributes -lightness (*L*), redness (*a*) and yellowness (*b*)- of mangoes stored up to 21 days at 12°C

Color parameter	Dose/Day	Control* (0.0 kGy)	Low (1.0 kGy)	Medium (1.5 kGy)	High (3.1kGy)
L	0	73.28^{ax}(3.18)	73.86^{ax}(3.81)	75.00^{ax}(1.20)	75.12^{ax}(1.74)
Lightness	5	69.54^{ax}(2.28)	71.25^{ax}(2.30)	69.10^{bx}(3.86)	65.92^{bx}(4.71)
(%)	10	72.51^{ax}(3.44)	72.07^{ax}(3.50)	71.53^{bx}(2.72)	72.06^{bx}(1.89)
	21	69.88^{ax}(1.72)	74.38^{ayz}(1.46)	75.68^{az}(0.43)	72.67^{ay}(1.21)
a	0	3.06^{ax}(5.44)	3.67^{ax}(5.23)	3.16^{ax}(3.38)	3.26^{ax}(1.70)
redness	5	12.84^{bx}(5.30)	10.57^{bx}(2.37)	9.25^{bx}(3.90)	9.74^{bx}(6.06)
(+red, -green)	10	4.20^{ax}(4.02)	3.79^{ax}(4.67)	6.20^{abx}(3.50)	3.50^{ax}(2.31)
	21	9.65^{bx}(1.51)	2.01^{ay}(0.64)	2.04^{ay}(1.28)	4.74^{az}(1.70)
b	0	49.78^{ax}(6.34)	48.86^{ax}(9.58)	49.31^{ax}(5.40)	43.13^{ax}(5.32)
yellowness	5	61.07^{bx}(9.18)	64.37^{bx}(2.23)	63.61^{bx}(5.05)	60.68^{bx}(10.58)
(+yellow, -blue)	10	51.03^{abx}(9.08)	53.78^{ax}(10.81)	59.36^{abx}(8.79)	52.47^{bx}(6.44)
	21	60.86^{bx}(2.07)	51.13^{ay}(2.88)	50.86^{ay}(5.11)	55.73^{bxy}(6.42)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-b}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
$^{x-z}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

Table 4-2
Effect of irradiation dose on color attributes-chroma (*C*), total color difference (*ΔE*) and hue (θ) - of mangoes stored up to 21 days at 12°C

Color parameter	Dose/Day	Control* (0.0 kGy)	Low (1.0 kGy)	Medium (1.5 kGy)	High (3.1 kGy)
Chroma	0	50.07^{ax}(6.80)	49.16^{ax}(10.03)	49.49^{ax}(5.58)	43.15^{ax}(5.34)
(C)	5	62.49^{bx}(9.97)	65.27^{bx}(2.23)	64.34^{bx}(5.58)	61.59^{bx}(11.38)
	10	51.29^{ax}(9.42)	54.06^{ax}(10.99)	51.74^{abx}(9.05)	52.61^{bx}(6.59)
	21	61.64^{bx}(2.18)	51.17^{ay}(2.90)	50.91^{ay}(5.16)	55.95^{bxy}(6.53)
Total color difference	0	88.78^{ax}(2.87)	89.02^{ax}(3.12)	89.79^{ax}(2.41)	86.56^{ax}(2.61)
(ΔE)	5	93.48^{ax}(4.76)	96.26^{bx}(2.71)	94.23^{ax}(1.56)	90.45^{ax}(4.48)
	10	88.70^{ax}(3.55)	90.10^{ax}(4.22)	93.02^{ax}(4.12)	88.86^{ax}(3.26)
	21	92.75^{ax}(2.08)	89.87^{ax}(1.94)	90.82^{ax}(3.02)	91.41^{ax}(3.57)
Hue angle	0	57.06^{ax}(1.21)	56.50^{ax}(1.23)	56.66^{ax}(1.23)	85.68^{ax}(1.68)
(θ)	5	78.57^{ax}(3.57)	80.67^{ax}(2.08)	81.90^{ax}(2.70)	81.51^{bx}(4.17)
	10	60.08^{ax}(1.15)	61.08^{ax}(1.40)	83.66^{ay}(2.30)	86.37^{bx}(1.98)
	21	81.00^{ax}(0.02)	87.77^{ay}(0.59)	88.05^{ay}(1.17)	85.27^{bz}(1.41)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-b}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
$^{x-z}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

attributed to an increased polyphenoloxidase activity and the consequent oxidation of phenolics giving rise to brown and dark pigmentation of the fruit (Thomas, 1986).

Compared with the control sample, redness (*a*) values showed a significant (P>0.05) decrease in all irradiated samples by the twenty-first day of storage, indicating a greener color. These reductions corresponded to 79.17%, 78.86% and 50.88% for samples treated with low (1.0 kGy), medium (1.5 kGy) and high (3.1 kGy) doses, respectively (Table 4-1). Mitchell et al. (1992) reported a reduction in *a* values of mangoes exposed to 75 and 300 Gy gamma rays. These results are associated with the potential effect of irradiation on delaying the ripening process. Normally, in climacteric fruits, such as mango, when ripening begin there are changes in pigments from green to yellow or red, due to chlorophyll degradation and carotenoid and/or anthocyanin development. It has been shown that irradiation can destroy pigments on fruit depending on the dose and the irradiation conditions (Hulme, 1971).

During storage, the *a* value for control and irradiated fruits did not follow a clear trend. However, for control samples a significant increase was observed in days 5 (76.16%) and 21 (68.30%). All irradiated samples showed a significant (P>0.05) increase in *a* values by the fifth day of storage. The increase corresponded to 65.3% for low dose (1.0 kGy), 65.84% for medium (1.5 kGy) dose and 66.63% for high (3.1 kGy) dose; this means that the fruit had a redder color instead of a greener color; therefore, an indication that the fruits were riper on this day than in the other times of evaluation. The formation of a red deep pigment was observed by Clarke (1959) to occur in irradiated pears during storage, while Maxie and Abdel-Kader (1966) observed an increase in red

pigmentation of irradiated peaches and nectarines. These authors did not find an explanation for this finding. However, Thomas (1986) observed that irradiated (0.15 to 0.75 kGy) 'Totapuri' mangoes developed a deep pink coloration around the shoulder due to formation of anthocyanins.

The yellowness (*b* values) was considerably higher for the control than for all the irradiated samples by the end of the storage time (Table 4-1). The difference became significant (P>0.05) in samples exposed to low (1.0 kGy) and medium (1.5 kGy) doses which had a decrease in *b* values of 16.0% and 16.5%, respectively. The pulp carotenoids continue to increase in the fruit as the ripening proceeds; therefore, these results suggest the ripening of the control samples and its delay in the irradiated fruits.

During the storage time, yellowness (*b*) values increased significantly (P>0.05) in all the treatments at the fifth day of storage. This increase accounted for 18.5% for control samples, 24.09% for low dose (1.0 kGy), 22.5% for medium dose (1.5 kGy) and 28.92% for high dose (3.1 kGy). Similar results were found by Lacroix et al. (1992) with mangoes exposed to gamma irradiation at dose levels between 0.6 and 0.9 kGy. These results are associated with an increase in carotenoids content and the effect of irradiation on delay of the ripening process as it was previously mentioned.

When compared with the control sample, chroma (*C*) values showed a significant (P>0.05) decrease by the twenty-first day of storage in samples treated with low (1.0 kGy) and medium (1.5 kGy) doses (Table 4-2) indicating that these fruits had more dull color. The *C* values were reduced by 17.0% and 17.5 %, respectively. The effect of storage, although significant (P>0.05) in all the treatments by the fifth day, didnot show

any specific trend. The increased value indicates a brighter color of the fruits and corresponds to 19.87% for control samples, 25.0% for samples treated with low dose (1.0 kGy), 23.0% for samples treated with medium dose (1.5 kGy) and 30.0% for samples treated with high dose (3.1 kGy). Similar results were observed in the sensory studies that indicated the non acceptance of the color of the samples exposed at higher dose by the end of the storage time (section 4.1.3).

The total color difference (ΔE) values were not affected significantly by irradiation dose (Table 4-2). During the storage time, this parameter increased slightly for all treatments; however, samples irradiated at low (1.0 kGy) dose showed a significant ($P>0.05$) increase (7.5%) by day five. This increase may be associated with the higher *a* and *b* values of these samples on that day which are associated with changes in carotenoids and anthocyanins content.

Irradiated mangoes had significantly ($P>0.05$) higher hue (θ) values than the control samples by the end of storage (Table 4-2) which means a change from the orange to yellow spectrum. Only the high dose (3.1 kGy) samples showed a significant ($P>0.05$) increase throughout the storage period. Lacroix et al. (1992) reported that irradiated mangoes exposed to gamma irradiation at dose levels between 0.6 and 0.9 kGy had significantly higher hue (θ) values than the control.

These results suggest that the low dose (1.0 kGy) is the best irradiation treatment to maintain the best fruit color quality attributes.

4.1.1.2. Texture (uniaxial compression)

The rupture force was determined as an indicator of firmness or softness of the fruits. Exposure to ionizing radiation induced a significant (P>0.05) softening of the mangoes throughout the entire storage time. The mangoes exposed to the higher dose level (3.1 kGy) were significantly (P>0.05) softer, requiring 82.7% less force to rupture than the control samples (Figure 4-4). These fruits were mushy and had increased moisture contents (1.35% more) (section 4.1.2.1). The firmness of the fruits exposed to low (1.0 kGy) and medium (1.5 kGy) doses, was reduced by 50.0% and 66.9 %, respectively (Table A-1, Appendix A). However, the rate of reduction was higher on day five; firmness decreased by 60.9 % at low (1.0 kGy) dose, 86.7 % at medium (1.5 kGy) dose, and 85.9 % at high (3.1 kGy) dose. According to Mitra (1997), ripening of the mango fruit is characterized by softening of the flesh. Skin color development is accompanied by ultrastructural changes associated with chloroplast to chromoplats transition. The thylakoid membrane of systems in the skin of the mangoes gradually breaks down, while osmiophilic globules enlarge and increase in number. The loss of membrane integrity is associated with chlorophyll degradation, while the appearance of the osmophilic globules accompanies an increase in carotenoid levels; therefore, it is possible that the reduction of firmness on day five may be associated with a more ripe stage of the fruits tested on that day. These results are in accordance with the increased *a* and *b* color values on that day.

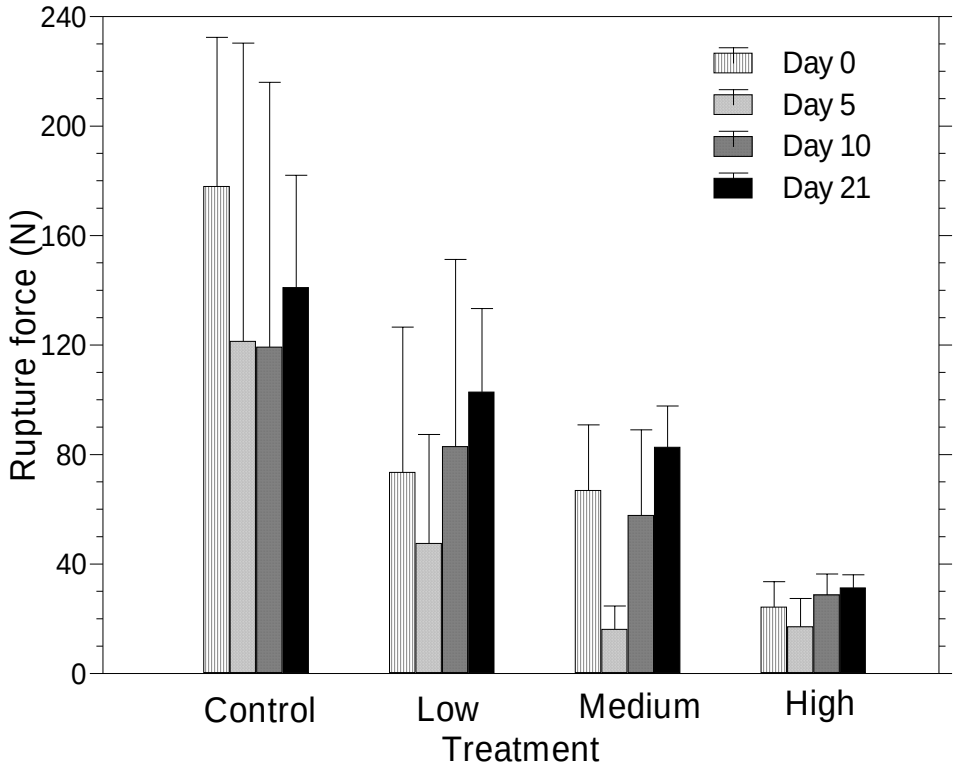

Figure 4-4. Effect of irradiation dose on texture (force to rupture in N) of mangoes stored up to 21 days at 12°C. (Control =non-irradiated, low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.1 kGy; uniaxial compression, 15% strain, room temperature 20˚C).

A significant (P>0.05) reduction on fruit firmness occurred in mangoes exposed to medium (1.5 kGy) and high (3.1 kGy) doses by the fifth day (Figure 4-4). The softening effect of irradiation and the loss of cell cohesion of fruit tissue can be associated with the degradation of cell wall polysaccharides and the solubilization of pectins, celullose, hemicellulose and starch (Kovacs & Keresztes, 2002; Kader, 1986). Similar findings have been reported by Lacroix et al. (1992) where mango samples irradiated (gamma rays) at 0.60 and 0.90 kGy showed a significant difference in the loss of texture when compared with unirradiated fruits. El-Samahy et al. (2000) also found a reduction of firmness of mango when exposed to gamma irradiation at dose levels between 0.5 and 1.5 kGy.

The same trend was observed for the values of toughness (Figure 4-5). Irradiated samples at 1.0, 1.5 and 3.1 kGy were 74%, 83% and 64% less tough than the control, respectively (Table A-1, Appendix A), indicating that these samples required less energy or work to fracture than the control fruits, therefore, when the fruit is consumed a perception of less force for biting the fruit and for the interaction between the teeth, tongue and the fruit would be felt. During storage, fruits exposed to medium (1.5 kGy) and high (3.1 kGy) doses showed a significant (P>0.05) decrease in toughness throughout the entire storage time. Again, in all the samples toughness decreased on day five as an effect of the reduction on firmness of the fruits on that day. However, the samples irradiated at low and medium doses were acceptable to the panelists (section 4.1.3).

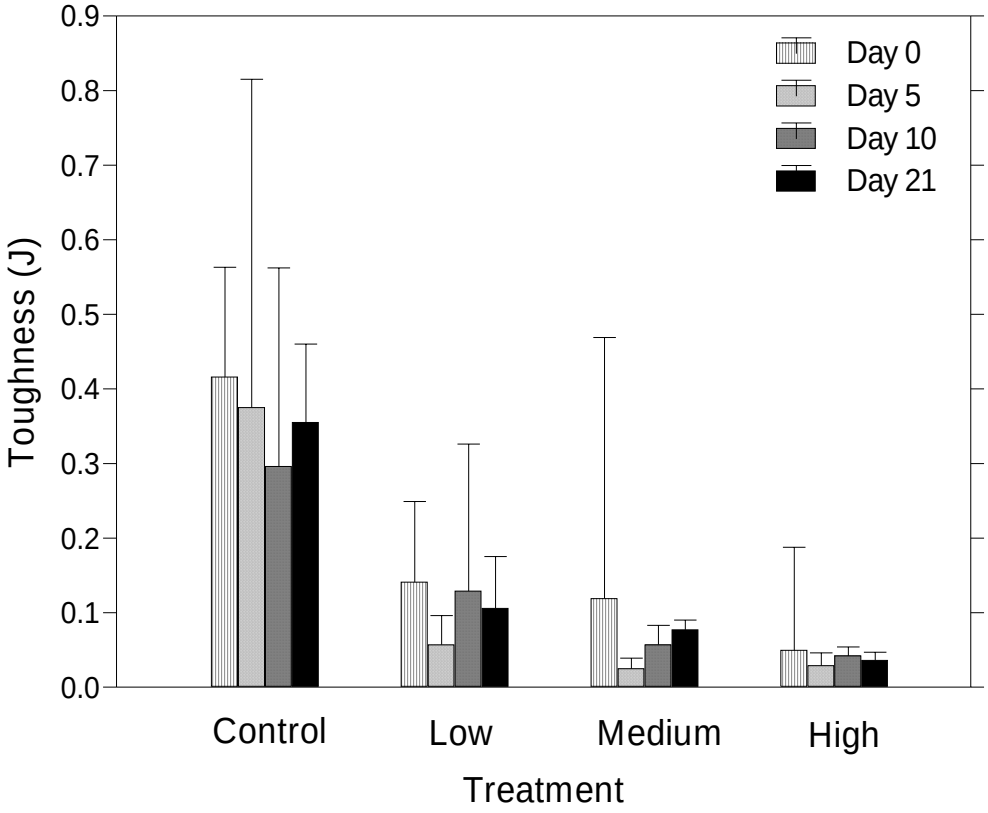

Figure 4-5. Effect of irradiation dose on texture (toughness in J) of mangoes stored up to 21 days at 12°C. (Control =non-irradiated, low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.1 kGy; uniaxial compression, 15% strain, temperature 20˚C).

There is a direct relationship between loss of texture (loss of firmness or softening) and irradiation dose (Figure 4-4). The loss of firmness of the fruits increased as a function of the dose and this trend followed a first order rate equation (Eqn 4-1) with exception of day five as,

$$F = F_o e^{-kD} \tag{4-1}$$

where F is the degradation on fruit firmness (rupture force) at dose D, F_o is the initial value of firmness in the non-irradiated mango and k is the dose dependent constant in kGy^{-1}. Table 4-3 presents the dose-dependent rate constants (k) for each storage time interval (Eq 4-1) obtained from the slope of the plot of ln F vs dose. The changes on day five did not follow the same trend and low correlation was found with this model (R^2= 0.72) therefore, they were considered separately.

Table 4-3
Dose-dependent rate constants (k) for degradation of texture (firmness) of mangoes stored up to 21 days at 12°C (Eq. 4-1)

Day	***k* (kGy^{-1})**	**R^2**
0	0.62	0.98
10	0.47	0.99
21	0.50	0.97

Although the obtained *k* value was higher on day 0 (at the beginning of the storage) than on the other days, no significant difference in rate of the loss of firmness was found for all mangoes; therefore, the loss of firmness occurs continuously throughout all the storage time. The following equations describe the loss of firmness with irradiation dose:

$$F_{\text{day 0}} = 162.52*e^{-0.62D},\ R^2 = 0.98 \quad (4\text{-}2)$$

$$F_{\text{day10}} = 122.50*e^{-0.47D},\ R^2 = 0.99 \quad (4\text{-}3)$$

$$F_{\text{day21}} = 148.61*e^{-0.50D},\ R^2 = 0.97 \quad (4\text{-}4)$$

The force versus dose data for day five was better fitted to an exponential model (Figure 4-6) described as:

$$F_{\text{day5}} = 110.1e^{-1.349D} + 11.83,\ R^2 = 0.98 \quad (4\text{-}5)$$

According to the above equations (4-2-4-5) the relationship between loss of firmness and dose follows a decreasing trend of each storage time interval, therefore, the higher the irradiation dose, the higher the loss of firmness of the fruits and the less the required force to rupture the samples.

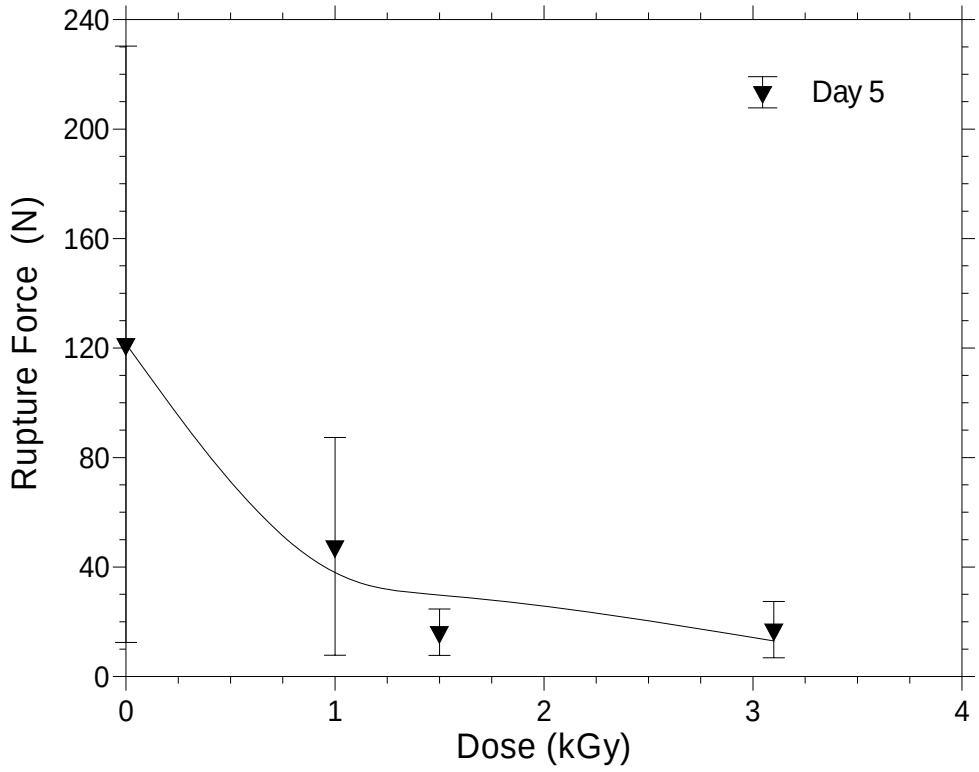

Figure 4-6. Effect of dose on the rate of firmness loss (force to rupture, FR) of mangoes on day five of storage at 12° C. (0 kGy means non-irradiated or control samples).

The effect of time on fruit firmness will be discussed in section 4.3.

The softening effect of ionizing radiation can be further explained by looking at the structure of the fruit (section 4.1.1.4).

Stiffness is related to the ability of the fruit to withstand a load before it breaks or impact during handling; it is an indication of how difficult it is to deform the fruit. The softening of the irradiated mangoes was also indicated by a significant ($P>0.05$) reduction (50 to 75%) in the values of the stiffness (Young's modulus) (Figure 4-7 and Table A-1). All irradiated samples were less stiff than the control fruits. Samples exposed to higher dose had the lowest values of stiffness, therefore these samples would deform easily. For all irradiated and non-irradiated samples an initial decrease in the fruit stiffness was observed from day zero until day five and then it increased at day ten. By the end of the storage, the stiffness of the all samples remained constant.

Overall, irradiation of mango fruits with doses higher than 1.0 kGy has a detrimental effect on the fruit's texture.

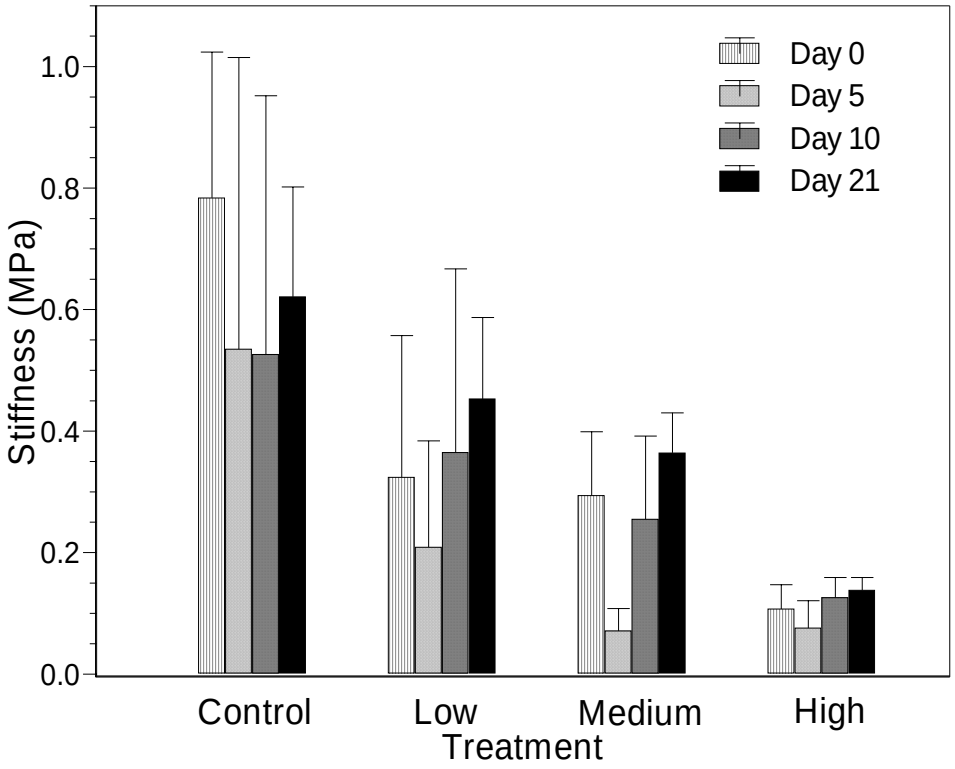

Figure 4-7. Effect of irradiation dose on texture (stiffness-Young's modulus (MPa)) of mangoes stored up to 21 days at 12°C. (Control =non-irradiated, low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.1 kGy; uniaxial compression, 15% strain, temperature 20˚C).

4.1.1.3. Fruit structure

One of the effects of ionizing radiation in plant cells is the interaction with atoms and molecules in the cell, especially with water, to produce free radicals which can diffuse far enough to reach and produce damage to important compounds of the plant cell (Kovás & Keresztes, 2002).

The cortex (skin) cells in mango irradiated at low, medium and high doses shrunk and collapsed (Figure 4-8). Large numbers of cells separated or were only slightly bound to the neighboring cells, due to the loss of pectin. The skin photomicrographs of the irradiated fruit showed the formation of fractures or cracks (c) and more depressions were noticeable. Micro-cracks were observed around the stomata (sto) (Figure 4-8 B). In samples irradiated at medium (1.5 kGy) and high (3.1 kGy) doses, wax platelets (wp) were seen in some areas covering the skin (Figure 4-8C and D) indicating the bleeding of the skin.

At a higher resolution, (Figure 4-9) the skin photomicrographs showed the control samples (4-9 A) to have a continuous cell wall on a parallel organized striation pattern, while for the irradiated samples the cell wall was fragmented and the striation became shorter. This effect was more intense in samples irradiated at medium and high doses (4-9 C & D). Additionally, the presence of microcracks in irradiated samples was observed as well as the amorphous surface and depressions that are characteristics of the brown spots mentioned in the visual changes (section 4.1.1.1). Fruit sensitivity to skin damage is related to the number of new fractures (Gazzola et al., 2004), meaning that in

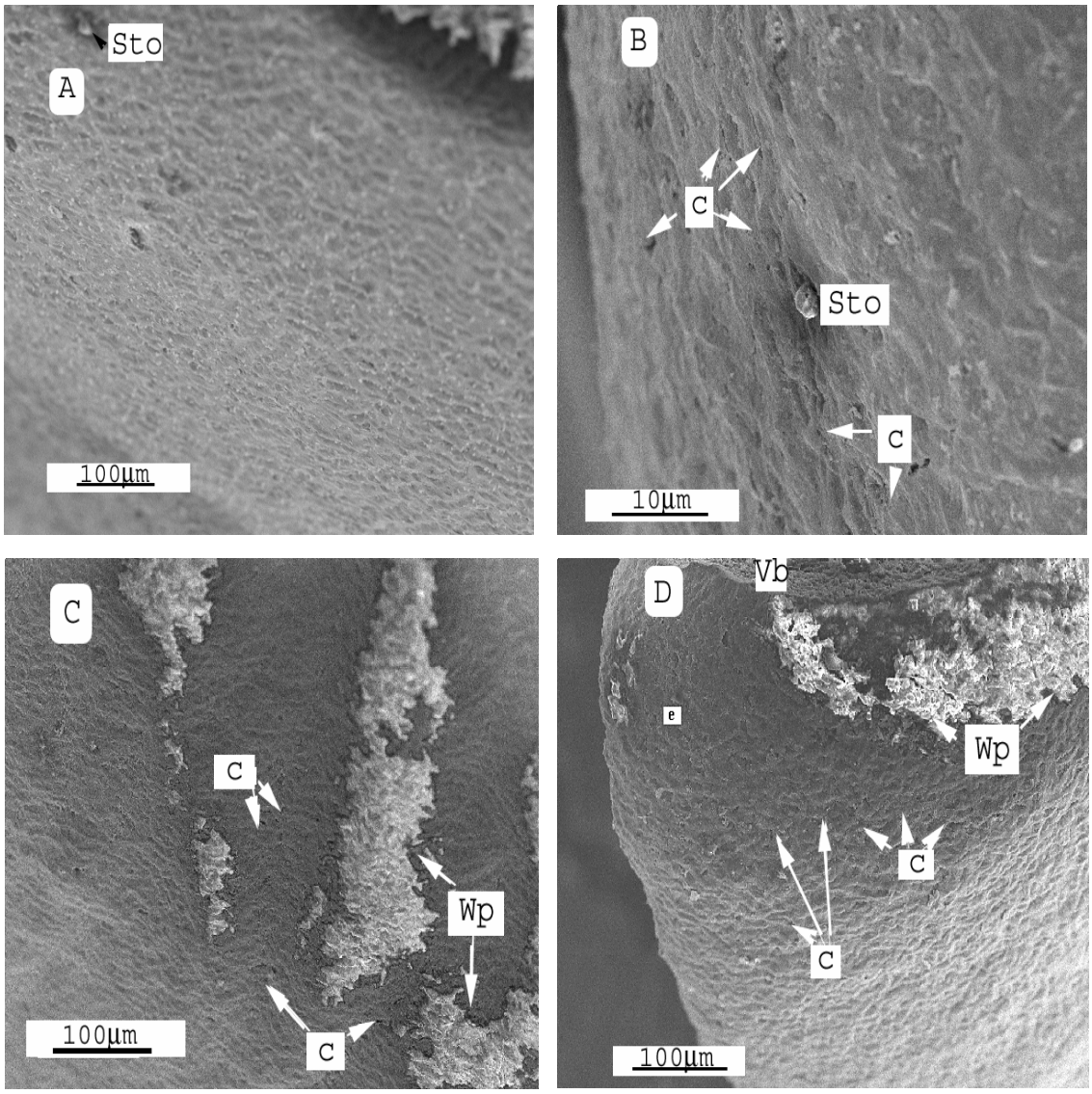

Figure 4-8. SEM photomicrographs of mango skin after irradiation treatment (10 days). (A) Control =non-irradiated, (B) low dose =1.0 kGy, (C) medium dose =1.5 kGy , (D) high dose =3.1 kGy, (c =cracks, sto =stomata, wp =wax platelets, Vb =vascular bundle, e =epidermis). Fruits were viewed at 15kV. Bars in (A), (C) and (D) represent 100µm; in (B), 10µm.

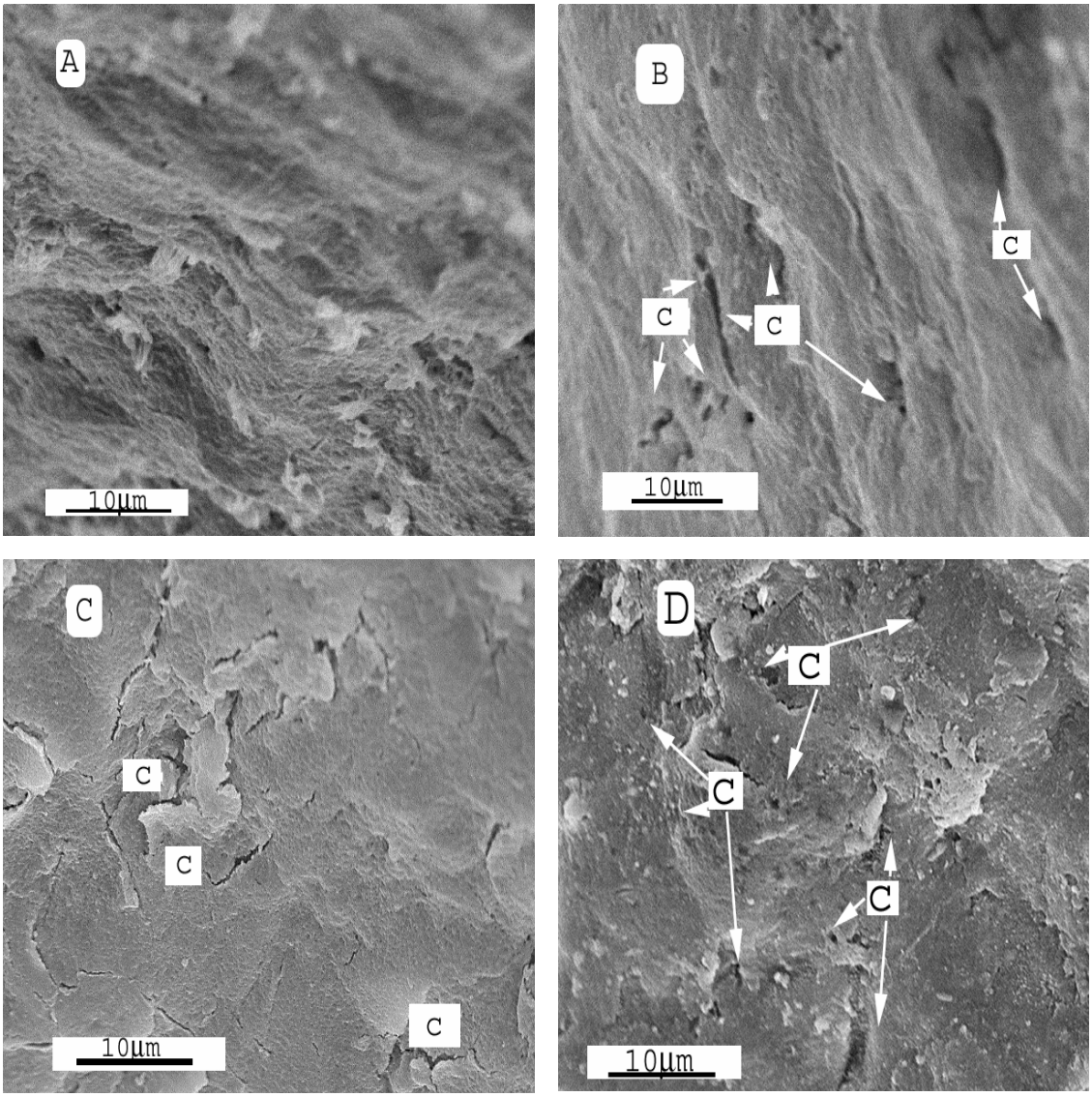

Figure 4-9. SEM photomicrographs of mango skin at higher resolution after irradiation treatment (10 days). (A) Control =non-irradiated, (B) low dose =1.0 kGy, (C) medium dose =1.5 kGy, (D) high dose =3.1 kGy, (c =cracks). Fruits were viewed at 15kV. Bars represent 10µm.

the fruit with cracks the susceptibility to skin spots would be higher. Similar findings were reported by Kovás et al. (1997) when apples were exposed to gamma rays at doses of 1.0 and 2.5 kGy. The authors also found a significant decrease in total pectin in cortex cells as an effect of irradiation. These results support the findings in the objective measurement of texture where the irradiated samples were less firm and tough than the controls. In addition, these results are in agreement with the changes in color, which also induce structural changes due to chlorophyll degradation, and the increase in carotenoids level.

Figure 4-10 presents the photomicrographs of mango flesh. The irradiated fruit exhibited a large number of cells that were separated and the presence of empty spaces without starch granules was also observed, demonstrating the mechanical damage of the mango fruits. At a relative low dose (1.0 kGy) the breakdown of the microstructure of mango was initiated. A similar trend was observed for the parenchyma cells (Figure 4-11). The irradiated samples showed more collapsed cells than the non-irradiated controls. These changes in cell structure were consistent with the texture characteristics (Section 4.1.1.2) where firmness and stiffness (Young's modulus) of the irradiated samples were reduced significantly, especially at higher doses. This result may be related to changes in the degree of polymerization of the cell wall constituents which also enhances changes in the chemical composition such as the increase in reducing sugars that could be associated with the starch degradation in irradiated samples and also with the accumulation of phenolic and volatile compounds (Keresztes & Kovács, 1991).

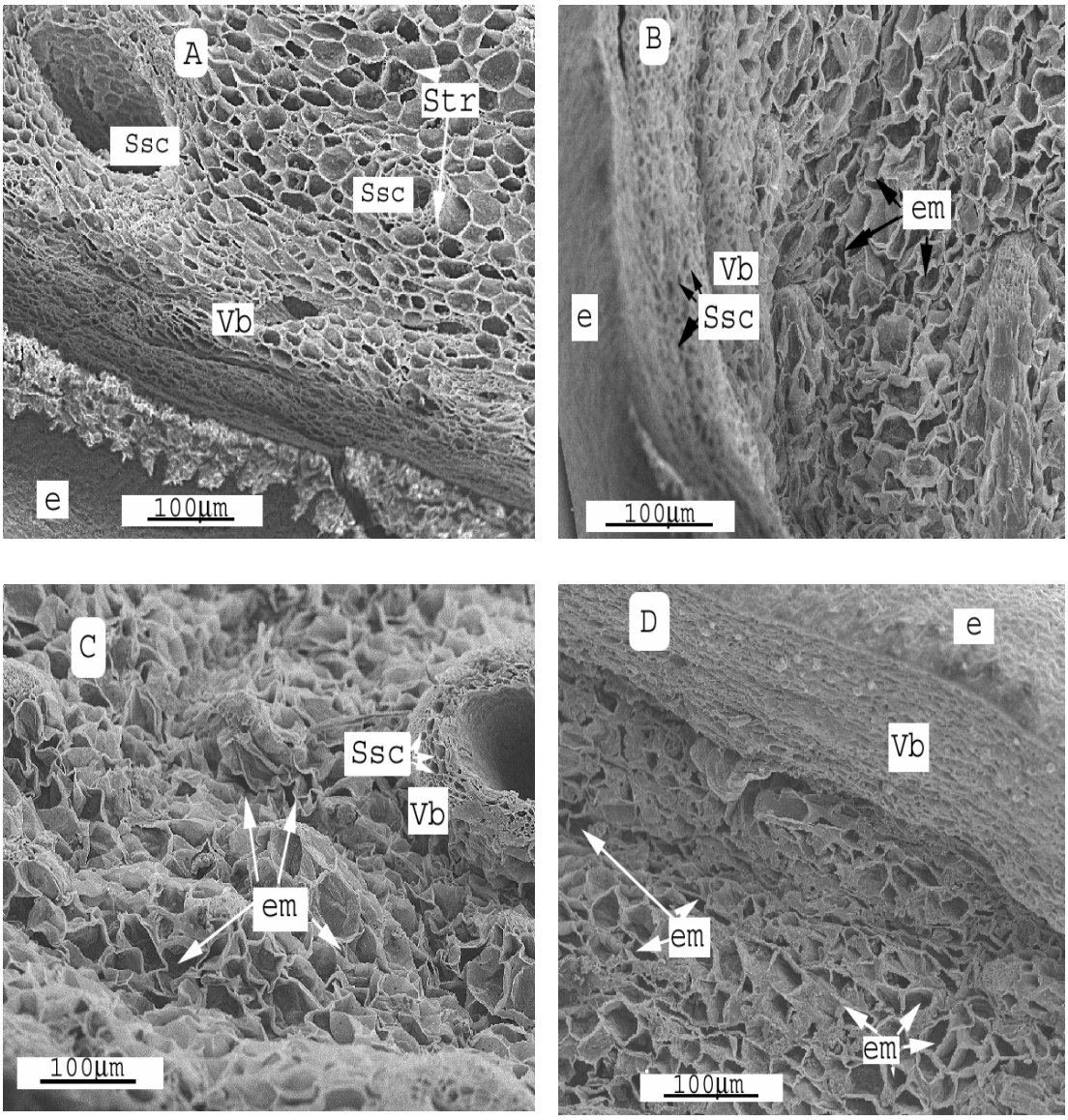

Figure 4-10. SEM photomicrographs of mango flesh after irradiation treatment (10 days). (A) control =non-irradiated, (B) low dose =1.0 kGy, (C) medium dose =1.5 kGy, (D) high dose =3.1 kGy, (e =epidermis, Vb =vascular bundle, Ssc =substomatal cavities, em =empty spaces, str =starch granules). Fruits were observed at 15kV. Bars represent 100µm.

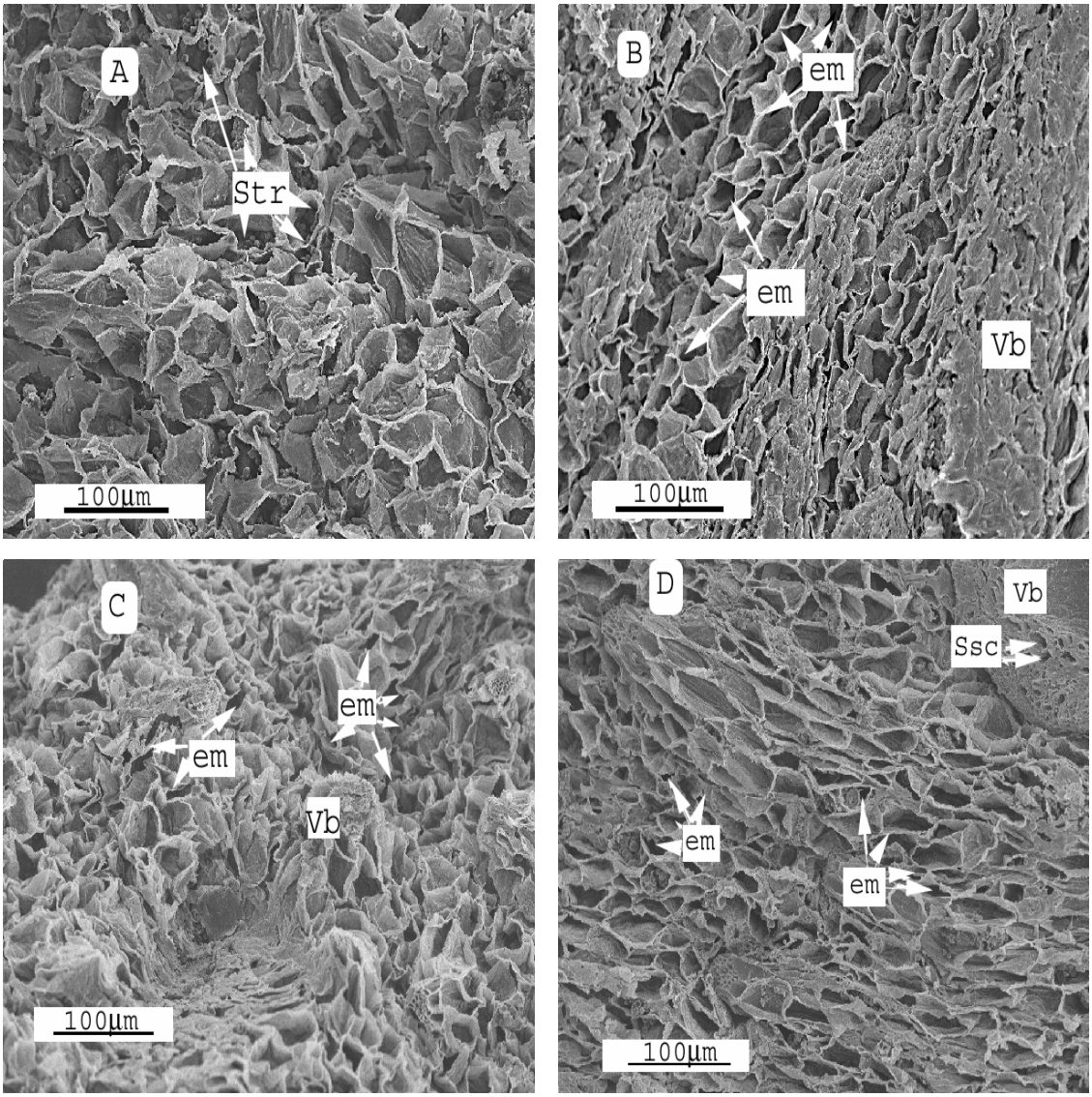

Figure 4-11. SEM photomicrographs of parenchyma cells of mango after irradiation treatment (10 days). (A) Control =non-irradiated, (B) low dose =1.0 kGy, (C) medium dose =1.5 kGy, (D) high dose =3.1 kGy, (e =epidermis, Vb =vascular bundle, Ssc =substomatal cavities, em =empty spaces, str =starch granules). Fruits were observed at 15kV. Bars represent 100µm.

On the other hand, these changes in structure are consistent with the increase in respiration rates and the phenolic compounds which were higher in the irradiated samples than in the control fruits (section 4.1.1.4 & 4.1.2.7).

The cell structure of mango was affected when the fruit was exposed to higher doses. Irradiation induced the softening of the fruit by causing the breakdown of the cells and its components. The results suggest that low dose (1.0 kGy) is an appropriate treatment to minimize changes in product textural attributes and higher doses are not recomended.

4.1.1.4. Respiration rate

The rate of respiration is often a good index to the storage life of horticultural products: the higher the rate, the shorter the life, and the lower the rate the longer the life.

The effect of ionizing radiation on respiration rate (Eq 3-5) of the mangoes is presented in Table 4-4. The CO_2 concentration of the irradiated samples stored at 12˚C ranged between 10.94 and 22.26 mg/Kg h. Compared with the control, the CO_2 concentration in the atmosphere increased significantly ($P>0.05$) in fruits exposed to low (1.0 kGy) and medium (1.5 kGy) doses right after irradiation (day 0) and by the end of storage. According to Hulme (1971) radiation induces an immediate increase in the respiration rate in most of the fruits. However, these differences according to dose may be associated with the variability in the ripening stage of the fruits treated at each dose. The rise in carbon dioxide depends on the ripening stage of the fruit at the time of irradiation. In general, for climacteric fruits the earlier the treatment is given in the pre-

climacteric stage, the greater is the stimulated rise in carbon dioxide production (Hulme, 1971). Therefore, the results suggest that the fruits treated at low (1.0 kGy) and medium (1.6 kGy) doses may be more immature than the fruits treated at high dose (3.1 kGy). This respiratory rise also suggests a premature start of the climacteric phase in samples irradiated at low (1.0 kGy) and medium (1.6 kGy) doses.

Table 4-4
Headspace gas (CO_2 in mg /Kg h) concentration for mangoes stored up to 21 days at 12˚C

Gas	Day/Dose	Control* (0.0 kGy)	Low (1.0 kGy)	Medium (1.5 kGy)	High (3.1kGy)
	0	12.85^{ax}(2.83)	22.26^{ay}(0.68)	18.27^{acz}(1.01)	11.64^{ax}(0.53)
CO_2	5	19.96^{ax}(5.45)	20.08^{bx}(0.56)	18.02^{ax}(0.28)	10.94^{ay}(0.20)
	10	17.45^{ax}(0.74)	17.98^{cx}(0.44)	16.23^{bx}(0.56)	11.08^{ax}(0.32)
	21	14.65^{ax}(0.34)	20.96^{dy}(0.18)	19.48^{cy}(0.94)	16.03^{bx}(2.75)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-d}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
$^{x-z}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

The samples treated at 3.1 kGy had decreased CO_2 concentration. However, only on day 5 is this reduction significant ($P>0.05$). This does not extend shelf-life. At low CO_2 levels some physiological disorders such as internal browning and pitting can be induced (Robertson, 1993). According to Eq 2-5 increasing the CO_2 levels in the atmosphere would reduce the respiration rate and extend the shelf-life by delaying the

senesce and retarding fungal growth. Therefore, the samples exposed to 1.0 and 1.5 kGy may have a longer shelf-life.

The increased carbon dioxide concentrations after irradiation also may be associated with the effect of radiation in the raise of ethylene production which is another phenomenon related to the climacteric stage of the fruit. A small amount of ethylene present in the fruit at harvest is sufficient to initiate the ripening (Mitra, 1997). This phenomenon could be explained by the effect of irradiation at the cellular level (structural changes) which induces the damage to the tissue and therefore physiological changes which include the fruit's respiratory mechanism that enhances the production of ethylene and carbon dioxide (section 4.1.1.3). In addition, these changes are correlated with the differences in color due to chlorophyll breakdown and carotenoids biosynthesis (section 4.1.1.1) which also induce changes in the ethylene production. The ethylene production continues as the fruit approaches color break (Seymour, 1993).

Paul (1996) found an increase in respiration rate of papaya irradiated at 250 Gy with lower concentrations in non-irradiated samples. Akamine and Goo (1971) reported that the respiration of green pre-climacteric mangoes exposed to gamma irradiation doses of 0.25, 0.50, 0.75 and 1.0 kGy showed an initial increase, reaching the peak one day after the treatment and then a final decrease.

The effect of time on fruit respiration rate will be discussed in section 4.3.

Overall, with the conditions used in this study, the irradiation treatment of mangos of doses up to 1.5 kGy keeps respiration at normal level (~ 20 mg CO_2 /Kg h) and extends the fruit's shelf life by approximately 3 days by increasing the CO_2 levels.

4.1.1.5. Density and specific gravity

Exposure to ionizing radiation did not induce a significant effect on the specific gravity of the fruits (Figure 4-12) (also see Table A-2, appendix A); though a slight reduction occurred in all irradiated samples. This finding could be associated with the structural changes in the tissue due to the mechanical damage produced by irradiation and the lyses of the cells that include the solubilization and degradation of pectin where the cells of the irradiated samples shrunk and separated (section 4.1.1.3).

No effect of storage was recorded for control and irradiated samples. In addition, the values obtained in this study are in agreement with the range of specific gravity values for mangoes which vary between 0.99 and 1.065 (Seymour, 1993). These findings suggest that no weight losses were induced by irradiation.

The bulk density of the mango is approximately 0.58 g/cc. When calculating the porosity (Eq 3-4) of the material no effect of the irradiation treatment was found. The porosity values were 0.48, 0.43, 0.43 and 0.44 for control, low, medium and high samples respectively, indicating that the fruits did not get more porous with the irradiation treatment.

Overall, irradiation of mangoes with doses up to 3.1 kGy does not affect the density and specific gravity of mangoes.

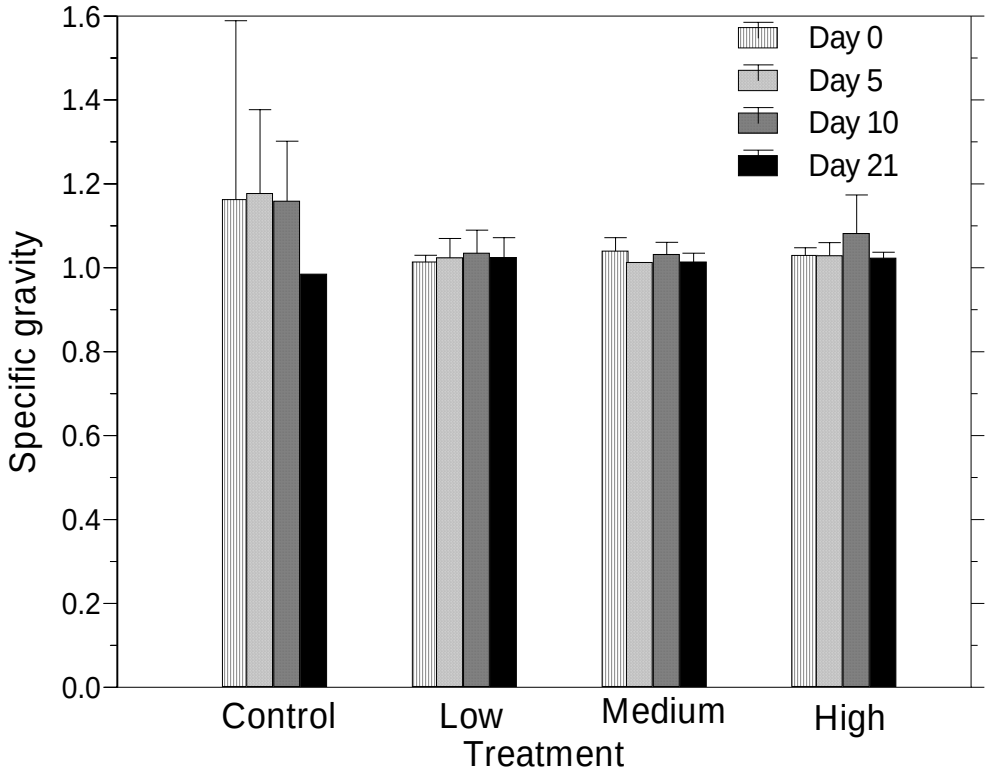

Figure 4-12. Effect of irradiation dose on specific gravity of mangoes stored up to 21 days at 12ºC. (Control =non-irradiated, low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.1 kGy).

4.1.2. Chemical properties

4.1.2.1. Moisture content

The moisture content of the fruit ranged between 84 and 86% (w.b). Irradiated mangoes had significantly higher moisture content than the control sample up to day ten, but a reduction was observed on the twenty-first day in all irradiated samples. Within the irradiated fruits the samples exposed at medium dose had higher moisture content on the 21st day (Table 4-5). This result could be associated with the effect and interaction of ionizing radiation with atoms and molecules in the cell, especially with water, to produce free radicals which can diffuse and produce damage in different compounds of the cell. Additionally, with the increase in respiration rate when the fruits exude water as a byproduct of respiration which could be accumulated as free water which increases the moisture content.

During storage time, the moisture content values of the control and the irradiated fruits did not follow a clear trend. However, for control samples and the mangoes exposed to a high (3.1 kGy) dose, a significant ($P>0.05$) decrease in moisture content was observed at days five and ten; while samples treated with a low dose (1.0 kGy) had increased values on these days. No significant change in the moisture content of the samples treated with medium (1.5 kGy) dose was observed. Even with these statistical differences, the changes in the moisture content of the mangos were too small to induce the water lost in the fruit; thus, this factor does not affect the quality of the fruit. Loss of moisture content is associated with the loss of juiciness of the fruit. Therefore, the

exposure of mangoes at irradiation up to 3.1 kGy does not affect the juiciness characteristics of the fruit.

Table 4-5
Effect of irradiation dose on moisture content (% w.b.) of mangoes stored up to 21 days at 12ºC

Day/Dose	Control* (0.0 kGy)	Low (1.0 kGy)	Medium (1.5 kGy)	High (3.1 kGy)
0	85.09^{aw}(0.17)	83.30^{ax}(0.16)	85.48^{ay}(0.04)	85.74^{az}(0.01)
5	84.59^{bx}(0.03)	85.48^{by}(0.29)	85.08^{az}(0.02)	85.52^{by}(0.20)
10	83.64^{cx}(0.13)	85.82^{cy}(0.04)	85.19^{ay}(0.67)	85.42^{by}(0.02)
21	85.13^{aw}(0.10)	84.12^{dx}(0.04)	84.89^{ay}(0.05)	84.57^{cz}(0.01)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-d}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
$^{w-z}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

4.1.2.2. Water activity

The water activity of the fruit ranged between 0.89 and 0.92. According to Fennema (1996) at ranges between 0.95-0.91 and 0.91-0.87 some bacteria (Salmonella, Lactobacillus, Serratia) and many yeast and mold are inhibited. Therefore, in the treated samples these microorganisms would not grow and the decay of the fruit would be reduced.

No significant changes in the water activity of the irradiated samples were observed. Only at day ten of storage the water activity of the irradiated fruit increased

significantly (P>0.05) by: 5.35% at low dose (1.0 kGy), 4.58% at medium dose (1.5kGy) and 4.30% at high dose (3.1 kGy) (Figure 4-13) (see also Table A-3). The rise in water activity may be attributed to the generation of hydrolytic reactions due to irradiation (Rosenthal, 1992). These results are consistent with the findings in moisture content which was higher for irradiated samples than for the control fruits (section 4.1.2.1) and with the softening of the irradiated samples.

During storage, the water activity of the fruits increased slightly for all treatments; the change became significant (P>0.05) on the twenty- first day of storage. For instance, samples exposed to medium (1.5 kGy) and high (3.1 kGy) doses had an increase of 2.85 and 2.16%, respectively. However, non-irradiated samples (control) had a significant (P>0.05) reduction in the tenth day of storage with a subsequent increase on the twenty-first day. Samples treated with low dose (1.0 kGy) increased by 2.42% throughout the time. This increase could be related to the reduction of total soluble solids.

Although significant differences in the water activity of the mangos were observed statistically, this factor does not affect the quality of the fruits. Therefore, exposure at dose levels up to 3.1 kGy would maintain the water content of the fruits. However, because of the softening of the samples treated at higher dose, irradiation at doses up to 1.5 kGy is recommended to maintain the quality of the fruit.

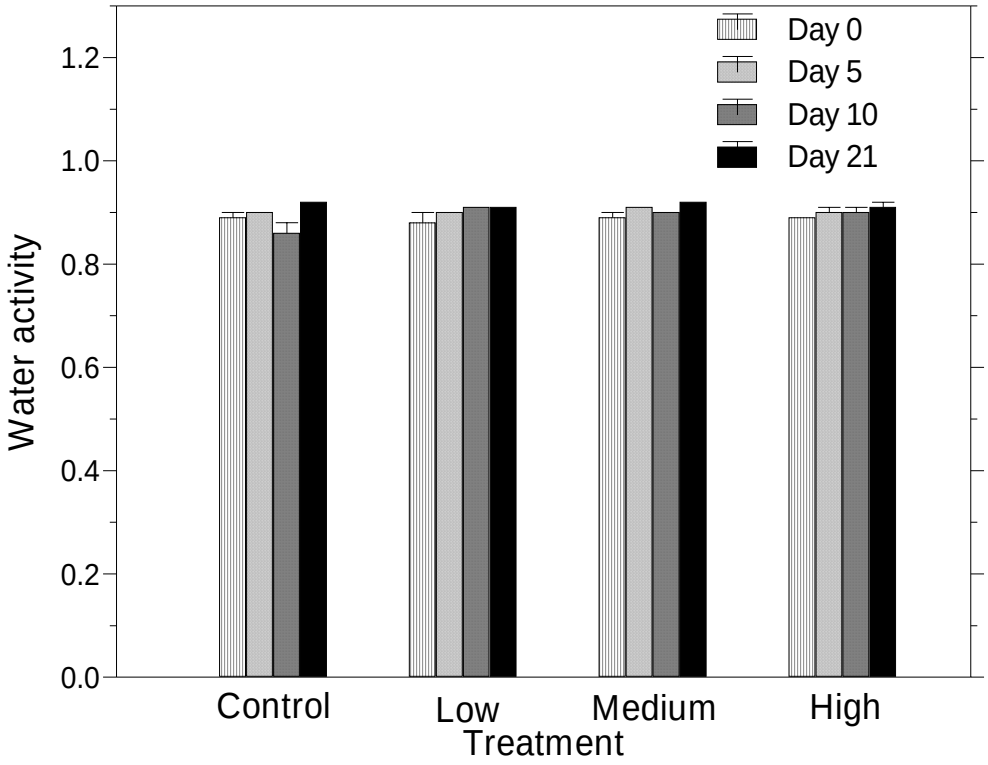

Figure 4-13. Effect of irradiation dose on water activity of mangoes stored up to 21 days at 12°C. (Control =non-irradiated, low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.1 kGy).

4.1.2.3. pH

There was not a clear effect of irradiation dose on the pH values (Figure 4-14). For instance, all irradiated samples showed a significant (P>0.05) increase (3.14-3.44) in pH values until the fifth day, with the exception of the samples exposed to high dose (3.1 kGy) which had a significant reduction (3.27-3.21) on that day. On the other hand, samples treated with low and medium doses decreased significantly (P> 0.05) on day ten and twenty-one, but the samples treated at high (3.1 kGy) dose had an increase in pH values by the last day (21) (see also Table A-4, Appendix A). These results are associated with the changes in acidity levels which were lower in samples treated with the highest dose by the end of the storage time.

According to Hulme (1971), changes in pH are associated with the acid content of the fruit which changes during the development of the fruit. Consequently, low pH (2.0-4.0) values correspond to high acid concentrations. The acid content is considerably greater in the greener young fruit than in the ripe fruit. Therefore, these results may be associated with the differences in the degree of ripening and the differences in color between the mango fruits. Krishnamurthy et al. (1960) evaluated the changes in physicochemical properties of Indian mangoes at different stages of maturity. The authors reported that for Badami variety a fruit with a pH of 2.68 corresponded to a green and hard stage of maturity with acidity of 3.41%, a pale white fruit color and no flavor. While a fruit with a pH of 2.85 corresponded to another stage of maturity with an acidity of 2.08%, the color was dull yellow and the fruit had a mild flavor.

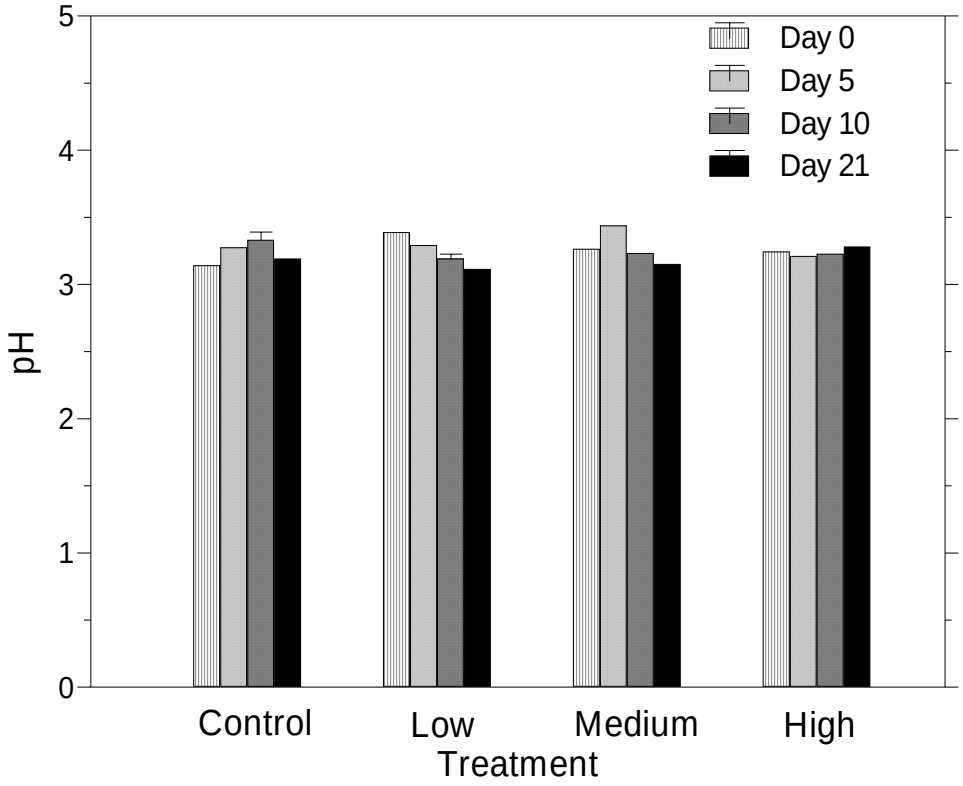

Figure 4-14. Effect of irradiation dose on pH of mangoes stored up to 21 days at 12ºC. (Control =non-irradiated, low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.1 kGy).

In the same study, they reported that for a Neeleman variety a pH of 3.06 corresponded to an acidity of 1.50%, pale white color and no flavor; but a mango with a pH of 3.10 and different stage of maturity, had acidity of 1.28%, dull yellow color and very mild flavor.

Throughout the storage time, the control samples had a significant (P> 0.05) increase (3.14-3.33, 4.0%) in pH while the samples treated with low (1.0 kGy) and medium (1.5 kGy) doses had a significant (P> 0.05) decrease (5.57% and 0.27%, respectively) in pH values. For samples exposed to a high dose (3.1 kGy) this reduction was significant on day five. These results could be associated with the effect of irradiation delaying the ripening of the fruit. The acidity in mango declines as the fruit ripens which means higher pH values as was observed in the control samples.

Overall, the changes in pH were associated more with the differences in the ripening stages of the fruits used in this study than with the effect of the irradiation dose. Therefore, the exposure of mangoes to irradiation levels up to 3.1 kGy does not affect the pH of the fruits.

4.1.2.4. Tritratable acidity

No effect of irradiation on the acidity values of the mangoes was observed up to day five. However, the acidity values of the fruits exposed to low (1.0 kGy) dose increased significantly (P>0.05) by days ten (8.7%) and twenty-one (10.5%); but for samples exposed to medium (1.5 kGy) and high (3.5 kGy) doses, a significant reduction was observed on day twenty-one (Table 4-6). Different findings have been reported about the effect of irradiation on acidity. For instance, Youssef et al. (2000) observed an

increase in acidity (21.0%) of gamma irradiated mango pulp at doses between 0.5 and 2.0 kGy.

However, opposite results were reported by Durigan et al. (2004) who observed a reduction of total tritratable acidity of mango irradiated with gamma rays at 0.8 and 1.0 kGy. This suggests that the effect of irradiation on acidity depends on the dose. In the present study, the reduction of acidity in samples treated at higher doses could be associated with the reduction in organic acids that are used in the respiration metabolism which increased at those dosages (section 4.1.1.3).

Table 4-6
Effect of irradiation dose on tritratable acidity (g citric acid/100g w.b.) of mangoes stored up to 21 days at 12ºC

Day/Dose	Control* (0.0 kGy)	Low (1.0 kGy)	Medium (1.5 kGy)	High (3.1 kGy)
0	1.14^{ax}(0.05)	1.03^{ax}(0.06)	1.00^{ax}(0.01)	1.15^{ax}(0.01)
5	1.15^{ax}(0.06)	1.20^{bx}(0.02)	1.09^{bx}(0.01)	1.19^{ax}(0.01)
10	1.00^{bx}(0.06)	1.24^{by}(0.02)	1.15^{cxy}(0.01)	1.08^{axy}(0.10)
21	1.23^{aw}(0.01)	1.26^{bx}(0.01)	1.13^{cy}(0.01)	1.06^{az}(0.01)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-c}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
$^{w-z}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

The ratio H^+/ acidity could be used as an index of maturity. The acidity may be useful as a reference to the stage of maturity or as objective information related to flavor. Therefore, a fruit with a lower acidity would have a sweeter flavor.

The range of the ratio sugar/acid of the irradiated samples (8.29-11.76) was lower than the ratio of the control samples (9.50-12.70) (Table A-5) suggesting the delay of ripening due to irradiation and the immature stage of the fruit.

During the storage period, the acidity values of the control samples showed a significant ($P>0.05$) reduction on day ten; while for the samples treated with low (1.0 kGy) and medium (1.5 kGy) doses, an increase of 21.7% and 1.6%, respectively was observed throughout storage. These results were consistent with the lower values (3.11 and 3.15, respectively compared with control 3.19) of pH for samples irradiated at these dosages by the end of the storage time (section 4.1.2.3). For samples exposed to a high dose (3.1 kGy) no effect of time was observed. The increase in acidity with time may be associated with a reduction (12.3%) in total soluble solids (section 4.1.2.5) as an indicator of the delay of ripening in all irradiated samples.

In summary, the effect of irradiation on the tritratable acidity depends on the irradiation dose, but at doses up to 1.5 kGy the flavor of the mango fruit may be less acid than the samples exposed to 3.1 kGy.

4.1.2.5. Total soluble solids

Irradiation treatment affected the total soluble solids (°Brix) content of mangoes. All irradiated samples showed a significant ($P<0.05$) decrease in the total soluble solids compared to the non-irradiated samples (control). These results indicate that irradiation

delays the ripening of mango. The decrease in total soluble solids is associated with the increase in tritratable acidity (section 4.1.2.4). However, the fruits exposed to low and high doses had a significant increase ($P<0.05$) on day zero (Table 4-7). This increase may be associated with the increase in reducing sugars (section 4.1.2.6) due to the starch breakdown (section 4.1.1.3).In addition, this difference may be associated with variation between the samples. Lacroix et al. (1992) reported that the level of soluble solids of mangoes irradiated with gamma rays at doses between 0.3 and 0.6 kGy was significantly higher than that of the control samples.

Table 4-7
Effect of irradiation dose on soluble solids (°Brix) of mangoes stored up to 21 days at 12ºC

Dose/Day	Control* (0.0 kGy)	Low (1.0 kGy)	Medium (1.5 kGy)	High (3.1 kGy)
0	10.83^{ax}(0.06)	13.17^{ay}(0.15)	10.93^{ax}(0.12)	13.40^{ay}(0.17)
5	12.17^{bw}(0.14)	11.42^{bx}(0.14)	12.50^{by}(0.00)	10.42^{bz}(0.14)
10	12.67^{cx}(0.14)	10.25^{cy}(0.00)	10.67^{cz}(0.14)	10.25^{by}(0.00)
21	12.08^{bx}(0.14)	11.25^{by}(0.00)	10.17^{dz}(0.14)	11.33^{cy}(0.14)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-d}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
$^{w-z}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

An increasing trend was observed in non-treated samples (control) during the storage time (11.5% by day 21). Overall, all irradiated samples had a significant (P>0.05) decrease over time, indicating the delay of ripening induced by irradiation. These findings are consistent with the higher values of yellowness (*b* values) observed in the control compared to all the irradiated samples by the end of the storage time, which was associated with an increase in carotenoids (section 4.1.1.1) due to the ripening process. Medlicott et al. (1986) reported an earlier increasing of carotenoids during ripening of mango fruits. However, on day five, samples treated with medium (1.5 kGy) dose had a significant (P>0.05) increase in the totals soluble solids. These results were in agreement with the reduction of acidity level and the increase in pH previously observed for the fruits treated at this dose level.

In summary, irradiation dose decreases the total soluble solids (°Brix) content of mangoes, but the exposure of the fruits at 1.0 kGy minimizes the decrease.

4.1.2.6. Sugars

4.1.2.6.1. Total sugars

The total sugars content (g sucrose/100g w.b) in irradiated samples did not show a consistent trend when compared with the control fruit (Table 4-8). Significant (P>0.05) differences were observed. For example, fruit treated at a low dose (1.0 kGy) had 28.1% more sugars than the control in day zero, but the samples exposed to a high dose (3.1 kGy) had a reduction of 26.32 % in day five. The fruit treated with medium dose (1.5 kGy) showed a decrease in total sugars of 16.82% on day twenty-one. However, within the irradiated samples, the low dose (1.0 kGy) treatment caused the higher (12.0%) level

of total sugars. Similar findings were reported by Mitchell et al. (1992) who found that the sucrose levels of gamma irradiated mangoes varied inconsistently with the dose. The authors observed that the samples irradiated at 300 Gy had the highest sucrose levels but those treated with 600 Gy had the lowest. However, in other studies, El-Samahy et al. (2000) found no effect of gamma radiation (0.5, 0.75, 1.0 and 1.5 kGy) on total sugars content of mangoes.

Throughout the storage time, there was a significant ($P>0.05$) reduction of the total sugars of the fruit irradiated at medium dose (1.5 kGy), approximately 14.13%. Samples exposed to low (1.0 kGy) dose showed a reduction (31.80%) on day ten (Table 4-8). On the other hand, the control (non-irradiated) and the high dose (3.1 kGy) samples showed an increase of 12.47% and 6.32%, respectively by the twenty-first day of storage. These differences in total sugars may be due to the variation in maturity level of the fruits used for each treatment in this study.

4.1.2.6.2. Reducing sugars

The content of reducing sugars (g glucose/100g w.b.) in irradiated mangoes was significantly ($P>0.05$) higher than in the control fruit (Table 4-8). This increase was 14.0% at low dose, 15.1% at medium dose and 10.0% at high dose. However, the highest content of reducing sugars was observed in the samples exposed at high dose on day zero and in those treated with medium dose in day twenty-one.

Table 4-8
Effect of irradiation dose on total (g sucrose/100g w.b.) and reducing sugars content (g glucose/100g w.b.) of mangoes stored up to 21 days at 12°C

Sugars	Dose/Day	Control* (0.0 kGy)	Low (1.0 kGy)	Medium (1.5 kGy)	High (3.1kGy)
Total sugars	0	6.95^{ax}(0.36)	8.90^{ax}(1.69)	7.69^{ax}(0.87)	7.63^{ax}(0.57)
(g Sucrose/100g w.b.)	5	8.50^{bx}(0.25)	8.94^{ax}(1.69)	7.76^{axy}(0.98)	6.73^{by}(0.12)
	10	8.46^{bx}(0.43)	6.07^{by}(0.23)	6.37^{by}(0.74)	6.10^{cy}(0.07)
	21	7.94^{cx}(0.24)	8.18^{ax}(0.26)	6.60^{bz}(0.23)	8.14^{dx}(0.26)
Reducing sugars	0	3.06^{ax}(0.05)	4.39^{ay}(0.06)	4.42^{ay}(0.09)	4.69^{az}(0.12)
(g Glucose/100g w.b.)	5	4.35^{bw}(0.03)	4.62^{bx}(0.10)	4.00^{by}(0.11)	3.53^{bz}(0.06)
	10	4.93^{cx}(0.07)	4.74^{by}(0.15)	4.98^{cx}(0.08)	5.10^{ax}(0.11)
	21	4.14^{bx}(0.44)	5.15^{cyz}(0.08)	5.69^{dz}(0.09)	4.80^{ay}(0.64)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-d}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
$^{w-z}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

This increase may be associated with the effect of irradiation in the depolymerization of large carbohydrate molecules (like cellulose and starch) which also induced degradation of the cell membranes and connective tissue (section 4.1.1.3) and therefore, softening of the fruit (section 4.1.1.2).

Mitchell et al. (1992) reported higher levels of glucose in irradiated samples at 300 and 600 Gy than the control samples. El- Samahy et al. (2000) also found that the reducing sugars content was higher in mangoes exposed to gamma radiation (0.5–1.5 kGy) than the controls at zero time.

Referring to storage, an increasing trend in the content of reducing sugars was observed in all fruits (Table 4-8). However, treatment at high (3.1 kGy) dose induced a significant ($P>0.05$) reduction (24.73%) by the fifth day. However, this dose had the higher level of reducing sugars on day ten. On day twenty-one, the samples exposed at low and medium doses presented the higher reducing sugars concentrations. Youssef et al. (2002) reported an increase after storage in reducing sugars of gamma irradiated (0.5-2.0 kGy) mango pulp. However, Soule and Harding (1956) reported different concentrations of reducing sugars according to starch content. According to Hulme (1971), the soluble sugars in ripening mango increases with corresponding decreases in the level of starch and organic acids. Therefore, the increase in reducing sugars during the storage could be associated with the fruit ripening and also with the generation of reducing sugars from starch. These results also suggest a variation between the degrees of ripening among the fruits used for each treatment.

In summary, the exposure of mangoes to ionizing irradiation does not show a consistent trend for its effect on total sugars, but it generally enhances the reducing sugars content of mangoes. Doses up to 1.5 kGy keep the sugars content of mangoes with a higher acceptable quality level (higher values of total sugars, and closer to the normal values, 9.9 g of sucrose/100g edible portion) than samples treated with 3.1 kGy.

4.1.2.7. Total phenolics and antioxidant activity index

The exposure of mangoes to ionizing radiation induced a significant ($P>0.05$) increase in the content of phenolic compounds for all dose levels studied (Figure 4-15 and Table A-6). Compared with the control (non-irradiated), concentrations (mg Gallic acid/100 g w.b.) were significantly higher in all irradiated samples on day zero and low dose (1.0 kGy) gave the higher concentrations. The same trend was observed by the end of the storage, but the samples irradiated at medium dose presented the higher phenolic concentrations (~27.44%). However, a significant ($P>0.05$) reduction of 25.0% and 19.0% was observed for mangoes exposed at low (1.0 kGy) dose on days five and ten of storage, respectively. These results are in agreement with those of El-Samahy et al. (2000) who reported an increase of 25.54% in the concentration of phenolic compounds in irradiated (0.5-1.5 kGy) mangoes compared to those in the untreated fruit. Tan Chye & Lam (1985) reported an increase (52.73%) of phenolics in mango irradiated at 0.25-1.0 kGy. In addition, Youssef et al. (2002) found a higher rate of increase (2.05%) on irradiated samples of mango pulp at doses between 0.5 and 2.0 kGy than in unirradiated fruits.

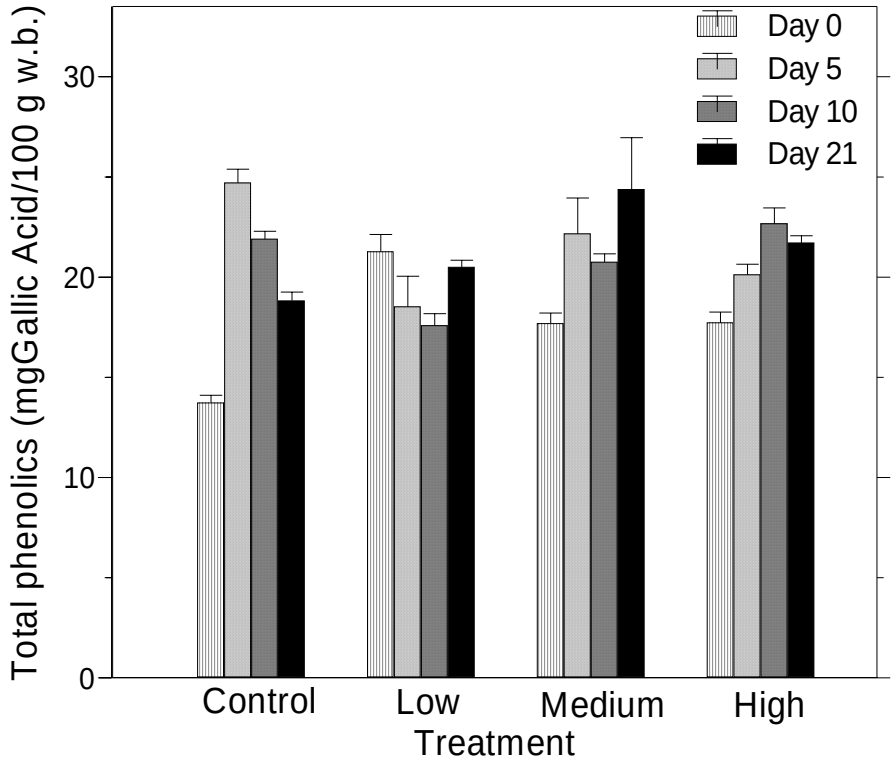

Figure 4-15. Effect of irradiation dose on total phenolics (mg gallic acid/100 g w.b.) of mangoes stored up to 21 days at 12ºC. (Control =non-irradiated, low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.1 kGy).

The accumulation of phenolic compounds after irradiation is associated with different factors such as the modifications occurring in cell structures as a response of the tissues to irradiation, reflecting the stress condition and also with the enhancement of enzymes activity due to radiation (Lorinda et al., 1987), the increase in extractability, and the variation between maturity levels among the samples. These results are in agreement with the browning color present in the irradiated samples (section 4.1.1.1) and with the changes observed in the fruit structure (section 4.1.1.4) where the irradiated fruits had a fragmented cell wall and separated cells, especially in samples exposed to medium (1.5 kGy) and high doses (3.1 kGy). This breakdown results in an increase of the cell permeability leading to increased contact between enzymes and substrates such as phenolics already present in the tissue. The raise in phenolic concentrations has a beneficial effect because of their antioxidant properties which are important in the prevention of different diseases. In addition, phenolics are essential components in the flavor and astringency of the fruit. Loss of astringency is associated with loss of phenolics content (Mitra, 1997). On the other hand, it has been reported by Tan et al. (1982), that there is a relationship between the phenolic compounds and fungal resistance in papaya; papaya fruits with higher phenolics content were more resistant to fungal infection. Therefore, the increase on phenolics content may be important in extending the shelf-life.

During storage time, an increase in phenolic compounds was observed in samples treated at medium (1.5 kGy) and high doses (3.1 kGy) (Figure 4-15). The control (non-irradiated) fruit had the higher concentrations on day five. The samples

treated with a high dose had the higher concentration of phenolics on day ten. On day twenty-one all the irradiated samples had significantly higher concentrations than the control. Variation of phenolic compounds in fruits has been found during growth and maturation. The concentration of phenolics decreases as fruit matures. This process is also affected by the modification of cell structures during ripening and also by the enzymes involved in the phenolics metabolism (Macheix et al., 1990). Therefore, results from this study suggest differences in the ripening stages of the samples.

The trend observed in phenolic compounds corresponded to changes in the antioxidant activity index of the fruits when the percentage of 2,2-diphenil-1-picrilhidrazil (DPPH) reduction was significantly ($P>0.05$) higher for irradiated samples by the twenty-first day of storage (Figure 4-16, Table A-6). This increase was about 3.73% for samples treated with low dose (1.0 kGy), 15.87% for medium dose (1.5 kGy) and 19.06% for high dose (3.1 kGy), so the higher the dose the higher the increase.

The increase in DPPH activity is a good indicator of the reduction of the oxidation reactions which induce the formation of compounds that may altered the flavor and odor of the fruit.

Throughout the storage time, the antioxidant activity index of the samples treated with a low dose (1.0 kGy) showed a significant ($P>0.05$) reduction (11.67%), while the control (non-irradiated) samples and those exposed to medium (1.5 kGy) and high (3.1kGy) doses showed a trend to increase. The control and the samples treated with a medium dose had the higher percentage of antioxidant activity on day five. On day ten, this value was higher for control and for samples exposed to a high dose.

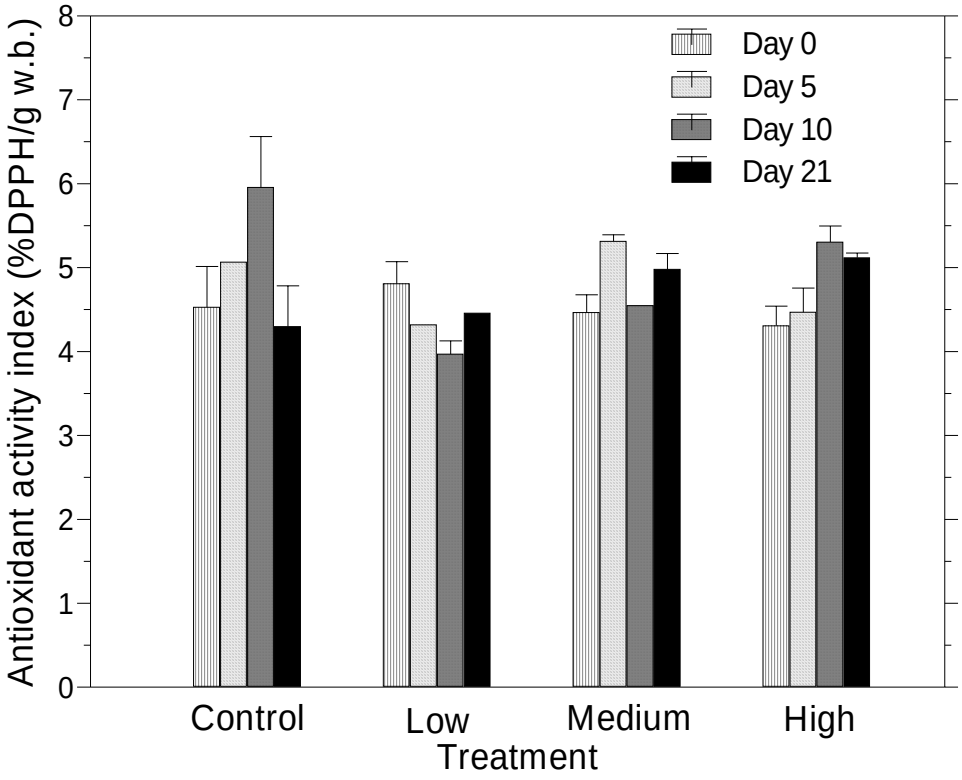

Figure 4-16. Effect of irradiation dose on antioxidant index (% of reduction of DPPH/g w.b.) of mangoes stored up to 21 days at 12°C. (Control =non-irradiated, low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.1 kGy).

All the irradiated samples had a significantly higher percentage of DPPH on day twenty-one. Again, these findings were consistent with the changes in phenolic compounds. It is possible that within the experimental conditions of this study some compounds (such as phenolics and ascorbic acid) were more easily oxidized which caused the creation of more free radicals. Consequently, this would be a reason for oxidation processing which induce the increase in the antioxidant activity index, and therefore, a reduction in the reactions that affect the quality of the fruit. Similar results were obtained by Reyes & Cisneros (2005) who found an increase in the antioxidant activity of mango irradiated under the same conditions used in this study. This increase was 11.56% for low dose (1.0 kGy), 5.58% for medium dose (1.5 kGy) and 2.78% for high (3.1 kGy) dose, by the end of storage time.

These findings are in agreement with an increased phenyalalanine ammonia lyase (PAL) activity found by Reyes & Cisneros (2005).

In summary, electron beam irradiation of mangoes with doses up to 3.1 kGy enhances the phenolics compounds and the antioxidant activity of the fruits.

4.1.2.8. Ascorbic acid

Irradiation had a significant ($P>0.05$) effect on the ascorbic acid content of mangoes. All irradiated samples had lower ascorbic acid content than the control (Table 4-9). By the end of the storage time, this decrease was 58.51%, 63.67% and 80.03% in samples treated with low (1.0 kGy), medium (1.5 kGy) and high (3.5 kGy) doses, respectively. However, the samples exposed to low dose had higher concentrations than the samples exposed to the other doses. This suggests that the higher the dose the higher

the decrease of the acid concentrations. On day five, control and samples exposed to medium dose had the higher concentrations; on day ten, samples treated with a medium dose and the controls had elevated ascorbic acid content. These results were consistent with the higher pH values and higher content of reducing sugars in the irradiated samples than in the control fruits (sections 4.1.2.3 & 4.1.2.6, respectively).

Similar findings were reported by Youssef et al. (2000) who found a marked decrease in ascorbic acid values of mango pulp upon gamma irradiation at doses between 0.5 and 2.0 kGy. In addition, Michell et al. (1992) observed that the application of gamma irradiation at 0.6 kGy on mangoes produced a significant reduction on total vitamin C (ascorbic acid). The reduction of ascorbic acid could be associated with the role that this organic acid plays as a substrate in the respiration rate, which increased in the irradiated samples evaluated in this experiment (section 4.1.1.4). Additionally, irradiation induces the oxidation of ascorbic acid as well as the synthesis of phenolic compounds as previously mentioned in the discussion on antioxidant activity (section 4.1.2.7).

All irradiated and non-irradiated mangoes had significant ($P>0.05$) decreased ascorbic acid content with time. On day five, control and samples exposed to medium dose had the higher concentrations, on day ten samples treated with medium dose and the controls had elevated ascorbic acid content. Normally, the ascorbic acid decreases during ripening of the fruit (Seymour, 1993) thus, reduction upon storage may be due to the ripening process.

Table 4-9
Effect of irradiation dose on ascorbic acid content (mg/100 g w.b.) of mangoes stored up to 21 days at 12°C

Dose/Day	Control* (0.0 kGy)	Low (1.0 kGy)	Medium (1.5 kGy)	High (3.1 kGy)
5	15.27^{ax}(1.44)	7.71^{ay}(2.50)	7.23^{ay}(0.04)	6.23^{ay}(1.31)
10	12.61^{bx}(0.72)	6.86^{ay}(0.86)	3.86^{bz}(0.56)	1.90^{bz}(0.26)
21	16.84^{ax}(0.55)	3.61^{by}(0.64)	0.59^{cz}(0.10)	0.68^{bz}(0.09)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-c}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
$^{x-z}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

The same trend was observed in the high performance liquid chromatography (HPLC) study of Reyes and Cisneros (2005) who found a reduction of 32.3%, 50.3% and 53.8% in ascorbic acid for samples irradiated at low (1.0 kGy), medium (1.5 kGy) and high (3.1 kGy) doses, respectively, by the end of the storage at 15°C (18 days).

In summary, the irradiation of mangoes up to 1.0 kGy minimizes the ascorbic acid reduction. Irradiation at higher doses causes detrimental changes in this parameter.

4.1.2.9. Volatiles

Volatiles in mango predominately contain a mixture of terpene carbons and oxygenated sesquiterpenoids, often mainly δ-3-carene. δ-3-carene was predominant in all treatments throughout the storage time and it accounted for 58% and 56% of the total volatiles on days zero and twenty-one, respectively (Table 4-10). MacLeod and Snyder

(1985) reported δ-3-carene as the major compound in the pulp of ripe mango of the Tommy Atkins variety.

Inspection of the chromatographs (Figures 4-17 & 4-18) indicates that the overall profiles are relatively similar. Only few differences were observed in the concentrations of α-humulene by the end of the storage time (Figure 4-18). Blakeskey et al. (1979) reported no significant change in the volatile profile of mango pulp irradiated with gamma rays at 0.75 kGy. Similar results were found by Gholap et al. (1990) where the effect of gamma irradiation at 0.25 kGy on mango fruits was similar in the control and the irradiated samples. However, compared with the control sample, some compounds increased and others decreased with the irradiation dose. For instance, α-pinene decreased significantly by approximately 29.5% in samples irradiated at medium dose on day zero and by 51.9% in samples irradiated at high dose (3.1 kGy) on day twenty-one. The same trend was followed by other terpenes such as trans-caryophyllene and α-humulene. However, limonene content was higher in samples treated with medium (1.5 kGy) and high (3.1 kGy) dose on day zero and twenty-one, respectively, but a reduction in the samples exposed to low dose was observed throughout the storage time. Moussaid et al. (2000) found a reduction of limonene in orange irradiated with gamma radiation at doses up to 2 kGy. The authors also reported that only linalool increased with storage time. These differences may be associated with the variation in the degree of ripening of the fruits and the harvesting conditions.

Table 4-10
Mean pick areas (%) of fourteen volatile compounds recovered by solid phase microextraction, GC-MS of mangoes stored up to 21 days at 12°C

	Day 0				Day 21			
Volatiles/Dose	**Control* (0.0 kGy)**	**Low (1.0 kGy)**	**Medium (1.5 kGy)**	**High (3.1 kGy)**	**Control* (0.0 kGy)**	**Low (1.0 kGy)**	**Medium (1.5 kGy)**	**High (3.1 kGy)**
Methyl buthanoate	0.13^{ax}(0.13)	0.53^{ax}(0.74)	bdl	0.29^{ax}(0.04)	0.13^{ax}(0.10)	0.05^{x}(0.03)	0.06^{ax}(0.04)	0.115^{ax}(0.05)
Ethyl buthanoate	2.74^{ax}(1.96)	0.28^{ax}(0.04)	bdl	3.73^{ax}(2.69)	0.085^{bx}(0.04)	0.65^{x}(0.09)	0.05^{ax}(0.02)	0.68^{bx}(0.08)
Ethanol	0.3^{ax}(0.05)	0.98^{ax}(0.37)	bdl	1.87^{ax}(0.83)	0.23^{ax}(0.05)	4.19^{y}(0.01)	2.63^{bz}(0.01)	0.58^{ax}(0.38)
Alpha pinene	15.09^{ax}(1.93)	13.92^{axy}(0.01)	10.64^{ay}(0.01)	14.195^{axy}(7.99)	19.45^{ax}(2.91)	12.89^{ax}(0.01)	10.13^{ax}(0.01)	9.34^{bx}(4.46)
E-hexenal	0.69^{aw}(0.45)	14.65^{ax}(0.01)	13.21^{ay}(0.01)	1.69^{az}(0.54)	2.84^{bx}(1.91)	2.66^{bx}(0.01)	5.08^{bx}(0.01)	31.64^{by}(14.94)
Methyl caproate	2.64^{ax}(0.20)	3.21^{ay}(0.01)	8.5^{az}(0.01)	2.49^{ax}(0.02)	5.33^{bx}(3.40)	3.46^{ax}(0.01)	5.66^{bx}(0.01)	6.05^{bx}(1.66)
Delta-3-carene	100^{ax}(0.00)	100^{ax}(0.00)	100^{ax}(0.00)	100^{ax}(0.00)	100^{ax}(0.00)	100^{ax}(0.00)	100^{ax}(0.00)	100^{ax}(0.00)
Alpha-terpinene	1.57^{ax}(0.31)	1.11^{ax}(0.01)	2.28^{ayz}(0.01)	1.82^{axz}(0.15)	0.70^{ax}(0.38)	0.63^{ax}(0.00)	0.65^{bx}(0.01)	0.51^{bx}(0.04)
Beta-myrcene	6.26^{ax}(1.55)	4.42^{ax}(0.01)	6.68^{ax}(0.01)	4.02^{ax}(0.04)	5.34^{ax}(3.40)	3.46^{ax}(0.01)	5.66^{ax}(0.01)	6.06^{ax}(1.66)
Limonene	8.14^{ax}(1.97)	5.26^{ax}(0.01)	8.68^{ax}(0.01)	6.25^{ax}(0.63)	6.64^{ax}(2.55)	4.37^{ax}(0.01)	6.59^{ax}(0.01)	14.84^{ax}(5.71)
P-cymene	7.64^{ax}(1.60)	5.40^{ax}(0.01)	3.82^{ax}(1.30)	5.56^{ax}(0.01)	3.78^{ax}(91.30)	4.72^{ax}(0.00)	2.70^{ax}(0.01)	7.19^{ax}(0.01)
Alpha-terpinolene	6.14^{ax}(1.34)	5.40^{ax}(0.01)	8.25^{ay}(0.00)	4.45^{ax}(0.24)	2.89^{bx}(2.12)	1.84^{bx}(0.01)	3.14^{bx}(0.00)	2.15^{ax}(0.33)
Trans-caryophyllene	6.52^{ax}(0.74)	5.40^{ay}(0.01)	3.26^{az}(0.01)	4.62^{ay}(0.05)	6.62^{ax}(2.96)	1.08^{ax}(0.31)	0.76^{bx}(0.01)	1.38^{ax}(0.13)
Alpha-humulene	3.31^{ax}(0.29)	2.27^{ay}(0.33)	1.60^{az}(0.31)	2.57^{ay}(0.07)	2.70^{ax}(0.92)	0.41^{by}(0.01)	1.53^{axy}(0.00)	0.69^{by}(0.04)

Bdl =below detection limit.*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence. Numbers in parenthesis are the standard deviation.
$^{a-b}$Means within days which are not followed by a common superscript letter are significantly different (P<0.05).
$^{w-z}$Means within treatments which are not followed by a common superscript letter are significantly different (P<0.05)

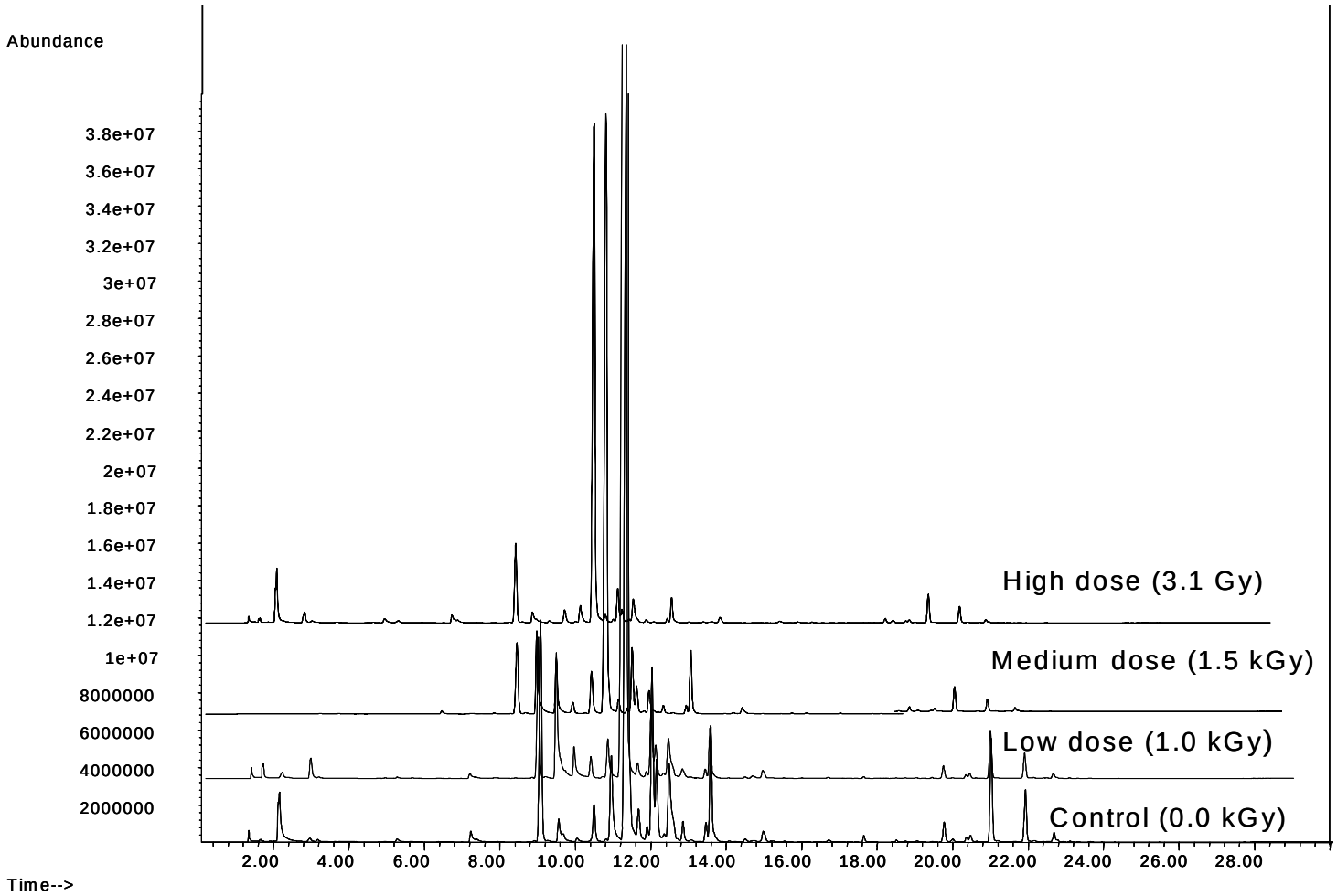

Figure 4-17. Gas chromatography/ mass spectrometry of head space volatiles extracted from irradiated and non-irradiated mangoes (day 0).

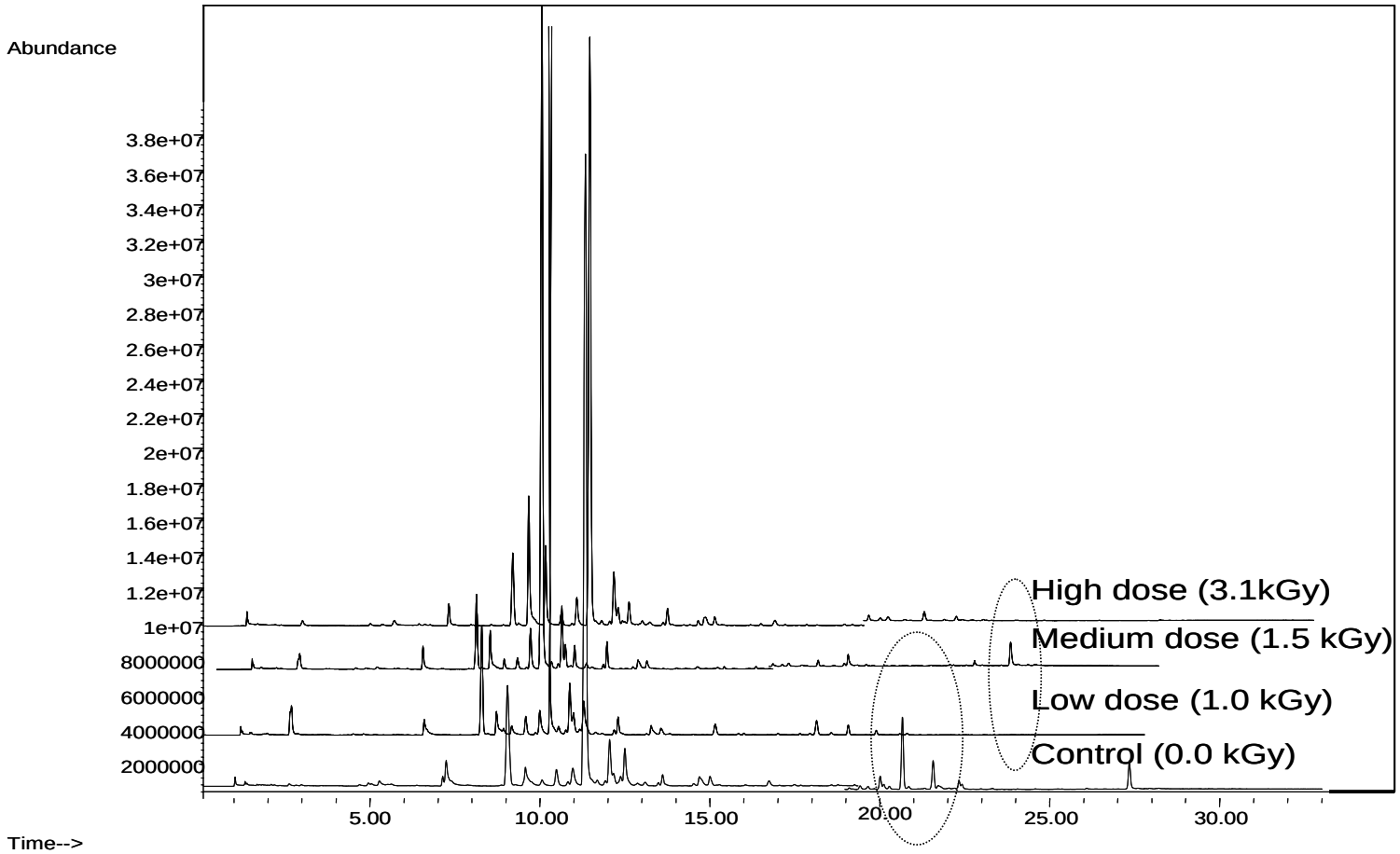

Figure 4-18 Gas chromatography/ mass spectrometry of head space volatiles extracted from irradiated and non-irradiated mangoes (day 21). Dot lines indicate the main differences.

According to Hulme (1971), the aroma of the climatecric fruit develops better quality if harvested after it starts ripening. In addition, the aroma of mango has been reported to be influenced by various factors including mango species, maturity stage, ripening and processing (Singh et al., 2004). Lalel et al. (2003) found that the production of the most terpenes (such as α-pinene, δ-3-carene, β-pinene, and α-terpinolene) during ripening of 'Kensington Pride' mango was parallel to the ethylene production, while the esters (such as ethyl acetate and ethyl butanoate) were related to the fatty acid biosynthesis. It is also important to consider that the aroma of mango could be affected by the storage temperature. Lakshiminarayana (1980) found a reduction in aroma volatiles of mango when they were stored below 15°C. The authors also reported a reduction in the production of mango aroma volatile compounds due to chill- injury and the increase in the concentrations of CO_2.

E-hexenal increased significantly ($P<0.05$) with dose throughout storage. Alcohols such as ethanol increased significantly ($P>0.05$) with the higher dose on day twenty-one. These results which were consistent with the increase in CO_2 concentration (section 4.1.1.4) occurred in irradiated samples but especially in those treated with the higher dose by the end of the storage time. They were also consistent with the changes in the cell structure where some of these compounds are released due to the cell disruption. Some of these changes were perceived by the panelists in the sensory evaluation when the fruits irradiated at medium and high doses were rated with stronger aroma than the control fruits (section 4.1.3).

No significant ($P<0.05$) changes in volatile compounds were observed with time. Only E-hexenal had a significant decrease in the control fruits and the samples treated with low and medium doses by the end of storage. However, there were some terpenes such as alpha pinene and alpha terpinolene that had a reduction on day twenty-one.

In summary, the exposure of mangoes to irradiation levels up to 3.1 kGy induced the formation of volatile compounds which are important components to the odor and flavor of the fruit.

4.1.2.10. Carotenoids

The carotenoids content (μg β-carotene/100g w.b) in irradiated mangoes did not show a consistent trend when compared with the control fruits (Table 4-11). Significant ($P>0.05$) differences were observed for irradiated samples. For example, an increase (47.7% and 21.16%) was observed on days 0 and 21 respectively, for fruits treated at low dose (1.0 kGy), but a reduction (49.10%) occurred on day10. These samples also had a decrease in acidity and an increase in sugars. Therefore, the variation in carotenoids is associated with the ripening stage of the fruits. According to Hulme (1971), the content of carotenoids in ripe mangoes is ten times more than the content of the partially ripe fruits. This increase is related to the structural changes associated with chloroplast to chromoplats transition. The samples exposed at high dose had the lower concentrations than the other treatments on day zero while the samples treated with medium dose had lower concentrations on day twenty-one. However, the maximum concentrations of carotenoids content were reached on the fifth day of storage but the rate of increase (48.5%) was higher in samples irradiated at low dose (1.0 kGy). This trend correlates

well with the values of redness and yellowness of the samples, which reached at maximum on day five (section 4.1.1.1).

Table 4-11
Effect of irradiation dose on carotenoids content (μg β-carotene/100g w.b.) of mangoes stored up to 21 days at 12°C

Dose/Day	Control* (0.0 kGy)	Low (1.0 kGy)	Medium (1.5 kGy)	High (3.1 kGy)
0	589.02^{ax}(1.50)	1126.41^{ay}(20.36)	713.60^{az}(26.99)	590.44^{ax}(9.25)
5	1222.64^{bx}(21.85)	1178.58^{bx}(51.15)	1300.85^{by}(77.73)	849.23^{bz}(21.85)
10	1050.74^{cx}(40.68)	704.69^{cy}(15.38)	531.00^{cz}(13.14)	710.21^{by}(27.79)
21	698.00^{dx}(13.77)	885.43^{dy}(17.74)	481.62^{cz}(11.32)	880.39^{dy}(18.36)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-d}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
$^{x-z}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

Beyers and Thomas (1979) reported a reduction in carotene levels in irradiated mangoes as compared with non-irradiated fruits. However, in a subsequent study, Beyers et al. (1983) recorded higher carotene levels for irradiated mangos at doses from 0.25 to 2.0 kGy than for non-irradiated samples. The authors explained the increase as an effect of irradiation on increasing the extractability of carotenoids due to the changes in the structure of the cells rather than an increase in their synthesis by enzymatic action. El-

Samahy et al. (2000) also recorded an increase in carotene levels of mango irradiated at low doses (0.5-1.5 kGy) using gamma rays.

Throughout the storage time, samples treated with low and medium doses had significantly higher carotenoids content than the control at day zero. On the fifth day, the concentrations were higher in samples exposed to medium dose. On day ten, all irradiated samples had significantly ($P>0.05$) lower carotenoid content than the control. By the end of the storage the samples treated with low and high doses had higher concentrations. The samples treated with low (1.0 kGy) and medium (1.5 kGy) doses had a reduction of 21.39% and 32.50%, respectively by the end of the storage. Carotenoids increase naturally with the ripening process of the fruits (Hulme, 1971); therefore, the variation during time is related to the different stages of ripening.

The color of the fruits is the base for categorization of many products in commercial scales but the concentration of pigments could be a good quality index. Color is more related to consumer perception of appearance; pigment concentration is more related to maturity (Abbott, 1999).

In summary, the effect of irradiation on carotenoids content depends on the fruit maturity level. The higher the concentration of carotenoids, the more mature the fruit and better the color and the appearance. These results suggest that irradiation up to 1.5 kGy may enhance the carotenoids content of mangoes and therefore, the overall appearance of the fruit.

4.1.3. Sensory evaluation

The sensory evaluation of mangoes indicated a stronger preference for the control (non-irradiated) and low dose (1.0 kGy) treated samples over those that were irradiated at higher doses. The overall acceptability of the fruits decreased as the dose level increased. The results of the sensory evaluation are shown in Table 4-12.

4.1.3.1. Overall quality

Differences in the acceptability of the overall quality of the fruits were observed during the sensory evaluation. For control samples a significant ($P>0.05$) preference was observed in day ten but, samples exposed at medium dose were more acceptable on day five. In general, the overall quality of the control samples was acceptable. However, fruits irradiated at low (1.0 kGy) and medium (1.5 kGy) doses were rated in the acceptability limit (1.0-3.0). The most unacceptable (higher scores) fruits were those exposed to a high dose (3.1 kGy).

4.1.3.2. Color

The results indicated differences for the acceptance of the irradiated and non-irradiated (control) samples. For instance, a significant ($P>0.05$) preference was observed on day zero for the color of samples treated with high (3.1 kGy) dose, but at day five the control and the samples exposed to low dose (1.0 kGy) received better scores. However, at day ten, the control samples showed more acceptability by the panelists.

Table 4-12
Sensory attributes (overall quality, color, texture and aroma) of irradiated mangoes stored up to 21 days at 12°C

Overall quality

Day	Control* (0.0 kGy)	Low dose (1.0 kGy)	Medium dose (1.5 kGy)	High dose (3.1 kGy)
0	2.72^{ax}(0.97)	2.72^{ax}(0.90)	2.52^{ax}(1.03)	1.86^{ay}(0.94)
5	2.22^{bx}(0.84)	2.80^{ay}(0.90)	2.08^{bx}(0.82)	3.14^{by}(1.04)
10	1.90^{bx}(0.90)	2.78^{ay}(0.88)	2.66^{ay}(0.68)	3.86^{cz}(0.92)
21	2.98^{axz}(1.16)	2.67^{axy}(1.04)	2.26^{aby}(0.97)	3.22^{bz}(1.04)

Color

Day	Control* (0.0 kGy)	Low dose (1.0 kGy)	Medium dose (1.5 kGy)	High dose (3.1 kGy)
0	2.70^{ax}(1.07)	2.72^{ax}(0.97)	2.54^{ax}(0.99)	1.94^{ay}(1.09)
5	2.18^{bx}(1.02)	2.78^{ay}(0.88)	1.84^{bx}(0.88)	2.89^{by}(1.02)
10	1.70^{cx}(0.81)	2.70^{ay}(0.93)	2.42^{ay}(0.83)	4.00^{cz}(0.90)
21	2.90^{ax}(1.01)	2.73^{ax}(1.13)	1.85^{by}(0.84)	3.08^{bx}(1.11)

Texture

Day	Control* (0.0 kGy)	Low dose (1.0 kGy)	Medium dose (1.5 kGy)	High dose (3.1 kGy)
0	1.70^{abx}(0.88)	1.90^{ax}(0.78)	2.78^{ay}(0.93)	3.88^{az}(0.96)
5	1.44^{ax}(0.73)	2.32^{by}(0.76)	2.62^{ay}(0.87)	3.83^{az}(1.04)
10	2.00^{bx}(1.10)	2.04^{abx}(0.85)	2.58^{ay}(0.62)	4.06^{az}(1.07)
21	1.34^{aw}(0.65)	3.30^{cx}(0.89)	2.98^{ay}(0.83)	3.85^{az}(0.76)

Aroma

Day	Control* (0.0 kGy)	Low dose (1.0 kGy)	Medium dose (1.5 kGy)	High dose (3.1 kGy)
0	3.66^{ax}(0.87)	3.38^{ax}(0.87)	2.86^{ay}(0.85)	2.22^{az}(1.05)
5	3.26^{bx}(1.06)	3.22^{ax}(0.79)	2.74^{ay}(0.92)	2.69^{by}(1.07)
10	2.66^{cx}(1.08)	3.30^{ay}(1.05)	3.32^{by}(0.94)	3.30^{cy}(1.03)
21	3.78^{ax}(0.91)	2.65^{by}(1.01)	2.69^{ay}(0.94)	2.77^{by}(1.15)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-c}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
$^{w-z}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$). On the hedonic scale for overall quality and color a score of 1= like extremely, 3= neither like nor dislike, 5=dislike extremely; for texture a score of 1= firm, 3= somewhat firm-soft, 5= soft; for aroma a score of 1= strong, 3= moderate, 5= none (see appendix A-A).

By the end of storage the color of the samples treated with high dose was less acceptable (higher scores) than the other treatments. Throughout time, only the scores of the samples exposed to low dose (1.0 kGy) did not show significant differences.

These results are in agreement with the measured color (section 4.1.1.1) where differences within doses and time were observed, due to the variation between the stage of ripeness and the variation of carotenoids content among the fruits used in this study. In general, both tests, the objective measurement of color and the sensory evaluation, indicated the low irradiation treatment as more appropriate to maintain the quality and acceptability of the color of the mangoes. The correlation factor was calculated between the yellowness (b values) previously evaluated and the scores of the sensory evaluation. The samples treated at low dose had the higher correlation factor ($r= 0.79$).

4.1.3.3. Texture

The panelists found a significant ($P>0.05$) difference between the texture of the control samples and the texture of the irradiated fruits. Control samples received better scores (firm) than the irradiated mangoes throughout the entire storage time. However, fruits irradiated at low (1.0 kGy) and medium (1.5 kGy) doses were rated in the acceptability range (1.0-3.0). Samples irradiated at high dose had the highest scores (3.83-4.06) indicating that the degree of softness was not acceptable. Throughout the time, no differences were observed for all treatments except for low dose samples which had higher scores on days five and twenty-one indicating a decrease in firmness, but still acceptable. Similar results were observed in the objective measurement of texture (compression test) which showed an increase in softening of the fruits irradiated at higher

dose, and the texture of the control was firmer (thus more acceptable) (r^2= 0.98) than the irradiated samples during the evaluation time.

4.1.3.4. Aroma

Significant (P>0.05) differences in the acceptability of the aroma of the control samples and the irradiated fruits were observed. The aroma of the control and the samples exposed to low (1.0 kGy) dose was less perceived by the panelists (higher scores) than the aroma of those fruits irradiated at medium (1.5 kGy) and high (3.1 kGy) doses, which were significantly rated better (moderate and moderate strong) especially those irradiated at a high dose. These results are supported by the objective evaluation of volatile compounds when the irradiated samples showed an increase in volatile compounds by the end of the storage.

In summary, irradiation of mangoes up to 1.5 kGy does not affect the sensory quality of the fruits. Irradiation at higher dose (3.1 kGy) did cause a detrimental change on the sensory quality of mangoes.

Overall, the previous results suggest that irradiation treatment of mangoes may lead to a stress condition which depending of the dose, may or may not cause the physicochemical changes that depend on the physiological parameters of the fruit. In general, irradiation of mangoes up to 1.5 kGy treatment maintains the overall quality of mangoes and may increase the shelf-life by three days (from 18 days to 21 days when stored at 12˚) by delaying the ripening.

4.2. Effect of irradiation on physical and chemical properties of blueberry

4.2.1. Physical properties

4.2.1.1. Color attributes

4.2.1.1.1. Visual changes

No obvious visual changes were noted in blueberry samples right after irradiation (day 0). However, in general, irradiated samples looked darker than the controls (Figure 4-19). By the end of the storage time (14 days), the fruits irradiated at medium (1.6 kGy) dose became more brownish-reddish (Figure 4-20) than the other samples. This darkening effect may be attributed to an increased polyphenoloxidase activity and the consequent oxidation of phenolics giving rise to brown and dark pigmentation of the fruit (Thomas, 1986). In addition, some of the control samples shrunk and spoiled due to the presence of molds (fungi) by the end of storage (Figure 4-21). This problem with molds did not occur in any of the irradiated samples.

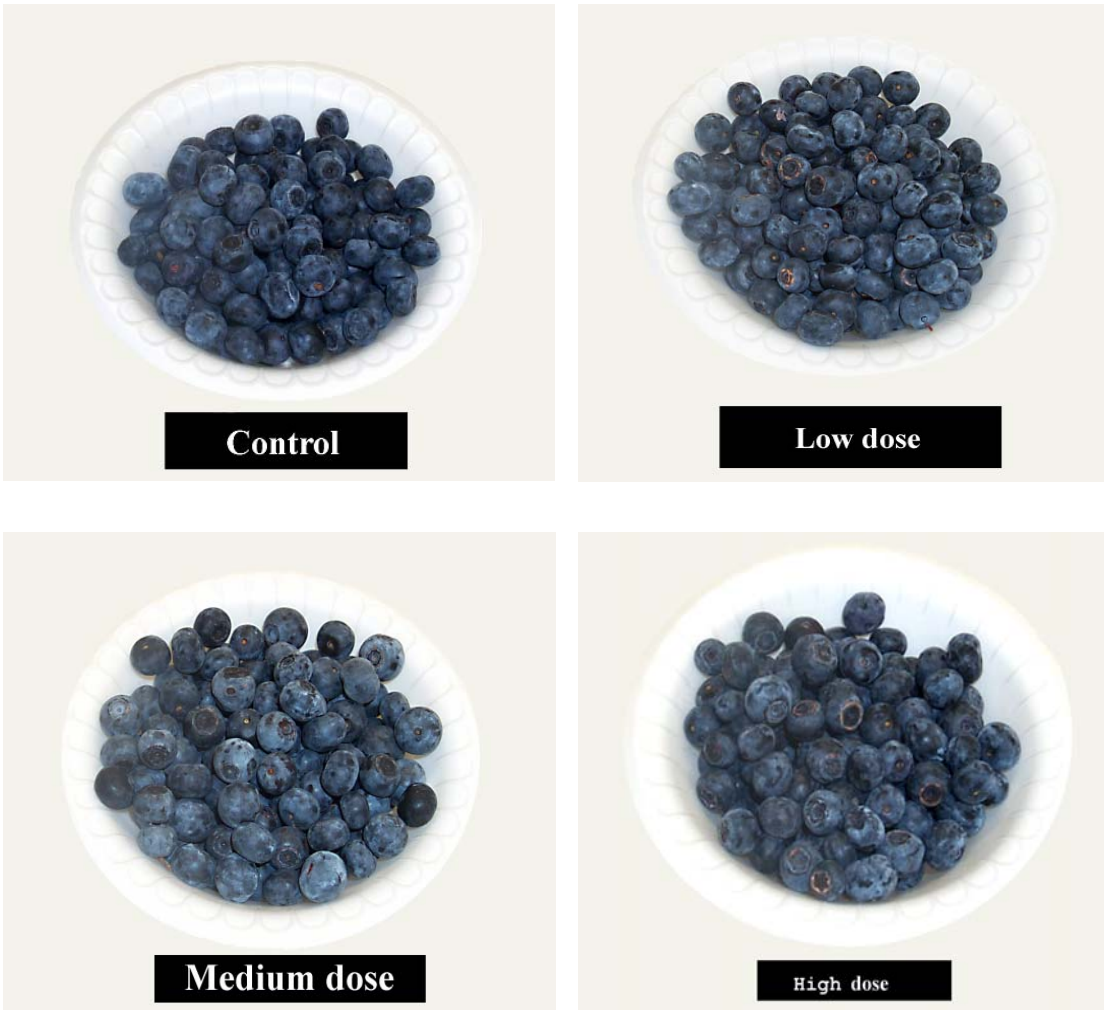

Figure 4-19. Irradiated and non-irradiated blueberries right after irradiation treatment (day 0). (Control =non-irradiated, low dose =1.1 kGy, medium dose =1.6 kGy, high dose =3.2 kGy).

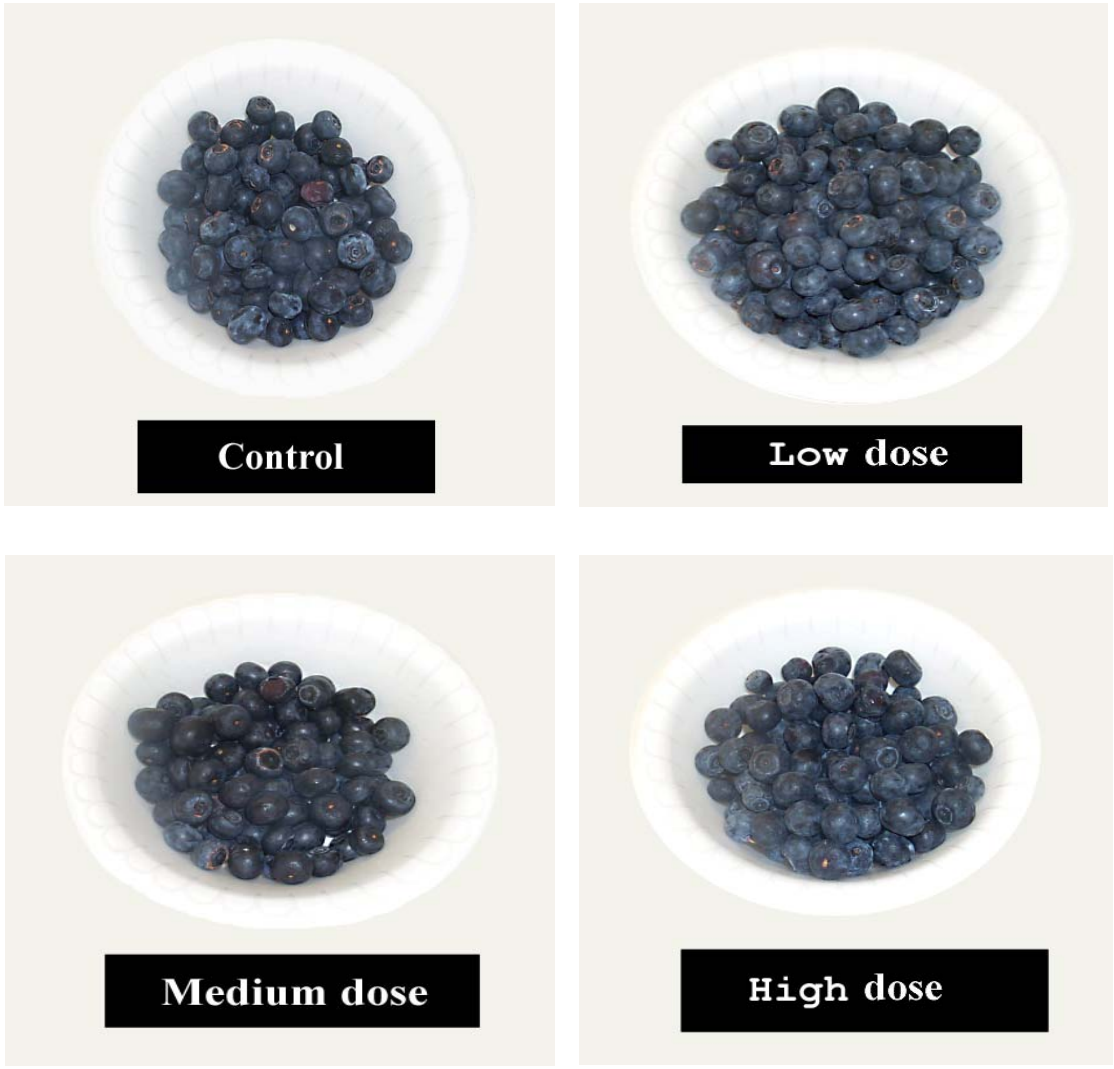

Figure 4-20. Irradiated and non-irradiated blueberries after 14 days of storage at 5°C. (Control =non-irradiated, low dose =1.1 kGy, medium dose =1.6 kGy, high dose =3.2 kGy).

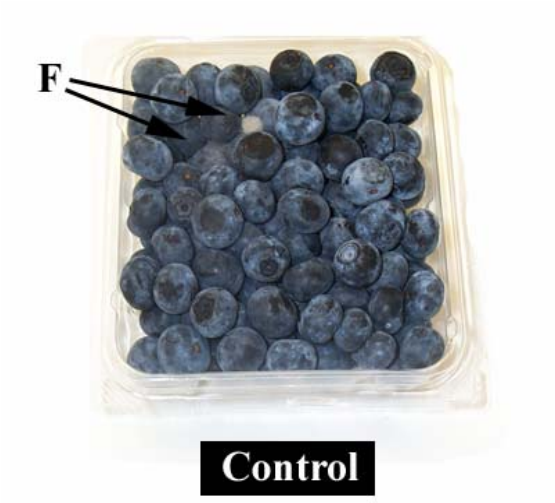

Figure 4-21. Blueberries (control) after 14 days of storage at 5°C. (F = fungi-molds).

4.2.1.1.2. Objective measurement

The values of lightness (*L*) of the fruits exposed to low (1.1 kGy) and medium (1.6 kGy) doses was significantly (P>0.05) lower than the control right after irradiation (day 0) which indicates a darkening of the fruits. The samples treated with high dose (3.2 kGy) were not significantly different from the control on that day (Table 4-13). The decrease in *L* values is associated with the effect of irradiation on enzymes (polyphenoloxidase) activity due to phenolics oxidation. According to Seymour et al., (1993) there is a correlation between Phenylalanine ammonia-lyase (PAL) activity and anthocyanin levels, which increase during the fruit ripening. Therefore, it is possible that at these doses the enzymes activity is enhanced and consequently changes in color may be observed.

The fruit exposed at low (1.1 kGy) and medium (1.6 kGy) doses became significantly (P>0.05) lighter (higher values of *L*) on days three and fourteen of storage. All irradiated samples were lighter than the control by the end of storage. The changes in *L* values with time are associated with the ripeness stage of the fruit and their anthocyanin content. Mustafa et al. (2001) reported that dark-red berries had four times more anthocyanin content compared with light-red berries. Yu et al., (1995) also reported an increase in *L* values of strawberries irradiated at 2.0 kGy using electron beam.

The redness (*a* values) of the samples exposed to low (1.1 kGy) dose was significantly (P>0.05) higher (more red) than the controls until day 7 (Table 4-13). However, all irradiated samples had decreased *a* values by the end of storage. This effect may be associated with the potential effect of irradiation on delaying the ripening proces; thus, with differences in the anthocyanin content which depends of the fruit maturity level. According to Seymour (1993), as the berry matures there is a gradual change from green to the characteristic color of the fruit, red or black depending on the variety. This suggests that the samples treated with a low dose were more mature on day 7 than the control and the fruits exposed to the other treatments.

Irradiation had an effect on the yellowness (*b* values) of the blueberries. The differences became significant (P>0.05) for samples treated with low dose (1.1 kGy) which had higher *b* values than the control on day zero. All irradiated samples had decreased *b* values (more blue than the control) by the end of the storage time (Table 4-13).

Table 4-13
Effect of irradiation dose on the color attributes-lightness (*L*), redness (*a*) and yellowness (*b*)- of blueberries stored up to 14 days at 5°C

Color parameter	Dose/Day	Control* (0 kGy)	Low (1.1 kGy)	Medium (1.6 kGy)	High (3.2 kGy)
L	0	18.14^{ax}(1.14)	15.62^{ay}(0.47)	16.87^{axy}(1.60)	18.28^{ax}(1.19)
Lightness	3	18.35^{axz}(0.72)	14.72^{aby}(0.99)	19.83^{bz}(0.39)	17.63^{ax}(1.25)
(%)	7	19.18^{ax}(1.19)	16.96^{bx}(1.53)	19.52^{bx}(1.07)	17.75^{ax}(0.58)
	14	14.27^{bx}(0.46)	19.58^{cy}(0.45)	18.95^{byz}(0.44)	17.58^{az}(1.85)
a	0	-0.38^{ax}(0.15)	-0.09^{ay}(0.04)	-0.31^{ax}(0.12)	-0.25^{axy}(0.18)
redness	3	-0.34^{ax}(0.15)	0.15^{by}(0.63)	-0.48^{ax}(0.57)	-0.43^{ax}(0.15)
(+red, -green)	7	-0.39^{ax}(0.10)	-0.19^{cy}(0.89)	-0.41^{ax}(0.45)	-0.30^{axy}(0.62)
	14	-0.21^{ax}(0.04)	-0.44^{dy}(0.02)	-0.45^{ay}(0.03)	-0.31^{az}(0.02)
b	0	-2.98^{ax}(0.16)	-2.13^{ay}(0.10)	-2.62^{az}(0.24)	-2.79^{axz}(0.30)
yellowness	3	-3.13^{ax}(0.17)	-1.94^{ay}(0.02)	-3.40^{bz}(0.13)	-3.53^{bz}(0.02)
(+yellow, - blue)	7	-3.61^{bx}(0.43)	-2.61^{by}(0.47)	-3.56^{bx}(0.14)	-3.21^{bxy}(0.13)
	14	-2.23^{cx}(0.10)	-3.83^{cy}(0.07)	-3.87^{cy}(0.62)	-2.65^{az}(0.18)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
[a-c]Means within a row which are not followed by a common superscript letter are significantly different (P<0.05).
[x-z]Means within a column which are not followed by a common superscript letter are significantly different (P<0.05).

This decrease in *b* values in irradiated samples may be associated with the strong effect of irradiation on the cell structure (section 4.1.2.3) which is related to the concentration of pigments. In addition, it is possible that a co-pigmentation occurs at these doses. For instance, anthocyanins may form complex with flavones, causing a color change from red to blue, therefore, an increase in color intensity is perceived (Asen et al., 1972). The samples treated with a low dose had significantly higher *b* values on days three and seven than the samples subjected to other treatments; therefore these samples would have more blue color. In some fruits the minor pigments such as carotenoids will be masked by the more intense pigments such as anthocyanins.

Throughout the storage time, different trends were observed in the yellowness (*b* values) of the non-irradiated and irradiated blueberries. Control samples had a significant ($P>0.05$) increase in *b* values on day fourteen indicating the ripening of the fruits. The samples treated with low dose (1.1 kGy) showed an increasing trend in *b* values during storage, while the samples exposed to medium dose (1.6 kGy) had decreased *b* values. The fruit exposed to high dose had decreased *b* values until day seven (half of storage time) with a subsequent increase on day fourteen. Again, these changes are related to the ripening stage of the fruit and to the effect of irradiation on delaying ripening especially in samples treated with medium (1.6 kGy) and high doses (3.2 kGy).

Table 4-14
Effect of irradiation dose on the color attributes -chroma (*C*), total color difference (*ΔE*) and hue (θ)- of blueberries stored up to 14 days at 5°C

Color parameter	Dose/Day	Control* (0 kGy)	Low (1.1 kGy)	Medium (1.6 kGy)	High (3.2 kGy)
Chroma	0	3.01^{ax}(0.17)	2.14^{ay}(0.10)	2.64^{az}(0.25)	2.81^{axz}(0.31)
(*C*)	3	3.16^{ax}(0.19)	1.95^{ay}(0.02)	3.44^{bz}(0.14)	3.56^{bz}(0.03)
	7	3.63^{bx}(0.44)	2.62^{by}(0.48)	3.58^{bx}(0.14)	3.23^{bxy}(0.14)
	14	2.25^{cx}(0.10)	3.86^{cy}(0.07)	3.90^{cy}(0.06)	2.67^{az}(0.18)
Total color difference	0	13.49^{ax}(0.80)	11.69^{ay}(0.32)	12.59^{axy}(1.12)	13.57^{ax}(0.82)
(Δ*E*)	3	18.51^{bxz}(0.72)	14.73^{by}(0.10)	20.02^{bx}(0.37)	17.88^{bz}(1.23)
	7	19.25^{bx}(1.25)	16.88^{cx}(1.58)	19.57^{bx}(1.08)	17.77^{bx}(0.59)
	14	14.21^{ax}(0.47)	19.73^{dy}(0.45)	19.12^{by}(0.43)	17.55^{bz}(1.82)
Hue angle	0	85.59^{ax}(2.83)	87.51^{ax}(1.08)	83.36^{ax}(2.36)	69.94^{ax}(1.24)
(θ)	3	83.84^{ax}(2.44)	44.51^{bx}(2.19)	81.93^{ax}(0.62)	83.00^{ax}(0.20)
	7	83.78^{ax}(1.12)	85.93^{ax}(1.36)	83.42^{ax}(0.75)	84.69^{ax}(0.88)
	14	84.57^{ax}(0.77)	83.38^{ax}(0.49)	83.29^{ax}(0.45)	83.22^{ax}(0.88)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-d}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
$^{x-z}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

Chroma (*C*) values of all irradiated samples were significantly (P>0.05) lower than the control samples after irradiation (day 0). However, by the end of the storage all irradiated samples had higher *C* values than the control (Table 4-14). This increase indicates brighter fruit color which is related to higher *L* values. However, the chroma of the samples treated with low dose (1.1 kGy) decreased significantly (P>0.05) until the seventh day of storage which suggests a more dull color. These samples had lower *L* values up to day seven.

The chroma values of the samples treated with low (1.1 kGy) and medium (1.6 kGy) doses increased significantly (P>0.05) with time. The samples exposed to high dose (3.1 kGy) had increased values until day seven with a subsequent decrease by the end of the study. Control samples had significantly lower chroma values on day fourteen which suggests that the color of these samples was less intense than the color of the treated fruits.

The total color difference (ΔE) was significantly lower for the samples exposed to low (1.1 kGy) dose after irradiation. However, by the end of the storage all irradiated samples had significantly higher ΔE values than the control (Table 4-14). This trend may be related to some physiological changes that involve differences in growth and ripening. Young (1952) reported that blueberries in any given cluster do not all ripen at the same time. Fruit from the medial portions of the cluster ripens first, followed by the fruit from the terminal and basal portions at about the same time. These changes in ripening induce variation in the anthocyanin levels which affect the color development. Ballinger et al. (1972) reported that the difference in color expression of blueberries was related to the

anthocyanin content. For instance, pink fruits had 2.5 mg while blue fruits had 49 mg of anthocyanin per 10 g of fruit.

Irradiation had no effect on the Hue (θ) angle of the blueberries. Throughout the storage time, only the fruit treated with low dose (1.1 kGy) showed a significant (P>0.05) decrease (49.13%) by the third day (Table 4-14) of storage.

In summary, the exposure of blueberries at 1.1 kGy seems to have an unfavorable effect in the color of the fruit. This effect was not observed at higher doses. Overall, treatment of blueberries with 1.6 kGy is the best to maintain the fruit color attributes.

4.2.1.2. Texture (Kramer shear test)

The texture of blueberries was significantly (P>0.05) affected by irradiation treatment at all doses applied (Figure 4-22, Table A-7, Appendix A). Shear force or the force required to cut the fruit decreased significantly (P>0.05) as the irradiation dose increased. The samples treated with higher dose (3.2 kGy) required much less force than the other treatments. These samples were also considerably less tough than all the other samples (see figure on p.175). The control samples were more resistant to shear throughout the storage time. The softening effect induced by irradiation may be associated with the changes in cell wall structure and the solubility of its pectin substances (section 4.1.2.3). These results are consistent with the reduction of color at higher doses due to changes in pigmented cells (section 4.1.2.1).

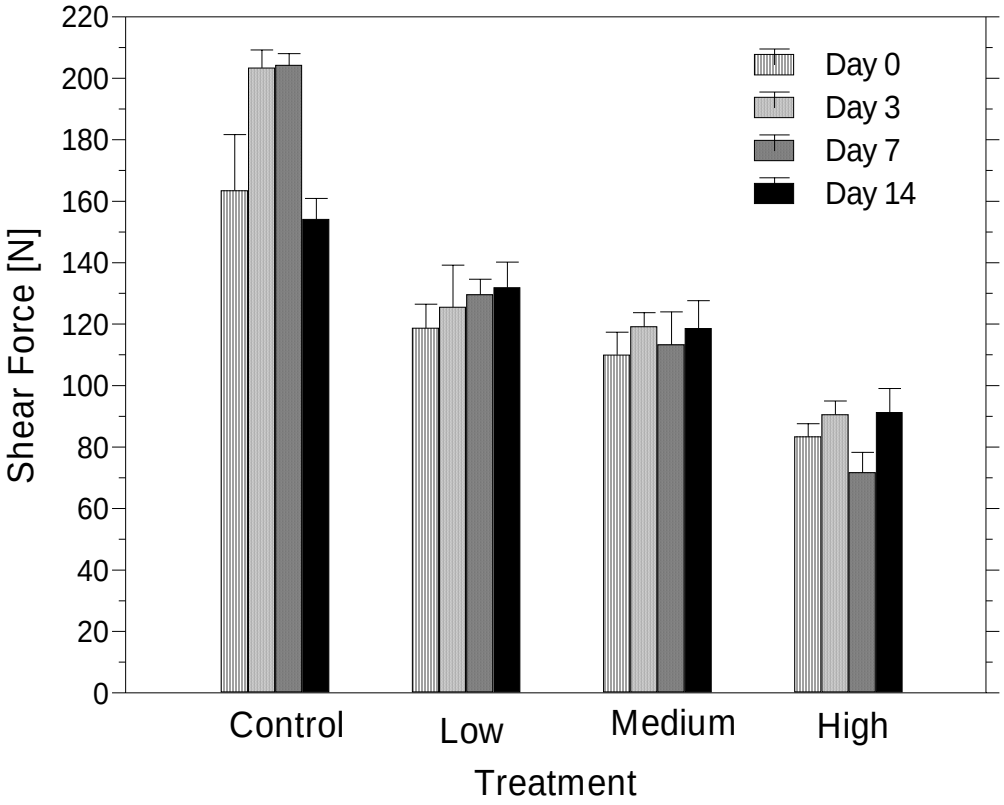

Figure 4-22. Effect of irradiation dose on texture of blueberries stored up to 14 days at 5°C (shear force (N)). (Control =non-irradiated, low dose =1.1 kGy, medium dose =1.6 kGy, high dose =3.2 kGy).

Similar findings were reported by Eaton et al. (1970) on irradiated Coville and Dixi blueberry varieties with gamma rays at dosages between 1.0 and 0.5 kGy. The irradiated samples were considerably softer than the non-irradiated fruits. Miller and McDonal (1996) observed that blueberries irradiated with gamma rays at 0.5 and 1.0 kGy were softer by 33% and 38%, respectively. Yu et al. (1995) showed that strawberries were significantly less firm after electron beam irradiation at doses of 0.5, 1.0 and 2.0 kGy.

Figure 4-23 shows the relationship between loss of texture (decreased shear force or softening) and the irradiation dose. The reduction on shear force of the fruits increased as a function of the dose. This effect can be described by an exponential model with the following equations:

$$SF(0) = 98.2 \exp^{(-0.517)D} + 64.99,\ R^2 = 0.997 \quad (4\text{-}6)$$

$$SF(3) = 118.1 \exp^{(-0.876)D} + 84.79,\ R^2 = 0.993 \quad (4\text{-}7)$$

$$SF(7) = 154.4 \exp^{(-0.5478)D} + 44.54,\ R^2 = 0.999 \quad (4\text{-}8)$$

$$SF(14) = 222.8 \exp^{(-0.1046)D} - 68.32,\ R^2 = 0.998 \quad (4\text{-}9)$$

where *SF* is the shear force in N at each storage time interval and *D* is the irradiation dose in kGy. According to Eqs 4-6 to 4-9 at any interval time, the higher the dose the higher the reduction on the shear force of the fruit.

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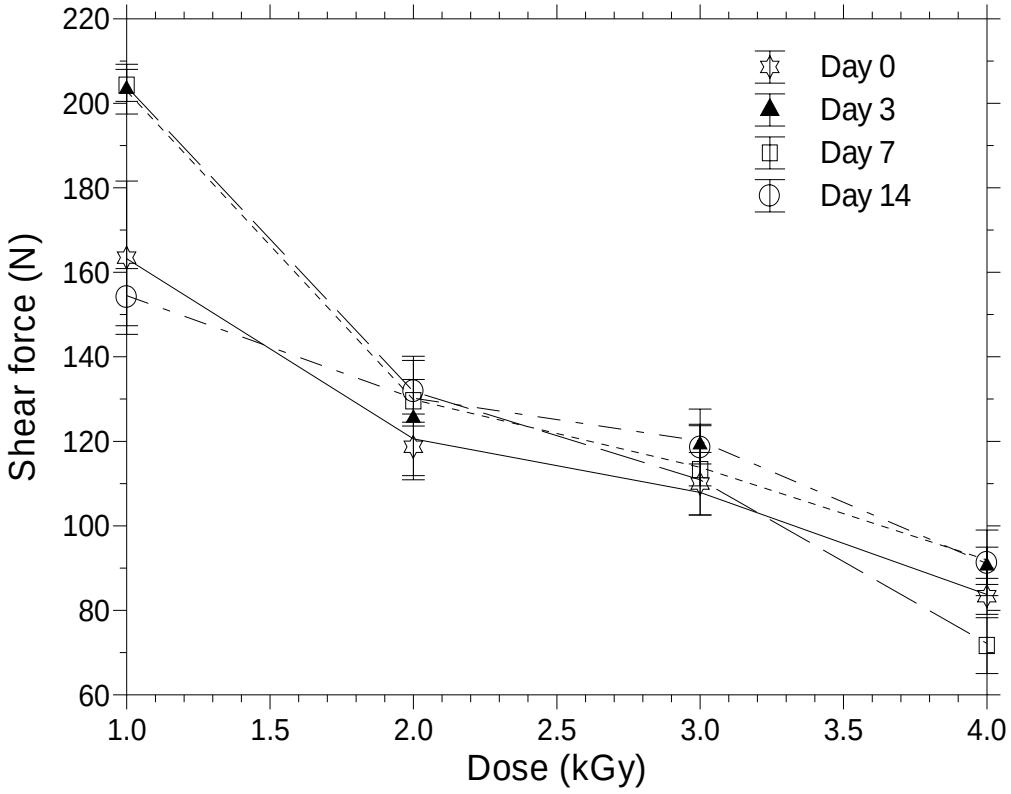

Figure 4-23. Effect of dose on shear force (N) of blueberries stored up to 14 days at 5°C.

The same trend was observed for the values of toughness (Figure 4-24). Irradiated samples at 1.1, 1.6, and 3.2 kGy were 25.6%, 33.6% and 48.7% less tough than the control fruit, respectively (Table A-7, Appendix A). The samples exposed to the higher dose were significantly less tough than the other treatments throughout the study. Therefore, when this fruit is consumed a perception of less force for biting the berry would be felt.

The softening effect of ionizing radiation can be more explained by examining the structural changes of the fruits which induce the degradation of the cell wall constituents such as polyssacharides, cellulose and hemicellulose, and therefore, changes in the cell structure are created reducing the firmness of the fruits . These changes will be discussed in section 4.1.2.3.

These results indicate that irradiating blueberries with doses as high as 3.2 kGy will yield unacceptable fruits in terms of texture. However, doses up to 1.6 kGy do not cause any detrimental change in texture.

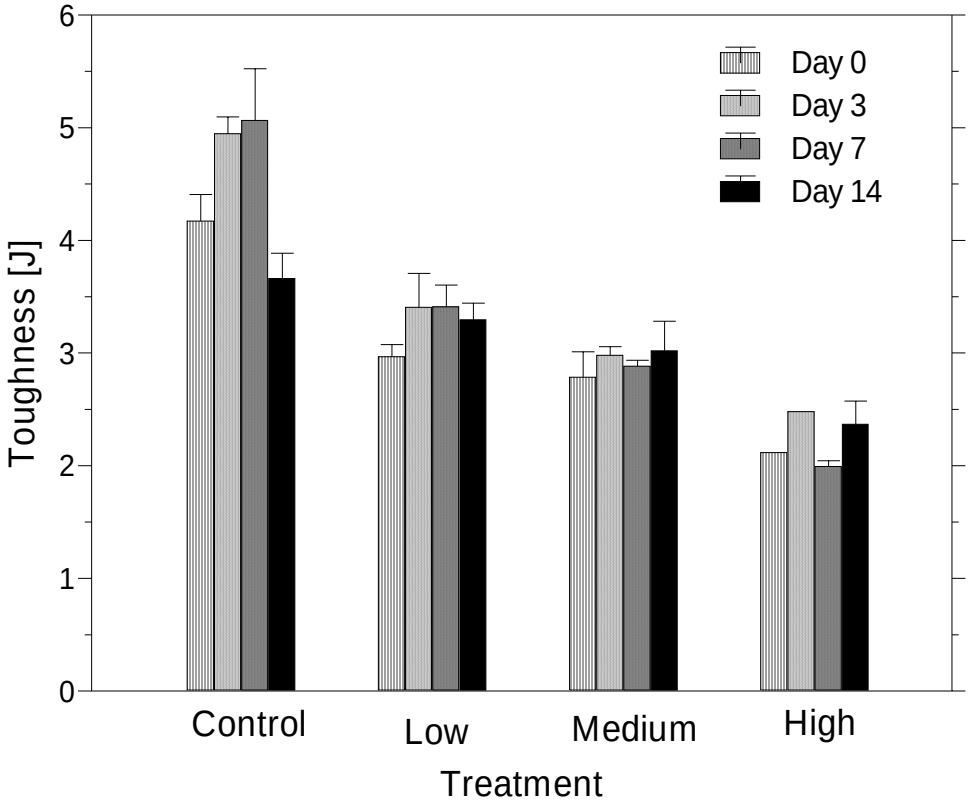

Figure 4-24. Effect of irradiation dose on texture (toughness in J) of blueberries stored up to 14 days at 5ºC. (Control =non-irradiated, low dose =1.1 kGy, medium dose =1.6 kGy, high dose =3.2 kGy).

4.2.2.3. Structural changes

Figures 4-25 and 4-26 present the SEM photomicrographs of blueberry skin. The skin of the control sample berries was found to be smoother that the skin of the irradiated fruit (Figure 4-25A). Dryness was observed in irradiated samples, especially in the fruits treated with medium (1.6 kGy) dose (Figure 4-25 C) which caused inconsistency in the cells shape and also closed stomata. Even though different findings were observed in the moisture content values, the dryness of these samples may be associated with changes in the cell compartments that cause the collapse of the structure of the hydrated cells (Allan-Wojtas et al., 2001). The methodology used in this study did not allow the observation of this effect. In samples irradiated at high (3.2 kGy) dose, micro-cracks and bleeding were observed in the surface (Figure 4-25 D).

At a higher resolution (Figure 4-26) the skin photomicrographs showed depressions in all irradiated samples (Figure 4-26 B to D) becoming more pronounced at medium (1.6 kGy) dose samples. In addition, the presence of fungi was noted in samples treated with high (3.2 kGy) dose (Figure 4-26D). According to Mustafa et al. (2001), the main path of entry of these microorganisms would be through the fractures or through the stomata. The authors reported the presence of fungal hyphae in the surface of cranberry in dark-red stage but not in white stage samples. Therefore, it is valid to assume that the presence of micro-cracks in the samples treated at a high dose facilitated the presence of fungus. These results are consistent with the increase in water activity observed for these samples (section 4.2.2.2).

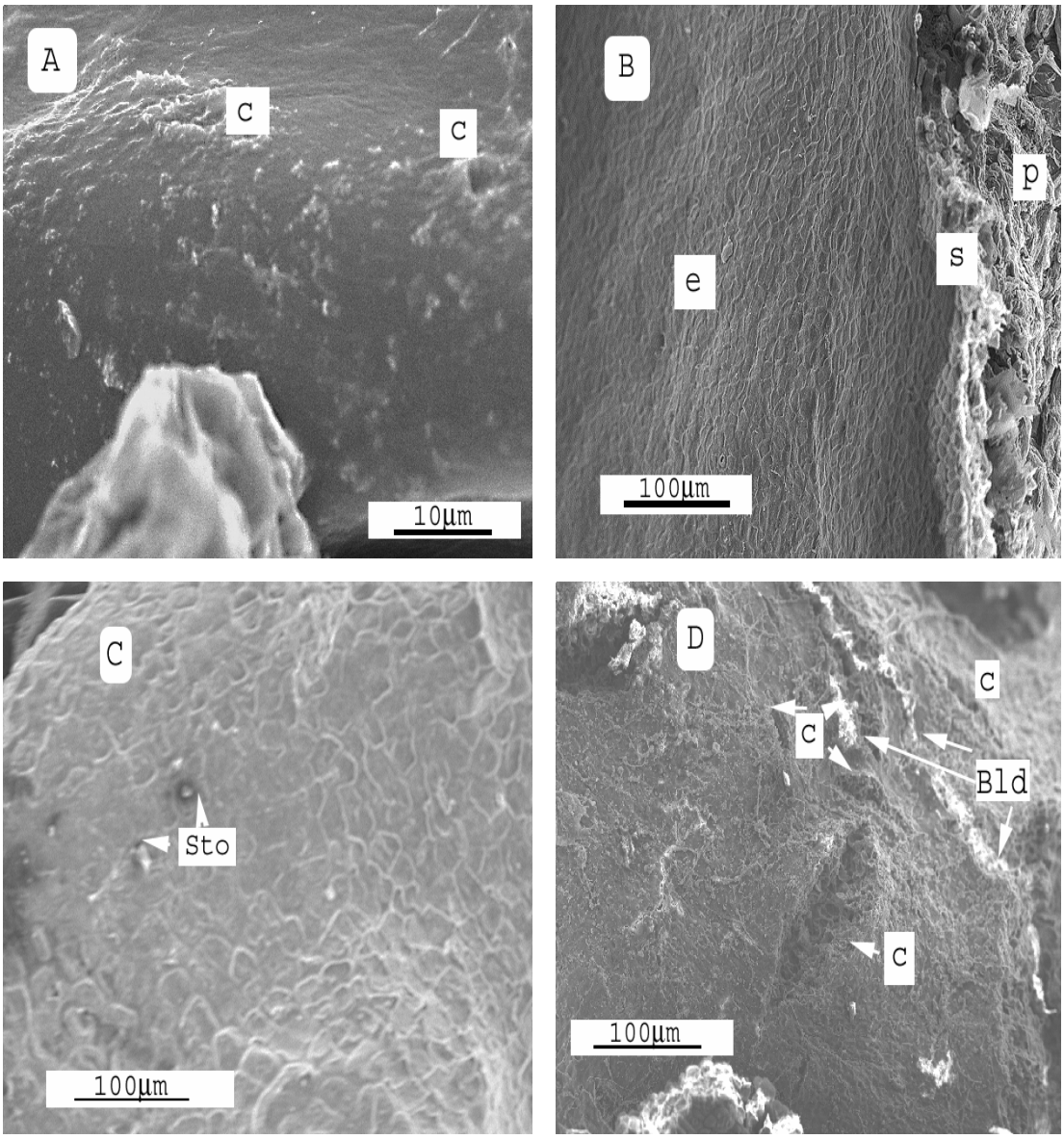

Figure 4-25. SEM photomicrographs of blueberry skin after irradiation treatment (10 days). (A) control= non-irradiated, (B) low dose =1.1 kGy, (C) medium dose =1.6 kGy , (D) high dose =3.2 kGy, (c =cracks , p =parenchyma, bld =bleeding, e =epidermis). Fruits were observed at 15 kV. Bars in (B), (C) and (D) represent 100µm; in (A), 10µm.

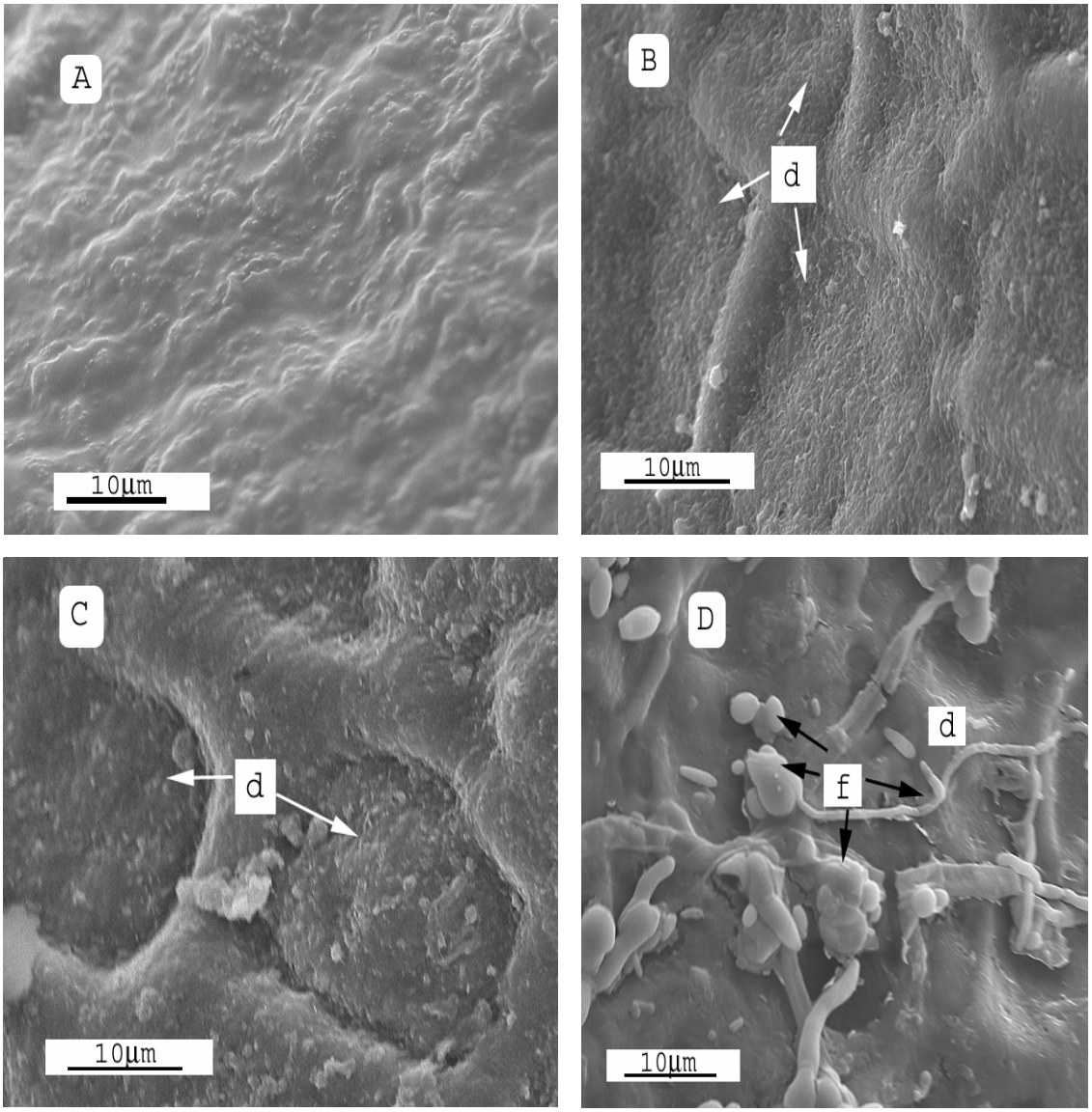

Figure 4-26. SEM photomicrographs of blueberry skin at higher resolution after irradiation treatment (10 days). (A) control =non-irradiated, (B) low dose =1.1 kGy, (C) medium dose =1.6 kGy , (D) high dose =3.2 kGy, (d =depressions, f =fungi). Fruits were observed at 15 kV. Bars represent 10µm.

The changes in structure observed in this study are in agreement with the texture changes monitored in the samples since the firmness of the fruit was significantly ($P>0.05$) reduced at all dosages (section 4.2.1.2). The samples exposed to 3.2 kGy required less shear force than the other treatments. These results may be related to the degradation of cell wall polysaccharides and the solubilization of pectins, cellulose, hemicellulose and starch (Kovacs & Keresztes, 2002; Kader, 1986). In addition, these physiological alterations induced by irradiation involve chemical changes that caused an increase in reduced sugars and respiration rate of treated samples (see sections 4.2.2.6 and 4.2.1.4, respectively).

Some relationship between changes in cell structure and the pigment concentration in the cells has been documented. Allan-Wojtas et al. (2001) related the susceptibility of epidermis cells of blueberry to bleeding with the sizes and arrangement of the cells in the cell wall and their pigment content. Pigmented cells were closer to each other. This is in agreement with the color results in this study where a reduction of color attributes (*a* and *b* values) was observed in samples irradiated at higher doses. This result suggests a stronger effect of irradiation in the cell structure of the samples exposed at such a high dose.

The observation of blueberry epidermis (Figure 4-27) showed shrinkage of the flesh tissue in irradiated samples, especially those subjected to high (3.2 kGy) dose where the cells were more compact and drier (Figure 4-27D). Thus, the cell structure was not preserved. In samples irradiated at a low dose, the presence of empty spaces (voids) (Figure 4-28B) occurred due to tissue shrinkage.

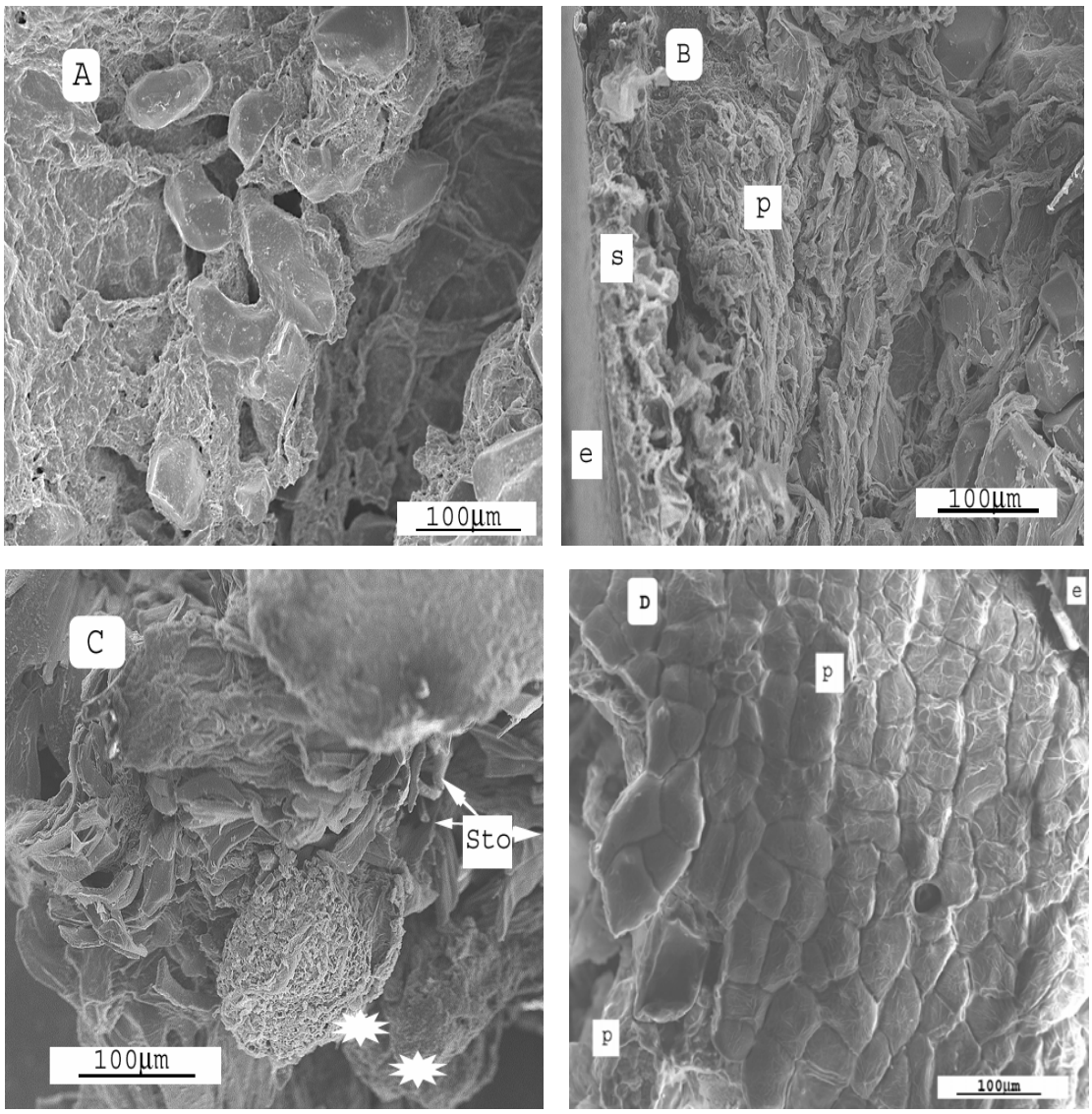

Figure 4-27. SEM photomicrographs of blueberry epidermis after irradiation treatment (10 days). (A) control =non-irradiated, (B) low dose =1.1 kGy, (C) medium dose =1.6 kGy , (D) high dose =3.2 kGy, (c =cracks, p =parenchyma,* =seed, stc =stone cells, e =epidermis). Fruits were observed at 15 kV. Bars represent 100µm.

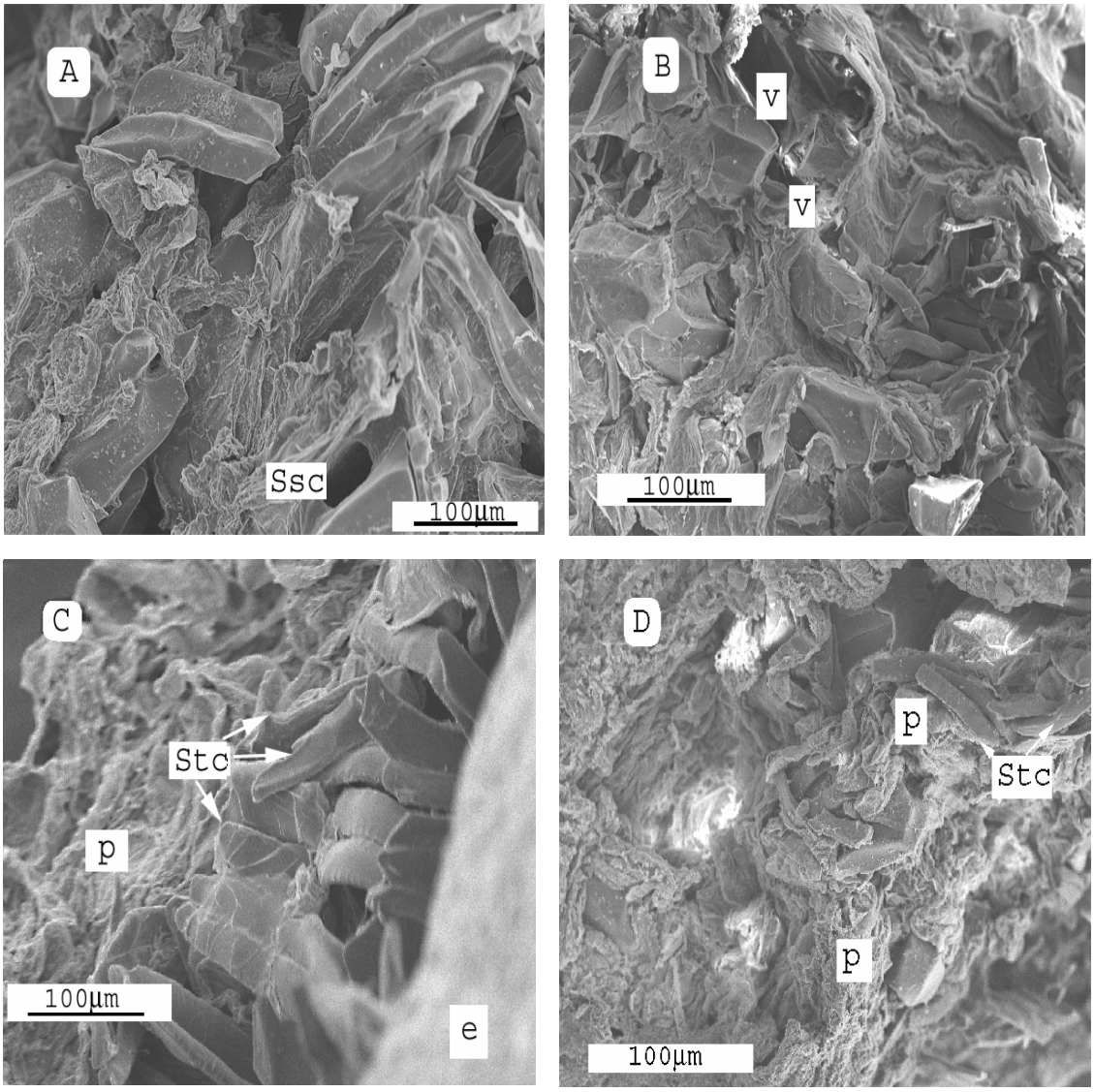

Figure 4-28. SEM photomicrographs of parenchyma cells of blueberry after irradiation treatment (10 days). (A) Control= non-irradiated, (B) low dose =1.1 kGy, (C) medium dose =1.6 kGy, (D) high dose =3.2 kGy, (p= parenchyma, e =epidermis, Ssc =substomal cavities, st =stone cells, v =void). Fruits were observed at 15 kV. Bars represent 100µm.

In the parenchyma cells (p) micrograph, samples irradiated at high dose showed more collapsed cells with loss of shape (Figure 4-28D). In all irradiated samples the arrangement of the stone cells changed and fracture of these cells was observed mainly in samples exposed to medium (1.6 kGy) and high (3.2 kGy) doses. The relative amounts and pattern of stone cells and vascular tissue in the fruit could determine its texture and response to instrumental test (Allan-Wojtas et al., 2001). These results are consistent with the findings from the compression test (toughness and Kramer shear) where the samples irradiated at higher doses were less tough (easier to crush) than the controls and less force was required to cut (shear) the fruits.

4.2.1.4. Respiration rates

Irradiation dose significantly increased ($P>0.05$) the respiration rate of blueberries in all irradiated samples right after irradiation. By the end of the storage time, the CO_2 concentrations in blueberries increased by 14.02% for low (1.1 kGy), 16.54% for medium (1.6 kGy) and 61.15% for high (3.2 kGy) doses, respectively (Table 4-15). Samples exposed to the higher dose had significantly higher CO_2 concentrations than the other treatments. Blueberries are considered non-climacteric fruits with a lack of rising in the respiration rate during ripening. Therefore, the increase in the CO_2 concentrations may be associated with the effect of irradiation on the changes of cell structure and the breakdown of substrate molecules for the respiration process normally present in the cells such as starch, sugars and organic acids.

Higher CO_2 levels in the atmosphere can reduce the respiration rate and also the physiological changes, specifically oxidation, with a beneficial effect of extending the

shelf-life. CO_2 in excess of 5% v/v inhibits the many food spoilage bacteria. This factor could explain the presence of mold observed only on the control samples by the end of the storage (section 4.2.2.1). Therefore, the irradiation at doses up to 3.2 kGy may extend the shelf-life of the fruits.

Table 4-15
Headspace gas (CO_2 in mg /Kg h) concentration for blueberries stored up to 14 days at 5°C

Gas	Day/Dose	Control* (0.0 kGy)	Low (1.1 kGy)	Medium (1.6 kGy)	High (3.2kGy)
CO_2	0	5.18^{ax}(0.41)	7.06^{ay}(0.26)	6.59^{ay}(0.72)	10.34^{az}(0.52)
	3	1.58^{bx}(0.26)	3.12^{by}(0.14)	3.24^{by}(0.82)	4.48^{bz}(0.72)
	7	2.78^{cx}(0.24)	3.29^{by}(0.18)	3.27^{by}(0.12)	4.07^{bz}(0.13)
	14	2.78^{cx}(0.08)	3.17^{bx}(0.02)	3.24^{bx}(0.47)	4.48^{by}(0.21)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
[a-d]Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
[w-z]Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

All samples had significantly ($P>0.05$) lower CO_2 concentrations by the end of the storage (Table 4-15). The decrease in CO_2 production could be associated with the beginning of senescence. Taylor and Brock (1998) reported no significant differences in CO_2 production over storage time of Laetitia plums irradiated at 150, 300 and 400 Gy while for Songold plums some changes were observed over time. In the same study, the

authors found that samples irradiated at higher dose showed the tendency to have elevated CO_2 production.

In summary, the irradiation of blueberries up to 3.1 kGy increases the respiration rate but still the CO_2 concentrations are acceptable (~10 mg/ Kg h) for the shelf-life preservation.

4.2.1.5. Density and specific gravity

No effect of irradiation dose on the specific gravity of the blueberries was observed (Figure 4-29, Table A-8, Appendix A). The value of the unit density of the blueberries ranged between 0.932 and 1.023 g/cc These results suggest no weight loss due to irradiation.

Normally, the specific gravity is an index of fruit maturity. Usually, specific gravity >1.0 means optimum maturity. For blueberries the normal specific gravity (Eq 3-2) ranges between 1.030 and 1.050. In this study, the average specific gravity of the samples was 0.994 suggesting a close but no mature stage of the fruit.

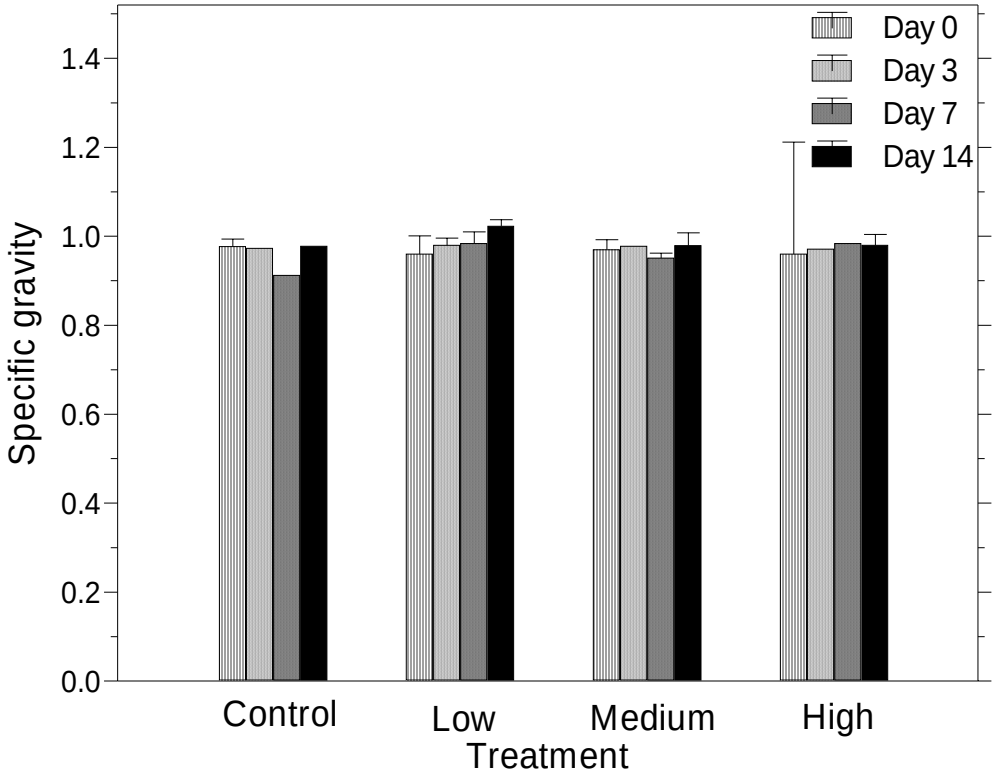

Figure 4-29. Effect of irradiation dose on specific gravity of blueberries stored up to 14 days at 5°C. (Control =non-irradiated, low dose =1.1 kGy, medium dose =1.6 kGy, high dose =3.2 kGy).

The bulk density (Eq 3-3) of the fruits was 0.59 g/cc. The porosity (Eq 3-4) of the samples was 0.39 for controls, 0.40 for 1.1 kGy-treated fruits, 0.40 for 1.6 kGy-treated fruits and 0.40 for 3.2 kGy-treated fruits, respectively. This suggests that the irradiated samples were not more porous than the control fruits. In addition, these results indicated that there is approximately 40% of air between the berries within the tray.

In summary, the exposure of blueberries to irradiation at dose levels up to 3.2 kGy does not affect the unit density and the specific gravity of the fruit.

4.2.2. Chemical properties

4.2.2.1. Moisture content

The moisture content of the blueberries ranged from 79.58% to 81.83% (Table A-9, appendix A). In comparison with the control, no differences in the moisture content of the irradiated blueberries were observed (Figure 4-30).

Throughout storage time, a slight increase of the moisture content of control fruits and samples treated with medium (1.6 kGy) dose was observed on day seven. This increase may be related to the differences in the ripening process of the blueberries. It has been found that there is an increase in moisture content in the early stage of development of berries with a tendency to stabilize as the fruit reaches maturity. (Hulme, 1971). No significant changes were found for samples treated with low and high doses.

Overall, the exposure of blueberries at dose levels up to 3.2 kGy does not affect the moisture content of the fruit.

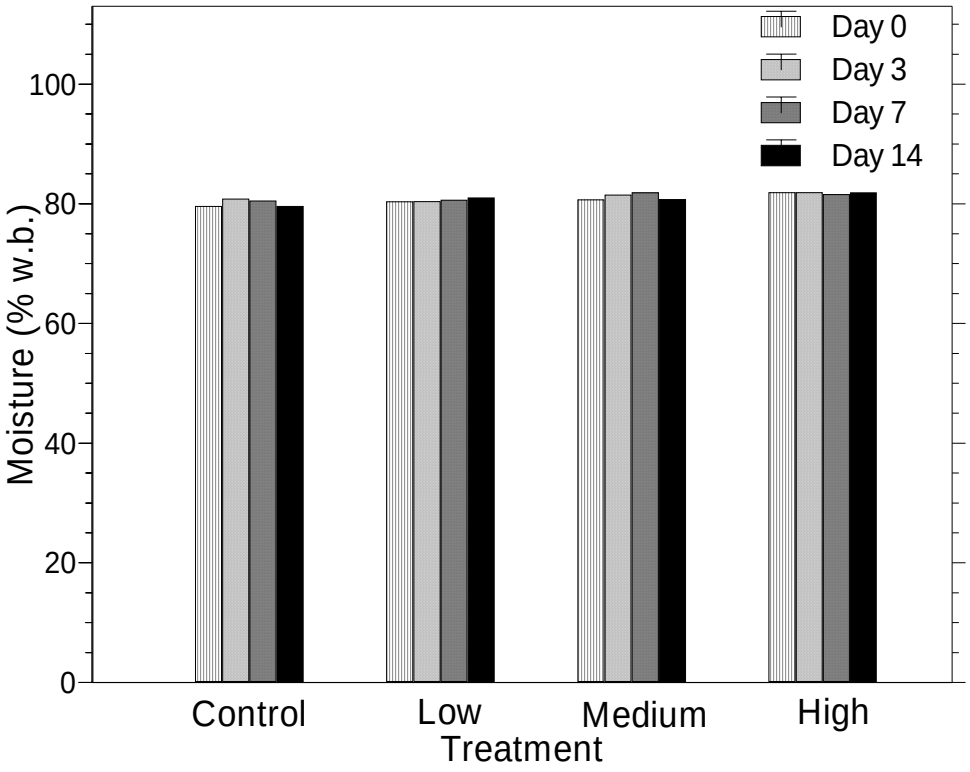

Figure 4-30. Effect of irradiation dose on moisture content (% w.b.) of blueberries stored up to 14 days at 5ºC. (Control =non-irradiated, low dose =1.1 kGy, medium dose =1.6 kGy, high dose =3.2 kGy).

4.2.2.2. Water activity

No effect of irradiation dose on the water activity of the blueberries was observed (Figure 4-31). The water activity of the blueberries ranged between 0.87 and 0.92 (Table A-10, Appendix A). Many yeast and mold are inhibited by water activities between 0.87 and 0.91 (Fennema, 1996). Therefore, in the treated samples the decay of the fruit would be reduced.

Throughout time, no differences were found for control samples. However, in the fruit treated with medium (1.6 kGy) and high (3.6 kGy) doses the water activity increased (0.87-0.91) during storage, but still this change does not affect the quality of the fruit, since it is within the range of mold inhibition. These results are consistent with the content of total soluble solids of the fruit at these doses that presented lower values than those fruit exposed to low dose.

Overall, irradiation of blueberries at doses up to 3.2 kGy does not affect the water activity of the fruit and may increase their shelf-life.

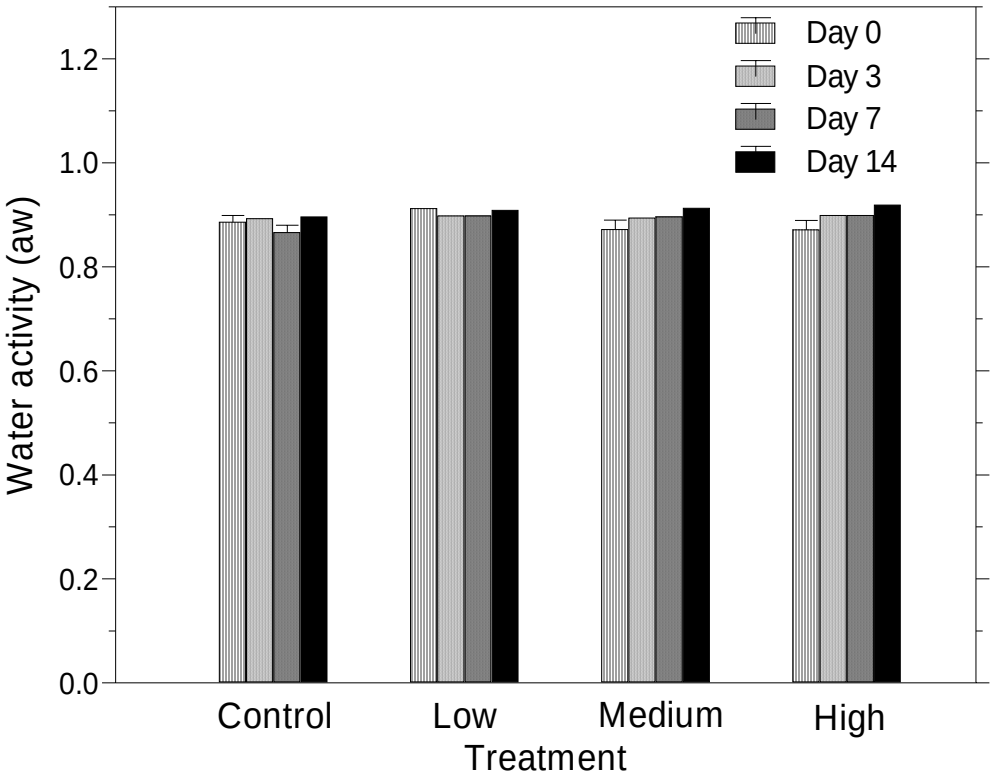

Figure 4-31. Effect of irradiation dose on water activity of blueberries stored up to 14 days at 5ºC. (Control =non-irradiated, low dose =1.1 kGy, medium dose =1.6 kGy, high dose =3.2 kGy).

4.2.2.3. pH

The effect of ionizing radiation on the pH of blueberries only showed a significant (P>0.05) decrease in fruits exposed to high (3.2 kGy) dose at day zero. By the end of the storage (day 14), no differences between the pH of the control and the pH of the irradiated samples were observed (Figure 4-32) (Table A-11). The samples exposed to high dose had the highest pH on day three and seven (3.19 and 3.21, respectively) of storage. These results may be associated with changes in acidity due to differences in the fruit ripening stage. The acid content is considerably greater in the greener young fruit than in the ripe fruit. Although there are differences, the pH of the irradiated samples ranged between the normal values (2.85 -3.49) reported for blueberries (Hulme, 1971).

Similar results were reported by Miller et al. (1994b) who found an increase in the pH (1.55% more) of blueberries irradiated at 2.25 and 3.0 kGy after seven and fourteen days of storage at 1°C. However, Miller and McDonal (1994a) reported no differences among the doses in the pH of sharpblue blueberries irradiated with electron beam at doses up to 1.0 kGy, but a slight increase in pH was found by the end of storage. Later, Miller and McDonal (1996) found no differences in pH (3.5-3.6) values of blueberries when irradiated with gamma rays at 0.5 and 1.0 kGy.

In summary, the exposure of blueberries to irradiation levels up to 3.2 kGy does not affect the pH of the fruit.

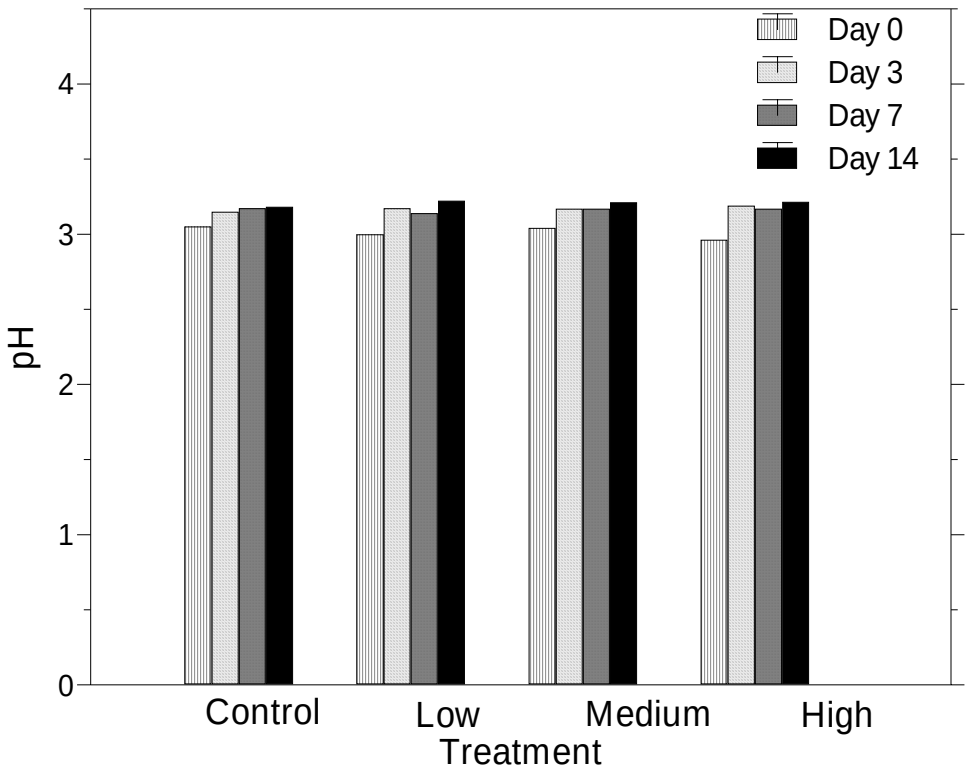

Figure 4-32. Effect of irradiation dose on pH values of blueberries stored up to 14 days at 5ºC. (Control =non-irradiated, low dose =1.1 kGy, medium dose =1.6 kGy, high dose =3.2 kGy).

4.2.2.4. Tritratable acidity

Although the acidity of irradiated samples showed a decreasing trend, only the fruit exposed to high (3.2 kGy) dose presented a significant (P>0.05) decrease in acidity on days three and seven of storage (Table 4-16). These samples had higher pH (3.19) than the other treatments on those days. No changes in acidity levels were observed between the control and the irradiated samples on day twenty-one. Throughout time, the samples exposed to a low (1.1 kGy) dose showed a significant (P>0.05) decrease in acidity on days seven and fourteen. In addition, the samples treated with high (3.2 kGy) dose had a decreased acidity during the entire storage period. Therefore, these fruits with lower acidity (0.55-0.60) would have a sweeter flavor. These differences may be associated with the different degrees of ripening between the samples.

These results are consistent with the lower pH values of the irradiated samples on day zero, and the higher values throughout the storage time. It has been documented that acids regulate cellular pH and may influence the appearance of fruit pigments within the tissue (Seymour, 1993). Yu et al. (1995) reported a decrease in tritratable acidity of strawberry irradiated with electron beam up to 2.0 kGy after two days of storage at 2°C. No effect of gamma irradiation on tritratable acidity of blueberry varieties has been reported (Miller et al., 1994; Miller & McDonal, 1996).

The range of the ratio sugar/acid of the irradiated samples (17.86-24.98) was similar to the ratio of the control samples (18.99-21.53) (Table A-12) suggesting the acceptability of the irradiated fruit.

In summary, the irradiation of blueberries at doses up to 3.2 kGy maintains the acidity levels of the fruit.

Table 4-16
Effect of irradiation dose on tritratable acidity (g citric acid/100g w.b.) of blueberries stored up to 14 days at 5ºC

Dose/Day	Control* (0 kGy)	Low (1.1 kGy)	Medium (1.6 kGy)	High (3.2 kGy)
0	0.73^{ax}(0.05)	0.81^{ax}(0.00)	0.72^{abx}(0.00)	0.76^{ax}(0.07)
3	0.78^{ax}(0.07)	0.74^{abx}(0.00)	0.74^{bx}(0.03)	0.60^{by}(0.02)
7	0.67^{ax}(0.04)	0.66^{bx}(0.01)	0.67^{abx}(0.03)	0.55^{by}(0.02)
14	0.72^{ax}(0.02)	0.69^{bx}(0.06)	0.65^{ax}(0.018)	0.66^{bx}(0.04)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-b}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
$^{x-y}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

4.2.2.5. Total soluble solids

Irradiation had an effect on the total soluble solids (°Brix) of blueberries. Compared with non-irradiated samples, the total soluble solids of all irradiated blueberries decreased significantly ($P<0.05$) with time. However, the fruit treated with the lower dose (1.1 kGy) had the highest values (Table 4-17). These results are consistent with the decrease in the acidity level of the samples irradiated at this dose level (section 4.2.2.2). The acid content of the fruits increases considerably during development and

ripening but it declines rapidly in the later stages of ripening which is accompanied by changes in pH, sugars and soluble solids. The reduction in soluble solids may be due to a delay in ripening induced by irradiation. Through time, a general increase (3.0%-12%) was observed in the content of soluble solids in all samples however, the irradiated samples presented a significant ($P<0.05$) decrease on day seven. This decrease is consistent with the reduction on total sugars for all irradiated samples on that day (see section 4.2.2.6).

Table 4-17
Effect of irradiation dose on soluble solids (°Brix) of blueberries stored up to 14 days at 5ºC

Dose/Day	Control* (0 .0kGy)	Low (1.1 kGy)	Medium (1.6 kGy)	High (3.2 kGy)
0	13.93^{ax}(0.11)	14.43^{ay}(0.12)	13.83^{ax}(0.29)	13.03^{az}(0.15)
3	14.50^{bx}(0.00)	14.92^{by}(0.14)	14.00^{az}(0.00)	14.33^{bx}(0.14)
7	14.92^{cx}(0.14)	14.08^{cy}(0.14)	13.75^{az}(0.00)	13.75^{cz}(0.00)
14	15.50^{dx}(0.00)	14.83^{by}(0.14)	14.75^{by}(0.00)	14.25^{bz}(0.00)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-d}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
$^{x-z}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

Different findings have been reported for the effect of irradiation on this quality parameter. Miller et al. (1994b) found no effect of gamma irradiation (0.75-3.0 kGy) on total soluble solids of rabbiteye blueberry. However, Eaton et al. (1970) reported an increase of soluble solids of blueberry with different responses according with the variety when the fruits were exposed to gamma radiation at levels between 0.10 and 0.5 kGy. In addition, Miller et al. (1995) reported a slight increase in total soluble solids of electron beam irradiated sharpblue blueberries at doses up to 1.0 kGy by the end of storage, but the authors found not differences between the doses.

In summary, irradiation at doses higher than 1.1 kGy causes a significant decrease in the total soluble solids (°Brix) content of blueberries.

4.2.2.6. Sugars

4.2.2.6.1. Total sugars

No differences in the total sugars content of irradiated and non-irradiated samples were observed on day zero. However, all irradiated samples had a significant ($P<0.05$) decrease by the end of the storage (Table 4-18). Throughout storage time, the samples exposed at high dose (3.2 kGy) had the higher content (11.65g glucose/100g wb) on day three. On day seven, the control and the samples treated with medium dose had the higher sugars content (11.85 and 10.15g glucose/100g wb., respectively). These results are consistent with the effect of irradiation on the reduction of soluble solids previously discussed (section 4.2.2.4).

Foa et al. (1980) reported a decrease in the total polysaccharides of cherries when subjected to gamma irradiation at dose levels between 0.1 and 1.0 kGy.

4.2.2.6.2. Reducing sugars

Irradiation dose induced a decrease in reducing sugars content of the fruit (Table 4-18). All irradiated samples had lower contents than the control. This decrease was 9.0%, 9.22% and 9.57% for samples exposed to low (1.1 kGy), medium (1.6 kGy) and high (3.6 kGy) doses, respectively, by the end of the storage. However, among the irradiated samples the fruit treated with low dose (1.1 kGy) had the higher concentrations on day three and fourteen. The decrease in reducing sugars could be associated with the effect of irradiation on delaying ripeness of the fruit.

In summary, the exposure of blueberries to irradiation doses up to 3.2 kGy decreases the total and reducing sugars of the fruit. However, the exposure to dose levels up to 1.6 kGy maintains an acceptable sugar content of the fruit.

Table 4-18
Effect of irradiation dose on total (g glucose/100g w.b.) and reducing sugars (g glucose/100g w.b.) of blueberries stored up to 14 days at 5ºC

Sugar	Dose/Day	Control* (0.0 kGy)	Low (1.1 kGy)	Medium (1.6 kGy)	High (3.2 kGy)
Total	0	11.55^{ax}(0.34)	11.87^{ax}(0.12)	11.87^{ax}(1.13)	10.50^{ax}(1.00)
(gGlucose/100g w.b.)	3	11.22^{ax}(0.99)	11.46^{ax}(1.38)	9.34^{bx}(0.57)	11.65^{ax}(1.69)
	7	11.87^{ax}(0.57)	9.77^{by}(0.11)	10.15^{by}(0.11)	9.87^{ay}(0.07)
	14	12.17^{ax}(0.57)	9.19^{by}(0.96)	9.92^{by}(0.25)	10.11^{ay}(0.41)
Reducing	0	13.94^{ax}(1.13)	12.78^{axy}(0.31)	11.87^{ayz}(0.52)	11.08^{az}(1.20)
(gGlucose/100g w.b.)	3	12.21^{bx}(0.79)	11.93^{bx}(0.58)	11.16^{abx}(0.64)	11.15^{ax}(0.32)
	7	10.70^{bx}(1.31)	9.87^{cx}(0.56)	10.22^{bx}(0.85)	9.55^{ax}(1.06)
	14	11.66^{bx}(0.53)	10.34^{cy}(0.23)	10.29^{by}(0.22)	10.16^{ay}(0.60)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-c}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
$^{x-y}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

4.2.2.7. Total phenolics and antioxidant activity index

The total phenolics content in irradiated blueberries was higher than the content of phenolics in the control fruit after irradiation treatment (Figure 4-33). By the end of the storage, the samples treated with 1.1 kGy had the higher concentrations but a significant ($P<0.05$) decrease was observed in samples irradiated at high dose. On day three, the samples exposed to low and medium doses had the higher concentrations. However, within the irradiated samples, those treated with low dose (1.1 kGy) had the higher phenolics content (10.43% more) (Table A-13, appendix A). The accumulation of phenolic compounds after irradiation is associated with different factors such as the increase in solubility due to the modifications in cell structures, the increase in extractability, and the variation between maturity levels among the samples. The increase in phenolics compounds has an important effect because they are considered powerful antioxidants and anti-inflammatory agents. In addition, they are associated with the bitter or astringent flavor of the fruit. Therefore, no loss of these properties was induced by irradiation treatment.

These results were accompanied by changes in the antioxidant activity index and were consistent when among the treatments, samples irradiated at low dose showed the higher percentage of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reduction. The samples exposed to medium dose had the highest percentage of antioxidant activity index on day zero. This increase corresponded to higher concentration of phenolics on this day. High correlation ($r^2= 0.97$) was found between the phenolic compounds and the DPPH percentage of the samples exposed at medium and high doses, indicating their high

antioxidant activity. The increase in DPPH is related to the decrease of oxidative reactions like the degradation of hydroperoxides from lipid oxidation that can affect the quality of the fruit.

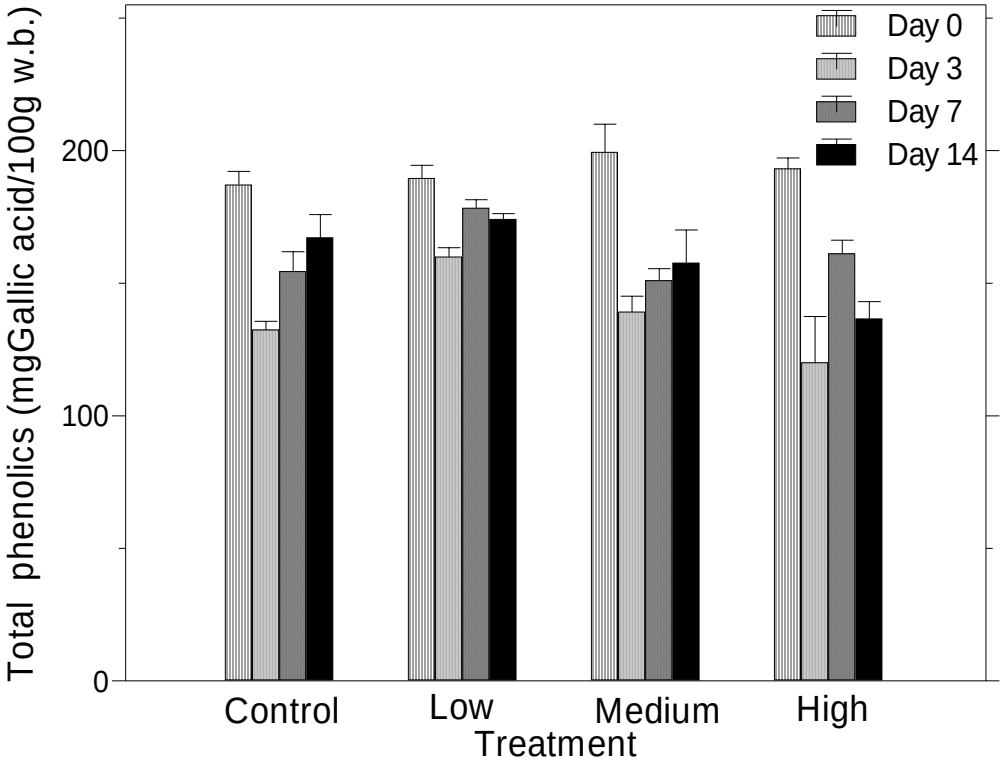

Figure 4-33. Effect of irradiation dose on total phenolics (mg gallic acid/100g w.b.) of blueberries stored up to 14 days at 5°C. (Control=non-irradiated, low dose=1.1 kGy, medium dose=1.6 kGy, high dose=3.2 kGy).

Throughout time, a reduction of the antioxidant activity index for all treatments was detected (Table A-13, appendix A) (Figure 4-34).

Lees and Francis (1972) reported that gamma irradiation at levels of 1.5 and 3.0 kGy showed an increase in flavonol pigments in full-red cranberries but when the berries were less colored the synthesis of flavonoids was reduced when storage at 34°F and 40°F. However, Breitfellner et al. (2003) found different behavior in phenolics compounds of strawberries exposed to gamma radiation at dose levels of 1.0 to 6.0 kGy. The authors reported the increase of some phenolics acids, the decrease of some flavonoids, and no effect of irradiation in other phenolic compounds.

In summary, the exposure of blueberries at dose levels up to 1.6 kGy may enhances the phenolics content of the fruits and maintains the nutritional and flavor properties of the fruits.

Similar findings were obtained by Reyes and Cisneros (2005) who found a slight increase in phenolics during the storage time, but within the irradiated samples blueberries exposed to low dose (irradiated in the same conditions used in this study) presented higher levels of total phenolics. In addition, the authors found that the volatile profile did not show significant changes, except for anthocyanins where its accumulation was induced by irradiation. The antioxidant activity was higher for samples irradiated at high dose by the end of the storage. The authors observed the same trend for the PAL activity.

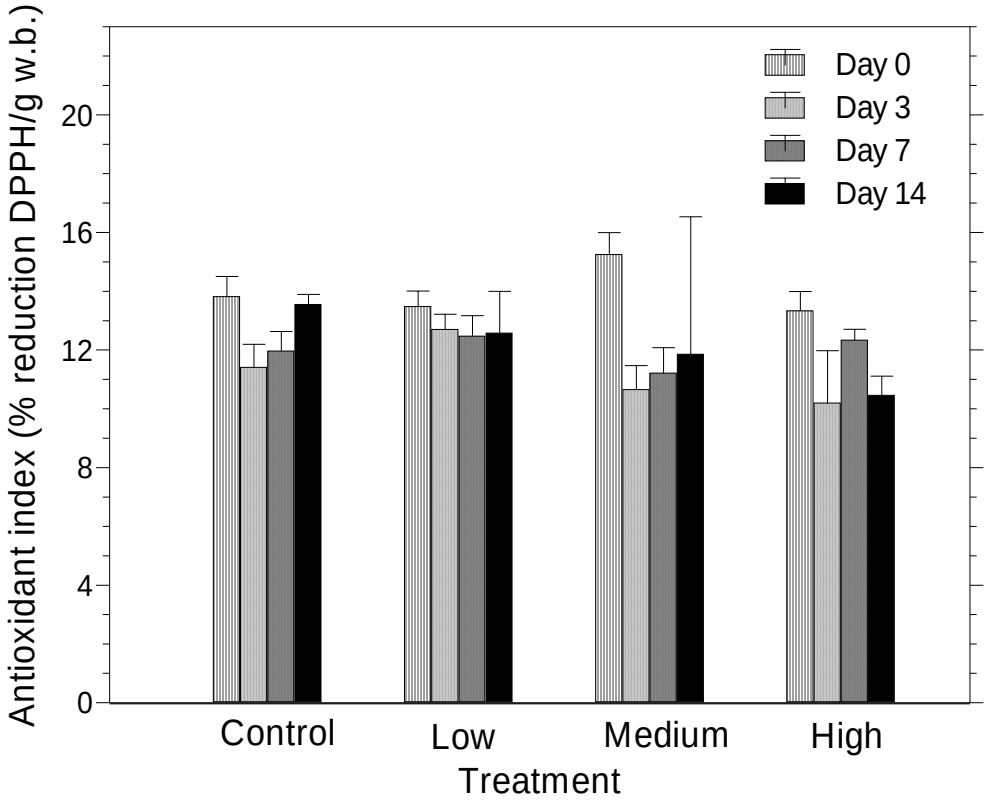

Figure 4-34. Effect of irradiation dose on antioxidant index (% reduction of DPPH/g w.b.) of blueberries stored up to 14 days at 5°C. (Control =non-irradiated, low dose =1.1 kGy, medium dose =1.6 kGy, high dose =3.2 kGy).

4.2.2.8. Ascorbic acid

Irradiation had a significant ($P<0.05$) effect on the ascorbic acid content of blueberries. Overall, compared with non-irradiated samples, a reduction (28.15%) of the ascorbic acid was observed by day three in all irradiated samples. By the end of the storage, the samples treated with low (1.1 kGy) and medium doses had the highest ascorbic acid concentrations. The samples treated with high dose (3.2 kGy) had the lowest ascorbic acid content (Table 4-19). These results are consistent with the increase in respiration rate in samples exposed to high dose (3.2 kGy) since ascorbic acid is one of the substrates available for respiration. They were also consistent with the elevated pH values in the samples treated with the higher dose indicating a reduction in the acid content of the fruit.

Similar results were obtained by Reyes and Cisneros (2005) who determined a decrease of the ascorbic acid content of the blueberries irradiated under the same conditions used in this study. By the end of storage, this decrease was 23.39% for control, 15.19% for low dose, 36.84% medium dose and 21.21% for high dose. However, between the treatments, just a reduction of 6-9% was observed in fruits treated at high dose.

In summary, the exposure of blueberries up to dose levels of 3.2 kGy decreases the ascorbic acid concentration but at doses up to 1.6 kGy the reduction is minimized to acceptable levels.

Table 4-19
Effect of irradiation dose on vitamin C (mg ascorbic acid/100g w.b.) content of blueberries stored up to 14 days at 5°C

Dose/Day	Control* (0.0 kGy)	Low (1.1 kGy)	Medium (1.6 kGy)	High (3.2 kGy)
3	14.01^{ax}(1.37)	11.56^{ay}(1.67)	9.26^{ay}(0.67)	9.36^{ay}(1.09)
7	10.09^{bx}(0.82)	7.83^{bx}(1.18)	12.66^{ay}(1.25)	9.68^{bax}(1.14)
14	9.93^{bx}(0.51)	12.51^{ax}(1.25)	12.86^{ax}(2.21)	9.160^{ax}(0.97)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-b}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
$^{x-y}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

4.2.2.9. Volatiles

The volatile constituents evaluated by head space are shown in Table 4-20.The flavor compounds that were determined included esters, hydrocarbons, aldehydes, alcohols and ketones. Hirvi and Honkanen (1983) found benzyl alcohol as the main volatile compound in bog blueberry, bilberry and high-bush blueberries. However, in this study the main compound in blueberry volatiles was (E) - 2-hexenal, which accounted for 45.24% and 23.9% of the total volatiles for days zero and twenty first, respectively.

Table 4-20
Mean pick areas (%) of fifteen volatile compounds recovered by solid phase microextraction, GC-MS of blueberries stored up to 14 days at 5°C

	Day 0				Day 14			
Volatiles/Dose	**Control (0.0 kGy)**	**Low (1.1 kGy)**	**Medium (1.6 kGy)**	**High (3.2 kGy)**	**Control (0.0 kGy)**	**Low (1.1 kGy)**	**Medium (1.6 kGy)**	**High (3.2 kGy)**
Acethaldehyde	0.04^{ax}(0.00)	0.09^{ax}(0.01)	0.13^{ax}(0.01)	Bdl	0.56^{ax}(0.07)	0.16^{ax}(0.02)	0.87^{ax}(0.01)	bdl
n-hexane	0.74^{ax}(0.01)	0.56^{ax}(0.07)	0.15^{ax}(0.21)	Bdl	1.43^{ax}(0.02)	2.24^{ax}(0.03)	0.83^{ax}(0.01)	bdl
Ethanol	1.15^{ax}(0.16)	1.77^{ax}(0.02)	0.98^{ax}(0.01)	0.25^{ax}(0.03)	1.44^{ax}(0.02)	7.67^{ax}(1.08)	6.40^{ax}(0.09)	0.41^{ax}(0.05)
n-hexanal	49.86^{ax}(7.05)	50.0^{ax}(7.71)	30.11^{ax}(4.25)	20.94^{ax}(2.96)	50.00^{ax}(7.07)	32.70^{ax}(4.62)	50.00^{ax}(7.07)	9.22^{ax}(1.30)
2-methyl-4 pentenal	1.31^{ax}(0.18)	1.81^{ax}(0.26)	0.22^{ax}(0.02)	1.07^{ax}(0.15)	2.83^{ax}(0.19)	2.66^{ax}(0.01)	5.08^{bx}(0.01)	31.63^{by}(1.49)
(E)-2-hexenal	100^{ax}(0.01)	97.54^{ax}(0.01)	100^{ax}(0.01)	100^{ax}(0.01)	86.90^{ax}(0.01)	90.63^{ax}(0.01)	95.98^{ax}(0.01)	100^{ax}(0.01)
Delta-3-carene	2.27^{ax}(0.03)	1.76^{ax}(0.28)	1.49^{ax}(0.21)	0.48^{ax}(0.06)	1.55^{ax}(0.21)	4.89^{ax}(0.69)	7.08^{ax}(1.00)	0.39^{ax}(0.05)
1-hexanol	3.37^{ax}(0.52)	1.80^{ax}(0.22)	1.98^{ax}(0.28)	2.00^{ax}(0.28)	11.40^{ax}(1.61)	28.47^{bx}(4.02)	32.88^{bx}(4.65)	11.13^{ax}(1.57)
Limonene	0.54^{ax}(0.07)	0.55^{ax}(0.08)	0.81^{ax}(0.01)	0.85^{ax}(0.12)	1.18^{ax}(0.16)	7.74^{ax}(1.094)	11.80^{bx}(1.66)	1.12^{ax}(0.59)
2-hexen-1-ol	6.41^{ax}(0.90)	1.67^{ax}(0.23)	2.25^{ax}(0.38)	2.31^{ax}(0.32)	9.52^{ax}(1.34)	29.93^{bx}(4.23)	26.33^{bx}(3.72)	0.70^{ay}(0.09)
Linalool oxide	0.21^{ax}(0.03)	0.18^{ax}(0.02)	0.19^{ax}(0.02)	0.39^{ax}(0.01)	1.20^{ax}(0.01)	1.55^{ax}(0.02)	1.45^{ax}(0.02)	bdl
Benzaldehyde	0.47^{ax}(0.06)	0.55^{ax}(0.07)	0.57^{ax}(0.08)	0.19^{ax}(0.27)	1.40^{ax}(0.09)	9.35^{by}(1.32)	13.85^{by}(1.95)	bdl
Linalool	0.48^{ax}(0.06)	0.75^{ax}(0.01)	1.13^{ax}(0.02)	1.19^{ax}(0.16)	1.23^{ax}(0.13)	3.03^{ax}(0.04)	5.98^{axy}(084)	13.14^{by}(1.85)
Alpha-terpinol	2.42^{ax}(0.34)	3.30^{ax}(0.46)	2.83^{ax}(0.40)	1.30^{ax}(0.83)	2.11^{ax}(0.28)	50.00^{by}(7.07)	39.37^{by}(5.56)	3.39^{ax}(0.47)
Beta-myrcene	0.84^{ax}(0.11)	1.43^{ax}(0.20)	1.26^{ax}(1.78)	0.73^{ax}(0.01)	3.80^{ax}(0.53)	16.88^{by}(2.37)	20.71^{by}(2.92)	3.56^{ax}(0.50)

Bdl= below detection limit. Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation. $^{a-b}$Means within days which are not followed by a common superscript letter are significantly different ($P<0.05$). $^{x-y}$Means within treatments which are not followed by a common superscript letter are significantly different ($P<0.05$)

Other compounds like n-hexenal, linalool, and 2-hexen-1-ol were significantly predominant in the flavor of blueberry. (E)- 2-hexenal, (E)-2-hexenol and linalool have been associated with the sensory characteristic flavor of blueberries (Simon et al., 1996; Parliment & Kolor, 1975).

No significant changes in the volatiles profile was observed after irradiation treatment (day 0). Only a slight reduction on the acethaldehyde concentration was detected in the samples treated with high dose (3.2 kGy) (Figure 4-35). The main differences were found after storage up to 14 days. Some of these compounds increased and others decreased with dose. The evaluation of the chromatograph (Figure 4-36) shows the reduction of compounds such as acethaldehyde and trans-caryophyllene at high dose. Other compounds like linalool had an increase of 3.8%, 9.6% in samples treated with medium and high doses, respectively; on day twenty-one. Alcohols such as 2-hexen-1-ol, had ($P<0.05$) significant increase in samples treated with low and medium doses by the end of the storage time, however, the concentration of this compound was less in samples irradiated at high dose. This reduction may be associated with the inhibition of ripening by irradiation. A similar trend was observed for compounds such as alpha terpinol, delta-3- carene and beta-myrcene with the samples treated at low and medium doses with higher concentrations than the other treatments on days zero and twenty-one. However, the fruit exposed to high dose had significantly higher concentration of 2-methyl-4 pentenal and linalool on day 14. These results are consistent with the sensory evaluation of aroma where the samples treated with the higher dose were rated better than the other treatments and the samples irradiated at medium dose had acceptable aroma.

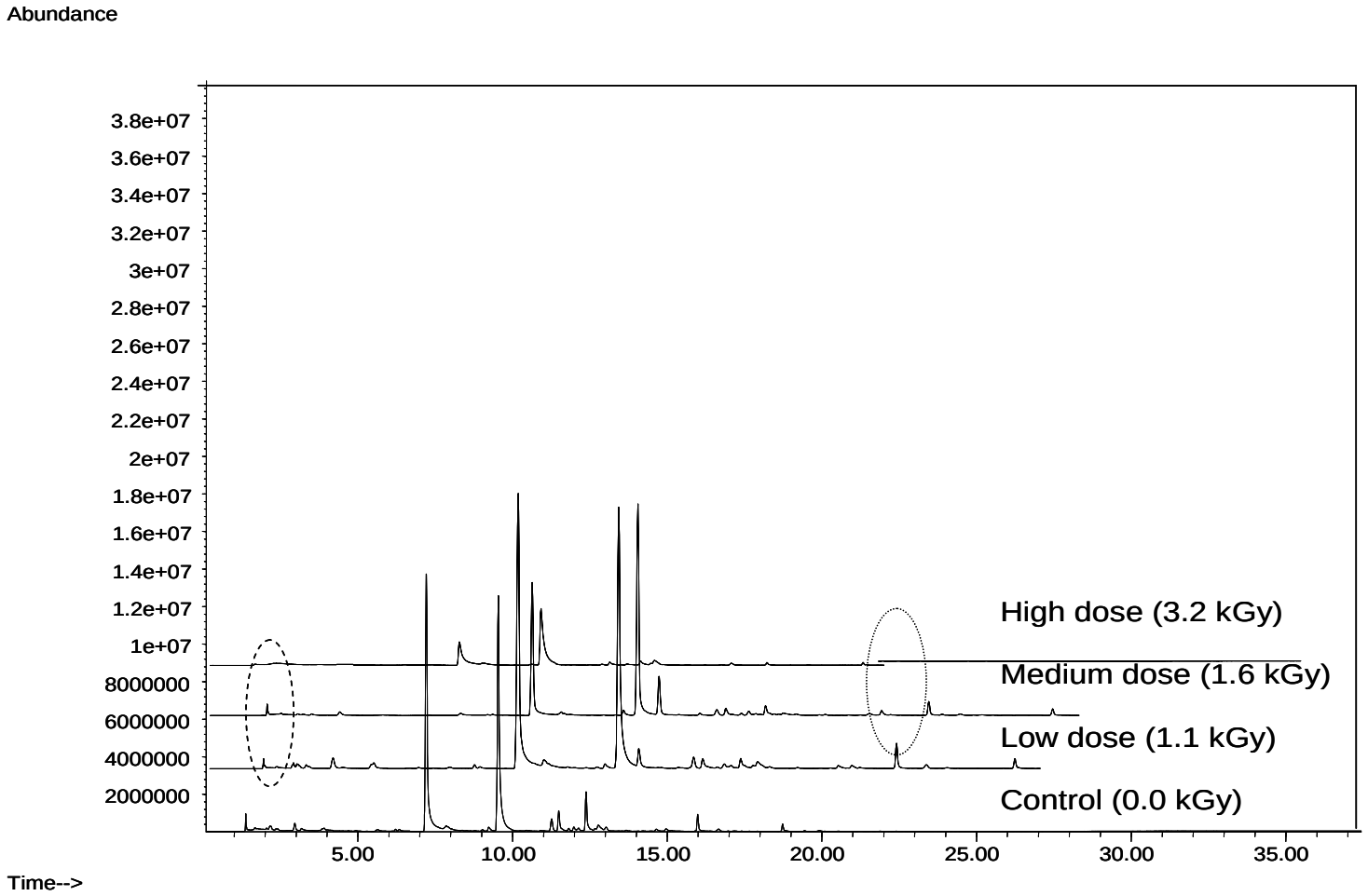

Figure 4-35. Gas chromatography/ mass spectrometry of head space volatiles extracted from irradiated and non-irradiated blueberries (day 0). Dotted lines indicate the main differences.

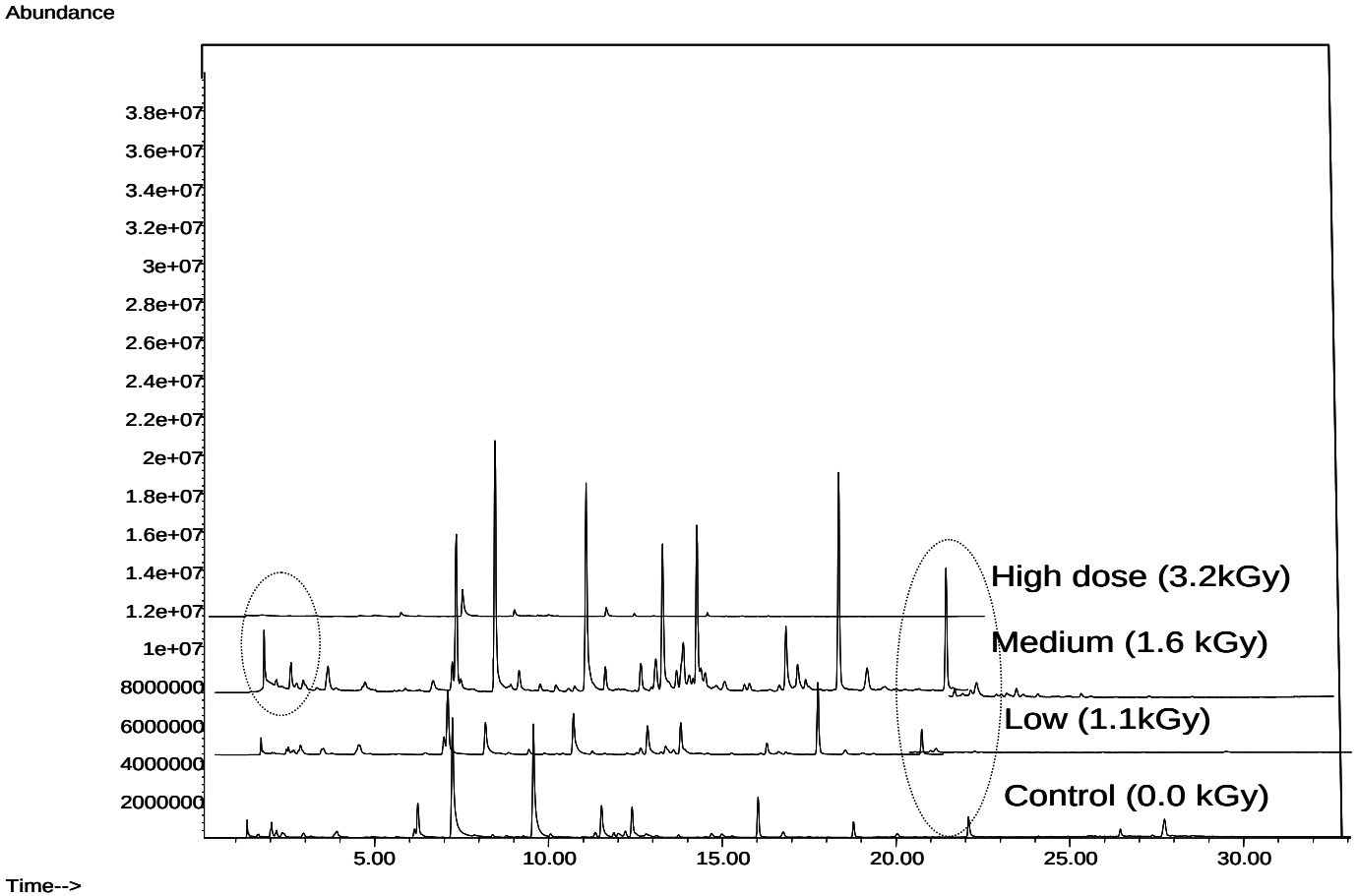

Figure 4-36. Gas chromatography/ mass spectrometry of head space volatiles extracted from irradiated and non-irradiated blueberries (day 14). Dotted lines indicate the main differences.

The production or the reduction of these compounds may be associated with the fruit maturity. Simon et al. (1996) reported an increase in concentration of volatiles as the fruit ripened. Horvat and Senter (1985) reported that during ripening of rabbiteye blueberries the concentration of low molecular weight volatiles tended to decrease while the higher molecular weight increased. The compounds trans-2-hexenal, trans-2-hexenol, α-terpineol, and β-caryophyllene all decrease in concentration as fruit progressed from green to midripe to fully ripe. However, linalool and geraniol were equal or greater in midripe and ripe than in green fruit.

Blakesley et al. (1979) did not find any significant differences among the volatile profiles of irradiated (2.0 kGy gamma rays) and non-irradiated strawberry pulp. Fan & Mattheis (2001) found an inhibition of volatiles in apple when the fruit was treated with 1- methycyclopropene and exposed to gamma radiation doses of 0.88 and 1.32 kGy. However, a consistent trend was not found when the samples were irradiated at 0.44 kGy.

It seems like the effect of irradiation on the aroma of blueberries depends on the dose but in general, the exposure of blueberries to irradiation levels up to 3.2 kGy enhances the production of the volatile compounds that characterize the aroma of the fruits.

4.2.2.10. Tannins

In general, the tannin content of blueberries increased with the irradiation treatment. The fruits treated with low (1.1 kGy) and high (3.2 kGy) doses had the higher concentrations of tannins after irradiation treatment and also by the end of the storage (Table 4-21). A significant ($P<0.05$) increase (56.48%) in tannins content was observed in samples exposed to medium (1.6 kGy) dose by the seventh day of storage. These results were consistent with the increase in the total phenolics observed for irradiated samples (section 4.2.2.7) and also with the effect on color where some modifications were observed for redness (*a*) values (section 4.2.2.1) of the irradiated fruits.

Referring to the storage time, the tannin content in all samples showed a decreasing trend. The differences became significant ($P<0.05$) on days seven and fourteen for fruits treated with low dose (1.1 kGy), on days three and fourteen for samples exposed to high dose (3.2 kGy), and on day fourteen for samples irradiated at medium dose (1.6 kGy). This finding may be related to the maturity stage of the samples. According to Hulme (1971) there are considerable changes in phenolic compounds as fruit mature and this are closely linked with the oxidative enzyme system.

These results are in agreement with the findings of Reyes and Cisneros (2005), who found changes in the total anthocyanin content of blueberries (irradiated at the same conditions used in this study) during the storage. In addition, an increase was detected in samples irradiated at high dose (3.2 kGy). The authors found variation in the anthocyanins profile of the irradiated samples.

Large variations of tannins in fruits have been reported according to the degree of maturation and also with their distribution on the fruit (Macheix et al., 1990). Lees and Francis (1972) showed that gamma irradiation at levels of 1.5 and 3.0 kGy had a beneficial effect on the pigmentation of full mature and full-red cranberries when stored at 34° and 40°F by causing more rapid rate of anthocyanin synthesis.

Table 4-21
Effect of irradiation dose on tannin content (mg catechin/100g w.b.) of blueberries stored up to 14 days at 5°C

Day/Dose	Control* (0.0 kGy)	Low (1.1 kGy)	Medium (1.6 kGy)	High (3.2 kGy)
0	20.66^{ax}(7.68)	26.08^{ax}(6.51)	19.87^{ax}(5.68)	37.08^{ay}(5.94)
3	24.66^{ax}(3.61)	26.18^{ax}(2.82)	26.08^{ax}(2.52)	17.51^{bx}(6.13)
7	11.20^{ax}(0.66)	14.39^{bx}(2.12)	25.73^{ay}(1.65)	29.95^{az}(1.14)
14	15.09^{axy}(1.30)	15.71^{bx}(1.82)	13.28^{bxy}(0.94)	18.86^{by}(2.90)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
$^{a-b}$Means within a row which are not followed by a common superscript letter are significantly different (P<0.05).
$^{x-z}$Means within a column which are not followed by a common superscript letter are significantly different (P<0.05).

4.2.3. Sensory evaluation

The sensorial evaluation of blueberries showed significant differences (P<0.005) between the acceptability of the irradiated and the non-irradiated samples (Table 4-22).

4.2.3.1. Overall quality

The control fruit had better scores (1.7-2.7) than the irradiated samples. However, within the irradiated fruits, samples exposed to a medium dose (1.6 kGy) were the most accepted while the fruits treated with a high dose (3.2 kGy) were less acceptable to the panelists.

4.2.3.2. Color

The objective color measurements presented differences among the treatments and these variations in the color of blueberries were detected by the panelists. A significant ($P<0.05$) preference was observed for control samples on day zero. However, by days three and seven the fruits treated with low and medium doses received scores closer to the control. The samples exposed to high dose (3.2 kGy) rated consistently higher than the other treatments but still between the acceptance limit (1-3). These results are in agreement with the objective evaluation of color where the samples irradiated at the higher dose had lower values of color attributes due to the differences in the anthocyanin content and in the fruit maturity levels. A correlation factor was determined between the redness (*a* values) previously measured in the samples and the values of the sensory evaluation of color. The higher association was observed for control fruits ($r = 0.79$).

Table 4-22
Sensory attributes (overall quality, color, texture and aroma) of irradiated blueberries stored up to 14 days at 5°C

Overall quality

Day	Control* (0.0 kGy)	Low dose (1.1 kGy)	Medium dose (1.6kGy)	High dose (3.2 kGy)
0	1.76^{ax}(0.82)	2.13^{axy}(0.87)	2.37^{ay}(0.91)	2.91^{az}(1.03)
3	2.26^{bx}(0.88)	2.72^{by}(1.01)	2.14^{ax}(0.77)	2.74^{ay}(0.89)
7	2.54^{bcx}(1.13)	2.54^{abx}(1.13)	2.54^{ax}(1.00)	3.17^{ay}(1.15)
14	2.78^{cx}(1.03)	2.14^{ay}(1.16)	2.38^{axy}(0.86)	3.20^{az}(3.20)

Color

Day	Control* (0.0 kGy)	Low dose (1.1 kGy)	Medium dose (1.6kGy)	High dose (3.2 kGy)
0	1.61^{ax}(0.82)	1.83^{axy}(0.88)	2.25^{ayz}(0.88)	2.38^{az}(1.02)
3	1.98^{abx}(0.92)	2.20^{abx}(0.78)	1.93^{ax}(0.78)	2.26^{ax}(1.00)
7	2.27^{bx}(0.98)	2.54^{bx}(1.09)	2.37^{ax}(0.98)	2.78^{abx}(1.12)
14	2.78^{cx}(1.13)	2.06^{ay}(1.00)	2.22^{ay}(0.85)	2.98^{bx}(1.23)

Texture

Day	Control* (0.0 kGy)	Low dose (1.1 kGy)	Medium dose (1.6kGy)	High dose (3.2 kGy)
0	1.94^{ax}(0.85)	2.33^{axy}(0.98)	2.60^{ay}(0.74)	3.58^{az}(1.03)
3	2.52^{bx}(0.99)	3.44^{by}(1.16)	2.39^{ax}(1.03)	3.14^{ay}(1.08)
7	2.52^{bx}(1.08)	2.92^{cx}(1.18)	2.60^{ax}(1.13)	3.40^{ay}(1.24)
14	3.38^{cx}(0.99)	2.69^{acy}(1.29)	2.59^{ay}(0.95)	3.34^{ax}(1.29)

Aroma

Day	Control* (0.0 kGy)	Low dose (1.1 kGy)	Medium dose (1.6kGy)	High dose (3.2 kGy)
0	3.23^{ax}(1.13)	3.36^{ax}(0.90)	3.51^{ax}(0.74)	3.13^{ax}(0.96)
3	3.18^{ax}(1.10)	3.30^{ax}(1.14)	3.14^{abx}(0.96)	3.08^{ax}(0.97)
7	3.23^{ax}(1.05)	2.98^{ax}(1.00)	2.96^{bx}(1.04)	3.17^{ax}(1.20)
14	3.42^{ax}(0.75)	3.10^{ax}(1.18)	3.22^{abx}(0.96)	3.08^{ax}(0.84)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
[a-c]Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).
[x-z]Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).On the hedonic scale for overall quality and color a score of 1= like extremely, 3= neither like nor dislike, 5=dislike extremely; for texture a score of 1= firm, 3= somewhat firm-soft, 5= soft; for aroma a score of 1= strong, 3= moderate, 5= none (see appendix A-A).

4.2.3.2. Texture

The sensory results indicated differences for the texture acceptance of the irradiated and the non-irradiated samples. The control samples had significantly lower scores than the irradiated samples on days zero and seven; however, the low and medium dose samples received better scores (more firm at $P<0.05$) than the control on day fourteen. The higher scores (more soft at $P<0.05$) were given to those samples exposed to higher dose (3.2 kGy) at days zero, three and seven, indicating less acceptability of the fruits. These results are in agreement with the objective evaluation of texture where the samples irradiated at high dose required less force to shear and were also less tough than the other samples. In addition, the changes in the cell structure were more severe at this dose level (section 4.21.3). A negative correlation between the objective measure of texture and the sensory evaluation was found. This suggests that higher values of shear force are related with very low values of the hedonic scale. The correlation factors between these variables were: -0.27 for control, 0.35 for samples treated at low dose, -.62 for medium dose and, -0.45 for samples irradiated at high dose.

4.2.3.3. Aroma

No significant differences in the average score of aroma between the control and the irradiated samples were detected by the panelists. However, the samples treated with a high dose (3.2 kGy) were rated with better scores (moderate aroma) on days zero, seven and fourteen. In addition, the samples exposed to medium dose were more acceptable on day seven. Different results were found in the volatile analysis of the samples (section 4.2.2.9) that indicated a decrease of some compounds in samples

exposed to high dose. It is possible that the difference in these compounds was not enough to affect the aroma of the fruits or that the panelist had difficulties in evaluating the blueberry aroma.

Miller et al. (1994) reported that the subjective evaluation of texture of blueberries irradiated with gamma rays at doses of 0.75, 1.5, 2.25 and 3.0 kGy showed that firmness was reduced with irradiation after seven days of storage at 1°C. They also indicated increase in internal damage of fruits irradiated at 2.25 and 3.0 kGy. In the same study, flavor was rated unacceptable at doses above 1.5 kGy. Yu et al. (1995) evaluated the sensory quality of strawberries exposed to electron beam irradiation at dosages of 0.5, 1.0 and 2.0 kGy, they found a decrease in sensory color and firmness of irradiated samples as irradiation dose was increased, and off-flavor intensity of strawberries was incremented after 6 days of storage at 2°C.

In summary, irradiation of blueberries up to 1.6 kGy does not affect the sensory quality of the fruits.

4.3. Kinetics of quality changes

The main effects of irradiation on the product quality attributes were in color, texture, ascorbic acid content, phenolics and respiration rate. These changes were quantified using principles of kinetics.

In general, the rate of change of a quality parameter can be represented by

$$\frac{dC}{dt} = -k(C)^n \tag{4-10}$$

where k is the rate constant, C is the concentration of a quality parameter C at time t and n is the order of the reaction (Chen & Ramaswamy, 2002).

For the greater part of foods, the time dependence relationship appears to be described by zero or first order models. By integrating Eqn (4-10), zero-order (Eqn 4-11), first order (Eqn 4-12) and fractional conversion (Eqn 4-13) kinetics models can be derived:

$$C = C_o + k\mathrm{t} \tag{4-11}$$

$$\frac{C}{C_o} = \exp(-kt) \tag{4-12}$$

$$\frac{C - C_e}{C_o - C_e} = \exp(-kt) \tag{4-13}$$

where C_o represents the initial quality value and C_e is the equilibrium value of the quality factor C (Chen & Ramaswamy, 2002).

4.3.1. Mangoes

4.3.1.1. Color changes

The variation in color of fruits with time has been reported to follow first order kinetics (Ochoa et al., 2001; Ahmed et al., 2001; Shin & Bhowmik, 1995)

Figures 4-37 to 4-39 present the changes in color attributes of all mangoes throughout the storage time. The changes in color parameters found in the present study for control and irradiated fruits did not follow a clear trend and they could not be fitted to any simple kinetics model. Therefore, the kinetics describing *a, b* and *L* values was not determined. However, redness *(a* values) (Figure 4-37) and yellowness (*b* values) (Figure 4-38) showed an increase from day zero until day five. This increase was associated with the significant increase on tannins and carotenoids on that day (section 4.1.2.10). It is possible that on these samples a rapid destruction of the chlorophyll occurs with chlorophyll a preferentially degraded to chlorophyll b. This degradation is accompanied by increases in carotenoids levels which affect the color development on the fruit. From days five to twenty-one, a decrease occurred for all treatments on day ten, followed by a significant ($P>0.05$) increase for control and samples treated with high dose. For samples exposed to medium and low dose a slight decrease was observed by the end of the storage. Discussion of these parameters is available in section 4.1.1.1.

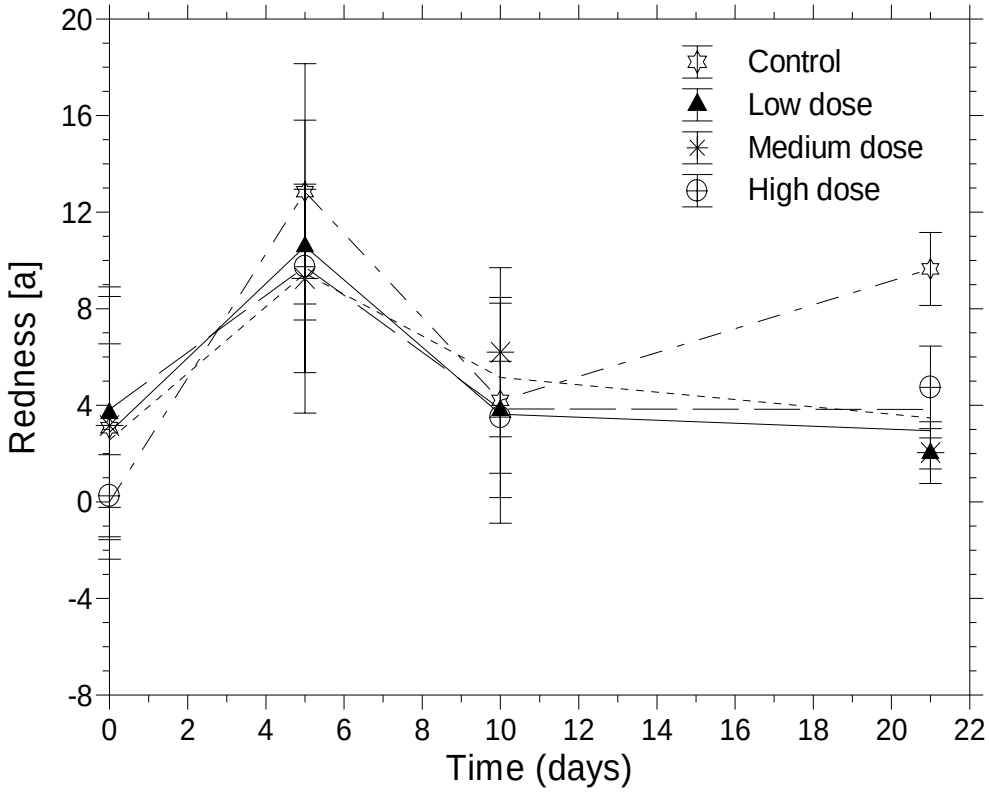

Figure 4-37. Changes in redness (*a values)* of irradiated and non-irradiated mangoes stored up to 21 days at 12°C. (Control=non-irradiated, low dose=1.0 kGy, medium dose=1.5 kGy, high dose=3.1 kGy).

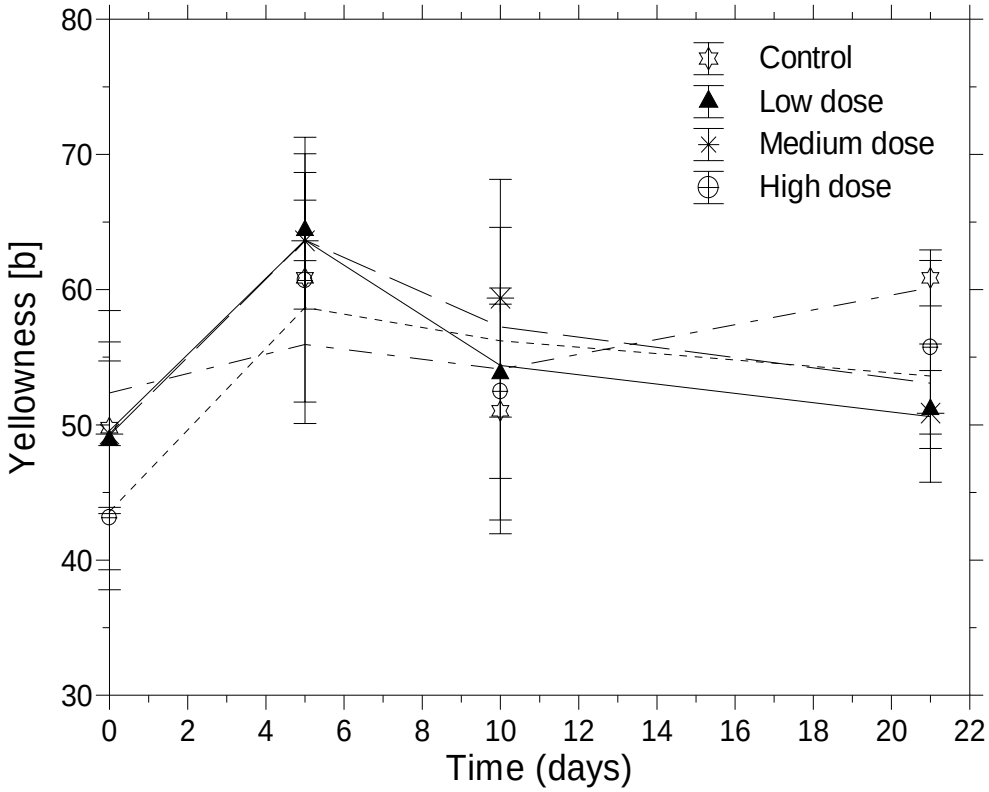

Figure 4-38. Changes in yellowness (*b values*) of irradiated and non-irradiated mangoes stored up to 21 days at 12°C. (Control= non-irradiated, low dose= 1.0 kGy, medium dose= 1.5 kGy, high dose= 3.1 kGy).

The change in redness (*a*) was fitted to different models as,

$$a(control) = \frac{t}{\left[-0.026t^2 + 0.787t - 2.89\right]}, R^2 = 0.96 \quad (4\text{-}14)$$

$$a\ (1.0\ kGy) = 17328[t^{(-3.366)}] + 2.88, R^2 = 0.96 \quad (4\text{-}15)$$

$$a\ (1.5\ kGy) = 66.66*[t^{(-1.41)}] + 2.57, R^2 = 0.89 \quad (4\text{-}16)$$

$$a\ (3.1\ kGy) = 2.92\ exp006*[t^{(-8.147)}] + 3.83, R^2 = 0.95 \quad (4\text{-}17)$$

The changes in yellowness (*b*) were described by the following equations:

$$b(control) = \frac{t}{\left[-0.124t^2 + 3.24t - 13.01\right]}, R^2 = 0.55 \quad (4\text{-}18)$$

$$b\ (1.0\ kGy) = 202*(t^{(-1.607)}) + 49.11, R^2 = 0.99 \quad (4\text{-}19)$$

$$b\ (1.5\ kGy) = 65.97*(t^{(-0.902)}) + 48.81, R^2 = 0.92 \quad (4\text{-}20)$$

$$b\ (3.1\ kGy) = 25.97*(t^{(-0.303)}) + 43.22, R^2 = 0.88 \quad (4\text{-}21)$$

The above equations (4-14 to 4-21) suggest that differences in the values of the color attributes (*a* and *b* values) would be observed with time at each irradiation dose. For instance, the redness of the 1.5 kGy samples (Eqn 4-16) would be lower at longer time, but for 3.1 kGy samples (Eqn 4.17) small changes would occur. The yellowness of the 1.0 kGy and 1.5 kGy samples (Eqn 4-19 and 4-20) would have a decreasing trend with time.

The changes in lightness (*L*) are shown in Figure 4-39. Differences among the samples were observed but not a clear trend was found. In general, all samples had a decrease in lightness by day five with a subsequent increase on day ten.

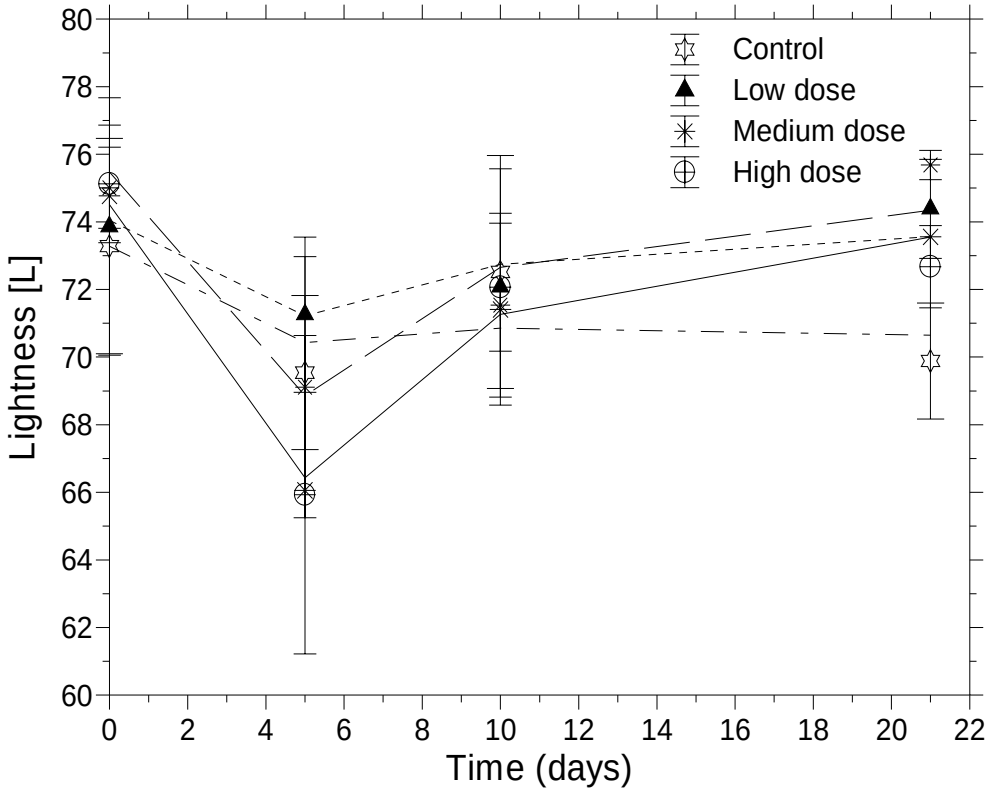

Figure 4-39. Changes in lightness (*L*) of irradiated and non-irradiated mangoes stored up to 21 days at 12°C. (Control= non-irradiated, low dose= 1.0 kGy, medium dose= 1.5 kGy, high dose= 3.1 kGy).

By the end of the storage, a reduction in lightness was observed for control samples while the samples treated with low (1.0 kGy) and medium (1.5 kGy) doses had increased value. The fruits treated with high dose (3.1 kGy) did not change by day twenty-one. These variations are associated with the changes in *a* and *b* values related to the pigments concentrations and also associated with the differences in fruit maturity levels. The changes in lightness were fitted using a power model and described as,

$$L \text{ (control)} = (-3.41)*(t^{(-0.1135)}) +73.28, R^2= 0.51 \quad (4\text{-}22)$$

$$L \text{ (1.0 kGy)} = (-20.04)*(t^{(-1.172)}) +74.13, R^2= 0.80 \quad (4\text{-}23)$$

$$L \text{ (1.5 kGy)} = (-47.78)*(t^{(-1.229)}) +75.80, R^2= 0.88 \quad (4\text{-}24)$$

$$L \text{ (3.1 kGy)} = (79.75)*(t^{(-1.374)}) +74.77, R^2= 0.97 \quad (4\text{-}25)$$

From Eq 4-22 to 4-25 an increasing but not significant trend in lightness of controls and fruits treated up to 1.5 kGy was observed through time. The samples treated with 3.1 kGy had a decreasing *L* values which imply the darkening of the fruits.

4.3.1.2. Texture changes

Texture degradation has been reported to follow a first order kinetic reaction (Lau et al., 2000; Ahmed et al., 2001) which is modeled by Eqn (4-12). By linearization of Eqn (4-12),

$$\ln C = \ln C_o - kt \quad (4\text{-}26)$$

where C is the textural characteristic (like firmness) at time t, C_o is the initial amount of the textural characteristic in the treated samples, k is the 'softening' rate constant in

(time $^{-1}$), which is the slope of the curve of the appropriate reaction vs time. The plot of ln (C/C_0) versus t would be a straight line, and the slope at a constant dose would be equal to $-k$.

Zero and first order kinetics models were used to evaluate the changes in texture parameters of mangoes but low correlation was found. Therefore, the fractional kinetics model (Eqn 4-13) best fitted the results of the changes in firmness and Young's modulus with time.

4.3.1.2.1. Firmness

The degradation of texture (loss of firmness) showed two distinct trends. There was a rapid loss of firmness in all treatments from day zero until day five, and then a continuous but not significant increase in the rupture force until the end of the storage, especially in samples irradiated at low and medium doses (Figure 4-40). The reduced firmness is associated with a more ripe stage of the fruits, which implies changes in the cell structure and in color. During the ripening there is a disruption of the cell wall with the release of many of its components (polysaccharides); therefore, the softening of the fruits increases. The changes in color involve the alteration of the cell structure associated with chloroplast to chromoplast transition that induces the breakdown of the thylakoid membrane in the peel of the fruit.

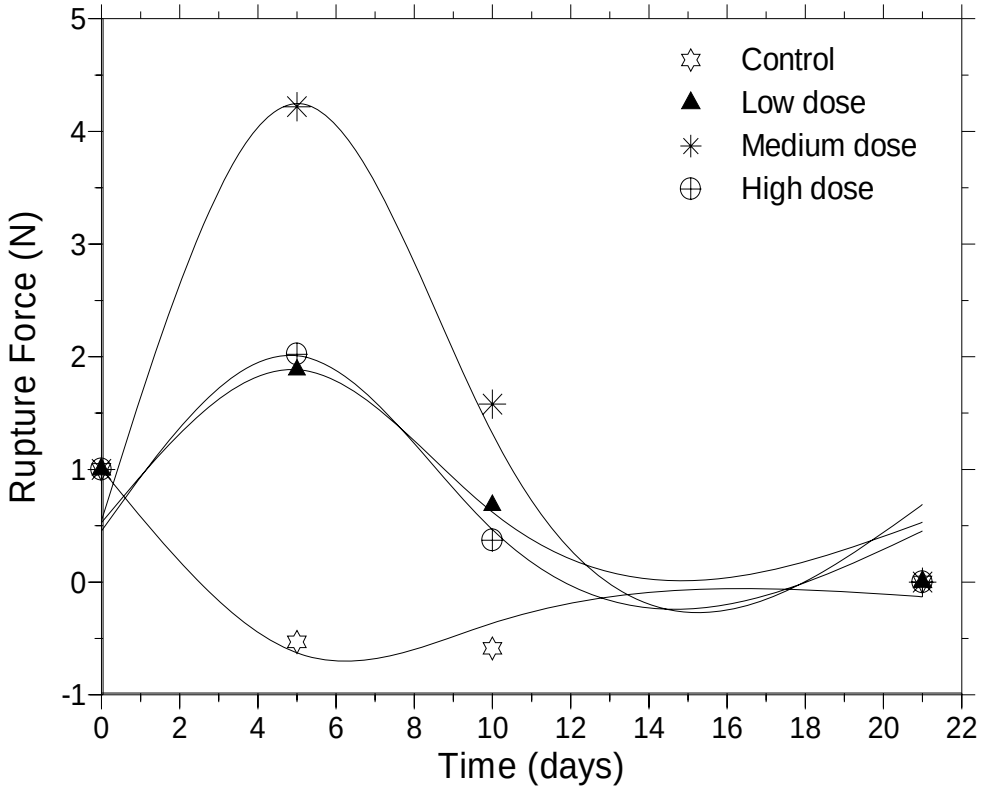

Figure 4-40. Changes in texture –rupture force (RF)-during storage of mangoes stored up to 21 days at 12°C. (Control= non-irradiated, low dose= 1.0 kGy, medium dose= 1.5 kGy, high dose= 3.1 kGy).

For fruits exposed at high dose firmness remained constant during time. It is possible that the variation on ripening stage among the fruits exposed to high dose was less than in the other treatments, therefore, minimum changes in texture with time were observed.

As it was expected, the rate constant (k) was higher at elevated doses, therefore, the higher the dose the greater the loss of firmness in the irradiated fruits (Table 4-23). The samples treated at low dose were firmer than those treated at medium and low dose.

Table 4-23
Rate constant (k) and R^2 values for changes in firmness of irradiated mangoes (Eqn 4-13)

Treatment	k (days^{-1})	(EMS)*	R^2
1.0 kGy	0.57	11.49	0.81
1.5 kGy	0.62	14.26	0.76
3.1 kGy	0.63	4.46	0.85

*Mean square error

Eqn 4-13 did not describe the changes in firmness of the control samples and the changes were described by,

$$RF \text{ (control)} = 173.9 + (-10.53)\, t + 0.4292 t^2,\ R^2 = 0.915 \quad (4\text{-}27)$$

and for irradiated samples: rupture force (RF) $= \dfrac{C - C_e}{C_o - C_e} = A\exp^{(-kt)}$ then:

$$RF \text{ (1.0 kGy)} = 1.702\exp^{(-0.57\, t)},\ R^2 = 0.81 \quad (4\text{-}28)$$

$$RF\ (1.5\ \text{kGy}) = 3.167 \exp^{(-0.62\ t)},\ R^2 = 0.76 \quad (4\text{-}29)$$

$$RF\ (3.1\ \text{kGy}) = 4.567 \exp^{(-0.63\ t)},\ R^2 = 0.84 \quad (4\text{-}30)$$

The influence of dose on the reaction rate constant (*k)* was assumed to follow an Arrhenius type relationship as:

$$k(D) = k_o e^{-Ea/RD} \quad (4\text{-}31)$$

where k_o is the frequency factor (day^{-1}), *Ea* is the activation energy (kcal/mol K), *R* is the universal gas constant (8.314 J/mol K), and *D* is the dose (kGy). Linearizing Eq.(4-31),

$$\ln\ k = \ln\ k_o - \frac{Ea}{RD} \quad (4\text{-}32)$$

By plotting ln(*k*) vs the reciprocal of dose (1/*D*), the following relationship was found

$$\ln k = -0.40\text{-}0.15\left(\frac{1}{D}\right),\ R^2 = 0.86 \quad (4\text{-}33)$$

From Eq. (4-33), k_o= 0.67 (day^{-1}) and *Ea*= 0.0003 kcal/mol (1.25 J/mol). Chen and Ramaswamy (2002) reported activation energy of 5.49 kcal/mol for the degradation of the axial puncture force of banana stored at 15°C. According to Karel et al. (1975) the activation energy for thermal degradation of texture of fruits and vegetables ranges between 13.0 and 41.0 kcal/mol. This suggests that firmness is more sensitive to temperature than it is to radiation dose.

The dependence of *k* on irradiation dose is given by:

$$k(D) = 0.67\ day^{-1} e^{(-1.25/D)} \quad (4\text{-}34)$$

and the changes in firmness due to irradiation dose at specific time, (*t*), are described by:

$$C(t) = C_o e^{[0.67\ e(-1.25/D)]*t} \quad (4\text{-}35)$$

Therefore, as the time increases the changes in firmness are more drastic or significant.

4.3.1.2.2. Toughness

The values of toughness of the control samples and the fruits treated with any irradiation dose remained relatively constant throughout the storage period. Only the samples exposed at medium (1.5 kGy) dose had a significant (P>0.05) decrease of this texture parameter on day 5 and it remained constant after that (Figure 4-41). Therefore, the kinetics of toughness changes was not determined. The decrease of toughness was associated with the increase in softening due to the changes in the cell wall structure.

The following equations describe the changes in toughness of mangoes,

$$T\,(\text{control}) = 0.43 + (-0.01823)\,t + 0.000703t^2,\ R^2 = 0.88 \quad (4\text{-}36)$$

$$T\,(1.0\ \text{kGy}) = (-6.03)^*\,(t^{-2.79}) + 0.129,\ R^2 = 0.83 \quad (4\text{-}37)$$

$$T\,(1.5\ \text{kGy}) = (-0.\ 24)^*\,(t^{-0.58}) + 0.119,\ R^2 = 0.99 \quad (4\text{-}38)$$

$$T\,(3.1\ \text{kGy}) = (-0.04)^*\,(t^{-0.53}) + 0.050,\ R^2 = 0.76 \quad (4\text{-}39)$$

The above equations (4-36 to 4-39) suggest that the toughness of fruits decreases with time independent of the dose.

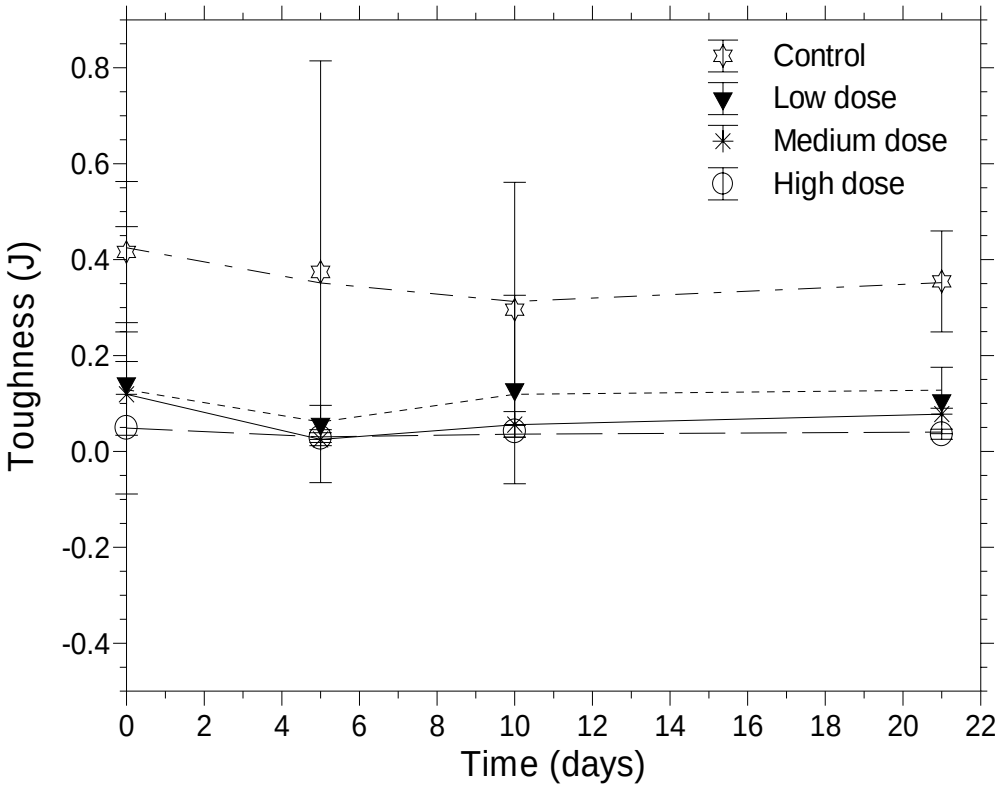

Figure 4-41. Changes in texture –toughness (T)-during storage of mangoes stored up to 21 days at 12°C. (Control=non-irradiated, low dose=1.0 kGy, medium dose=1.5 kGy, high dose=3.1 kGy).

4.3.1.2.3. Stiffness (Young's modulus)

For all irradiated and non-irradiated samples an initial decrease in the fruit stiffness was observed from day zero until day five and then it increased at day ten. The decrease (75.5%) was significant ($P<0.05$) in samples treated with medium dose (1.5 kGy) (Figure 4-42). These results are related with the loss of firmness due to structural changes. By the end of the storage, the stiffness of the all samples remained constant.

The effect of time on the Young's modulus (*E)* of the fruits was described by the fractional conversion kinetics model (Eqn 4-13). The rate constants (*k*) (Table 4-24) were determined from the ln (C-C_e/C_o-C_e) versus time plots.

Table 4-24
Rate constant (*k*) and R^2 values for changes in stiffness of mangoes (Eqn 4-13)

Treatment	*k* (days^{-1})	EMS*	R^2
0.0 kGy	0.28	2.35	0.84
1.0 kGy	0.36	3.63	0.82
1.5 kGy	0.35	5.31	0.73
3.1 kGy	0.30	1.76	0.86

*Mean square error

The value of the degradation rate constant (*k*) was higher for the irradiated samples than for the control indicating faster reduction of the stiffness with the irradiation treatment.

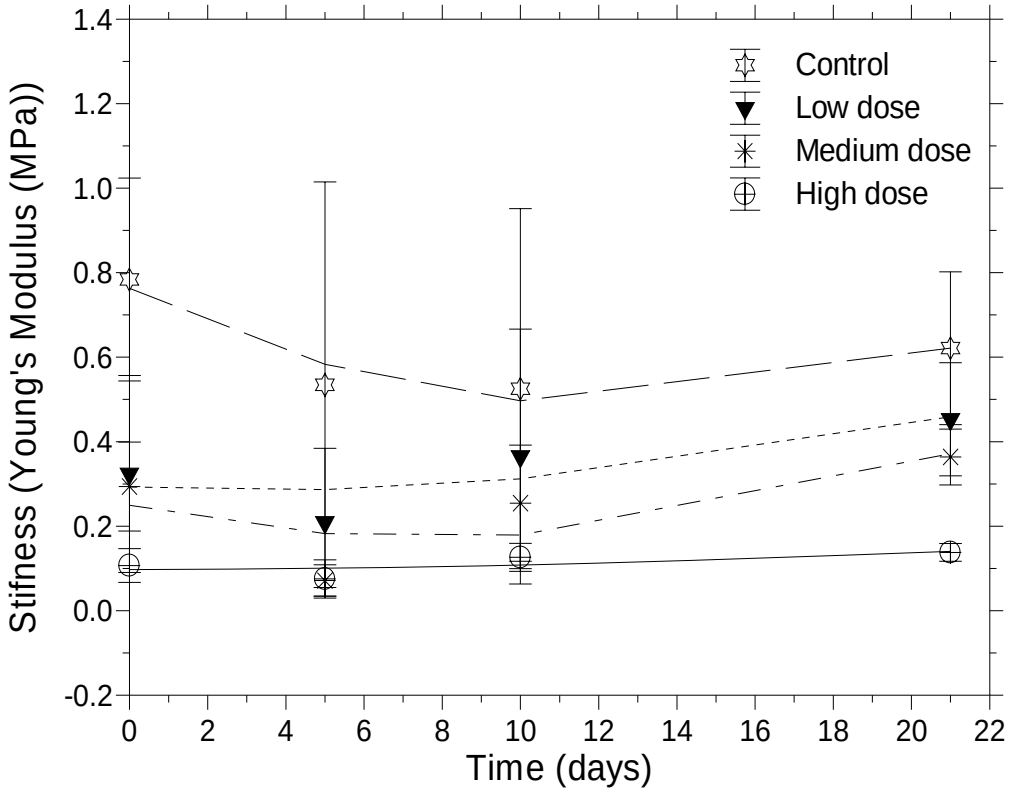

Figure 4-42. Changes in texture –Young's modulus (*E*) or stiffness- during storage of mangoes stored up to 21 days at 12°C. (Control= non-irradiated, low dose= 1.0 kGy, medium dose= 1.5 kGy, high dose= 3.1 kGy).

The low value of *k* for the samples treated with the higher dose may be related to the constant stiffness of these samples during the storage time. These variations may be associated with the differences in the degree of ripening of the fruits used for each treatment which increases the softening because of the cell wall depolymeration.

The changes in stiffness were described by the following equations:

$$E \text{ (control)} = 0.94\exp^{(-0.32\,t)},\ R^2 = 0.84 \quad (4\text{-}40)$$

$$E \text{ (1.0 kGy)} = 1.57\ \exp^{(-036\,t)},\ R^2 = 0.81 \quad (4\text{-}41)$$

$$E \text{ (1.5 kGy)} = 1.95\exp^{(-0.35\,t)},\ R^2 = 0.73 \quad (4\text{-}42)$$

$$E \text{ (3.1 kGy)} = 1.19\ \exp^{(-0.30\,t)},\ R^2 = 0.86 \quad (4\text{-}43)$$

The results implied that for all treatments, as the time increases the stiffness of the fruits decreases.

The influence of dose on the reaction rate constant (*k)* was assumed to follow an Arrhenius type relationship (Eq 4-31), where k_o is the frequency factor (day^{-1}), *Ea* is the activation energy (kcal/mol K), R is the universal gas constant (8.314 J/mol K), and *D* is the dose (kGy).

By plotting ln(*k*) vs the reciprocal of dose (1/*D*) (Eq 4-32, Figure 4-43), the following relationship was found

$$\ln k = -1.29 + 0.30\left(\frac{1}{D}\right),\ R^2 = 0.95 \quad (4\text{-}44)$$

From Eq. (4-44), k_o= 0.28 (day^{-1}) and *Ea*= 2.49 J/mol. The activation energy (*Ea*) is higher compared with the activation energy calculated for firmness (1.25 J/mol).

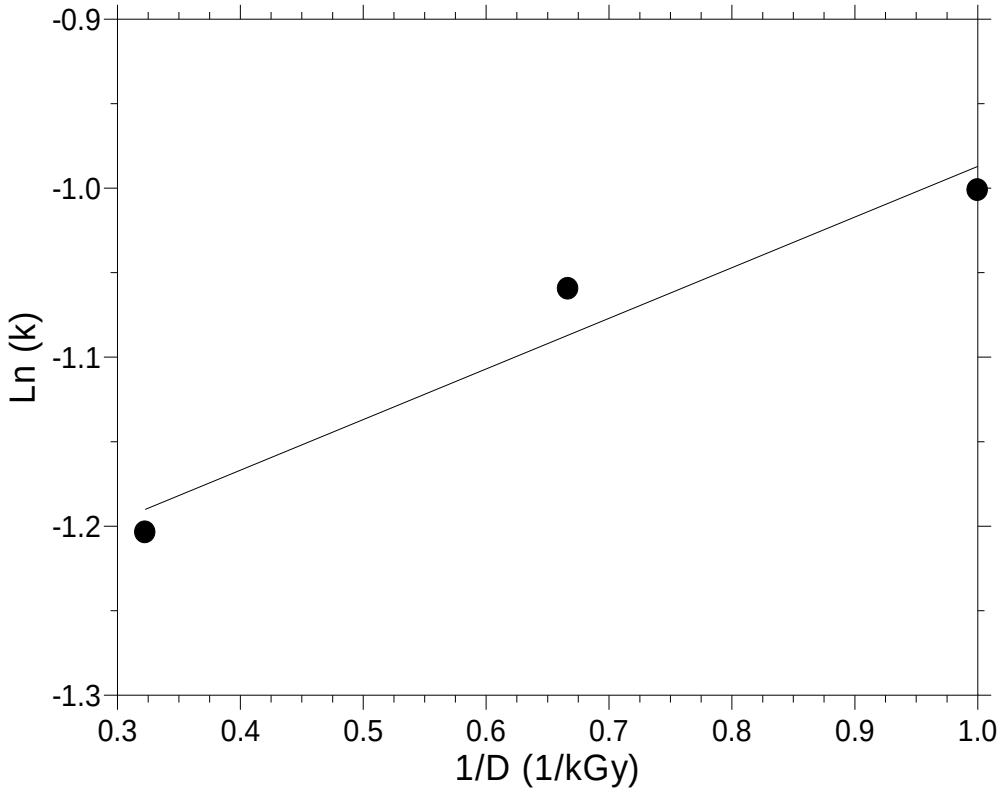

Figure 4-43. *k* describing changes in stiffness (Young's modulus) of irradiated mangoes stored at 12°C.

This suggests that the changes in firmness occurred at higher rate than the changes in stiffness.

The dependence of *k* on dose in the irradiation treatment of mangoes for 1.0-3.1 kGy is given by:

$$k(D) = 0.28 \text{ day}^{-1} e^{(-2.49/D)} \quad (4\text{-}45)$$

and the changes in stiffness due to irradiation dose at specific time, (*t*), are described by:

$$C(t) = C_o e^{[0.28\, e(-2.49/D)]*t} \quad (4\text{-}46)$$

The changes in stiffness suggest that the higher irradiation dose the higher the reduction of the stiffness of the fruits, and the longer the time the higher the reduction.

4.3.1.3 Degradation of ascorbic acid (Vitamin C)

The average concentration of ascorbic acid in the control (non-irradiated) fruits was 15.27±1.44 mg/100g w.b. while for the irradiated samples it was 6.77±0.74 mg/100g w.b (Figure 4-44). All irradiated and non-irradiated mangoes had a significant ($P>0.05$) decrease of ascorbic acid content with time. This reduction accounted for 31.5%, 47.0%, 91.0% and 89.0 % for control, low (1.0 kGy), medium (1.5 kGy) and high (3.5 kGy) doses, respectively by the end of storage. Lacroix et al. (1990) reported a decrease in the ascorbic acid content of gamma irradiated (0.5 – 0.95 kGy) and unirradiated mangoes during storage with significant differences in days 0, 9 and 15. Normally, the ascorbic acid decreases during ripening of the fruits (Seymour et al., 1993) thus, reduction upon storage may be due mostly to the effect of ripening process.

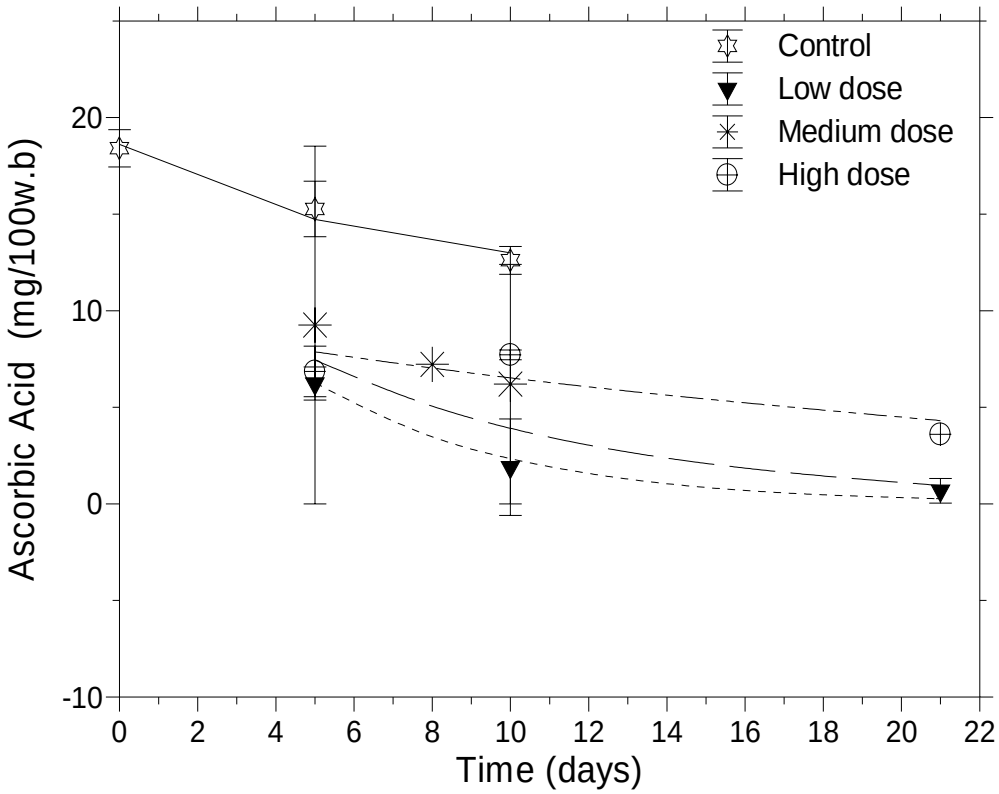

Figure 4-44. Effect of time of storage on ascorbic acid (AA) concentration of mangoes stored at 12°C. (Control= non-irradiated, low dose= 1.0 kGy, medium dose= 1.5 kGy, high dose= 3.1 kGy).

The reaction rate constants (*k*) and the initial concentration of ascorbic acid were calculated using the first order kinetics model (Eqn. 4-12) (Figure 4-45) and are presented in Table 4-25.

Table 4-25
Rate constant (*k*) and initial ascorbic acid concentration for degradation of ascorbic acid in mangoes stored up to 21 days at 12° C

Treatment	k(day^{-1})	ESM*	Co (mg/100g w.b)	R^2
Control	0.03	6.6E-05	2.91	0.99
1.0 kGy	0.13	0.156	2.27	0.94
1.5 kGy	0.16	0.015	2.84	0.99
3.1 kGy	0.05	0.010	2.34	0.97

* Mean square error

The reaction rate constants (*k)* confirm the influence of irradiation on the degradation of vitamin C. However, within the irradiated samples the fruits treated with medium dose degraded faster than the other treatments. This factor may be associated with different concentrations of solute and pH between the irradiated samples. Barr and King (1956) found that the rate of gamma induced oxidation radiation was inversely proportional to the concentration of solute. In solution the ascorbic acid is easily oxidized to a dehydorascorbic acid, and the liability to oxidation increases with increasing pH.

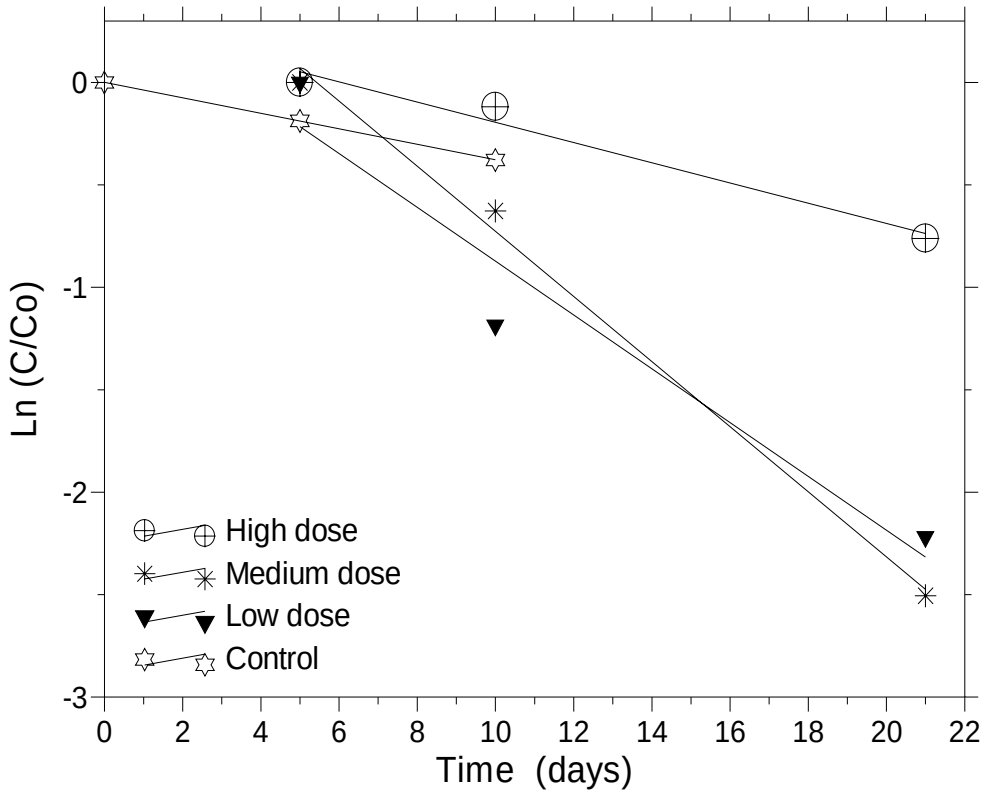

Figure 4-45. Relationship between ascorbic acid content and time of storage on mangoes stored up to 21 days at 12°C. (Control =non-irradiated, low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.1 kGy).

Since, these samples treated with medium dose had high antioxidant activity during storage; it is possible that at medium dose (1.5 kGy) these chemical reactions occur first.

The changes in ascorbic acid were described by the following equations:

$$AA \text{ (control)} = 2.91 \exp^{(-0.03\,t)},\ R^2 = 0.99 \quad (4\text{-}47)$$

$$AA \text{ (1.0 kGy)} = 2.27 \exp^{(-0.13\,t)},\ R^2 = 0.94 \quad (4\text{-}48)$$

$$AA \text{ (1.5 kGy)} = 2.84 \exp^{(-0.16\,t)},\ R^2 = 0.99 \quad (4\text{-}49)$$

$$AA \text{ (3.1 kGy)} = 2.34 \exp^{(-0.05\,t)},\ R^2 = 0.97 \quad (4\text{-}50)$$

The influence of dose on the reaction rate constant (*k)* was assumed to follow an Arrhenius type relationship (Eqn 4-6). By linearization and plotting ln(*k*) vs the reciprocal of dose (1/*D*) (Figure 4-46), the following relationship was found:

$$\ln k = -1.62 + 1.67\left(\frac{1}{D}\right),\ R^2 = 0.98 \quad (4\text{-}51)$$

From Eq. (4-51), k_o= 0.2 (day $^{-1}$) and *Ea*= 0.00331 kcal/mol (13.88 J/mol). Vikram et al. (2004) reported activation energies values between 9.52 and 15.48 kcal/mol for thermal degradation of ascorbic acid in orange juice treated with different heating methods. In addition, Karel et al. (1975) reported that the activation energy for thermal degradation of liquid vitamin C in food components was 23.1kcal/mol. This suggests that heat treatment has more detrimental effect on ascorbic acid than irradiation. With small changes in temperature ascorbic acid degrades faster than with small changes in dosage.

The dependence of *k* on dose in the irradiation of mangoes is given by:

$$k(D) = 0.2 \text{ day}^{-1} e^{(-13.88/D)} \quad (4\text{-}52)$$

and the changes in ascorbic acid concentrations due to irradiation dose at specific time, (t), are described by:

$$C(t) = C_o e^{[0.20e-13.88/D]t} \quad (4\text{-}53)$$

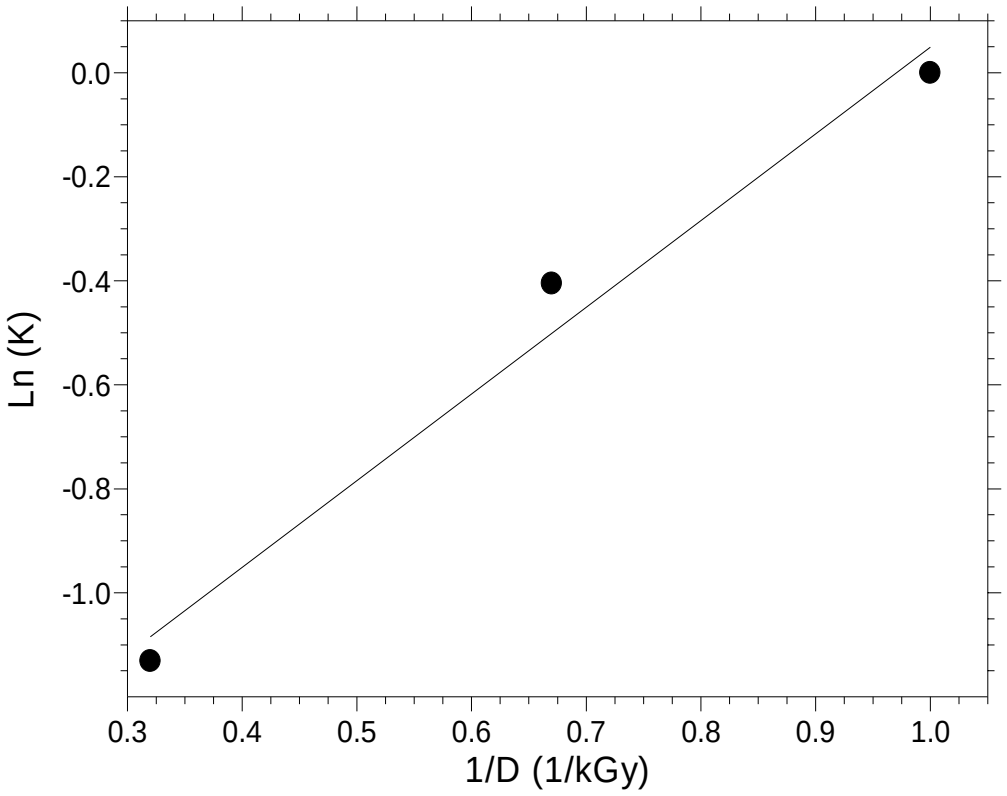

Figure 4-46. Effect of dose on the rate constant (*k*) for change in ascorbic acid in irradiated mangoes stored up to 21 days at 12°C.

4.3.1.4 Respiration rate changes

The changes in respiration rate (mg CO_2/Kg h) showed distinct trends but in general, all the samples had two phases: one of increase of respiration and other one of decrease (Figure 4-47). For instance, the samples treated at medium (1.5 kGy) and at low dose (1.0 kGy) had a rapid loss of respiration from day zero until day ten (phase 1), and then a continuous increase until the end of the storage (phase 2). The control fruits had a rapid decrease in respiration until day 5 (phase 1) with a subsequent increase until day twenty-one (phase 2). The samples exposed to high dose (3.1 kGy) did not have significant ($P>0.05$) changes in the respiration rate until day 10 (phase 1) but an increase was observed by the end of storage (phase 2). The variations in the CO_2 concentrations are associated with the differences in the stage of ripening of the fruit. Immature fruit have high respiration rates while mature fruits have relatively low rates (Salveit, 1997).

The rate constant k was calculated from the slope (dCO_2/dt) of each of the increasing and decreasing phases observed from each treatment. In phase 1, the values of k indicate that the changes in CO_2 concentrations occurred faster in the control samples than the irradiated samples (Table 4-26). It also suggest than the changes in the respiration rate of the samples treated with a high dose (3.1 kGy) are very slow. Contrarily, in phase 2 the CO_2 concentrations increased faster in high dose samples than in the other treatments.

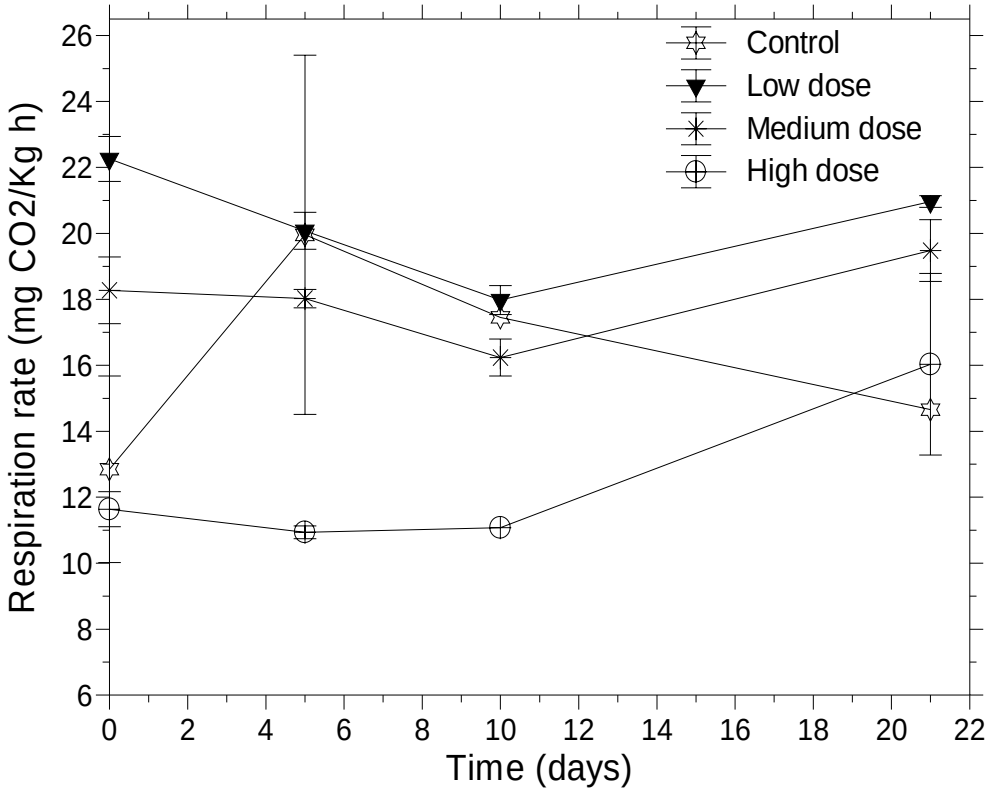

Figure 4-47. Effect of time of storage on respiration rate (mg CO_2/kg h) of mangoes stored at 15°C. (Control= non-irradiated, low dose= 1.0 kGy, medium dose= 1.5 kGy, high dose= 3.1 kGy).

Table 4-26
Rate constant (k) and R^2 values for changes in respiration rate of mangoes stored up to 21 days at 12°C

	Phase 1		Phase 2	
Treatment	***k*(day^{-1})**	**R^2**	***k*(day^{-1})**	**R^2**
Control	1.73	0.91	0.31	0.97
1.0 kGy	0.42	0.99	0.27	0.96
1.5 kGy	0.20	0.84	0.29	0.99
3.1 kGy	0.06	0.57	0.44	0.99

4.3.2 Blueberries

4.3.1.1 Color changes

4.3.1.1.1. Redness

Color values (*a, b* and *L*) of the berries did not follow a specific trend and therefore were not suitable for monitoring color changes during irradiation treatment. Previous discussion of these color parameters is available in section 4.2.2.1.

Control (non-irradiated), medium and, high doses did not cause significant changes in *a* values (redness) with time (Figure 4-48). Only the samples treated with the low dose had a significant (P>0.05) increase in *a* values until day three with a subsequent decrease at the end of the storage time. This variation could be due to the increase of anthocyanins and tannins content on that day according to the ripeness and pH of the evaluated fruit.

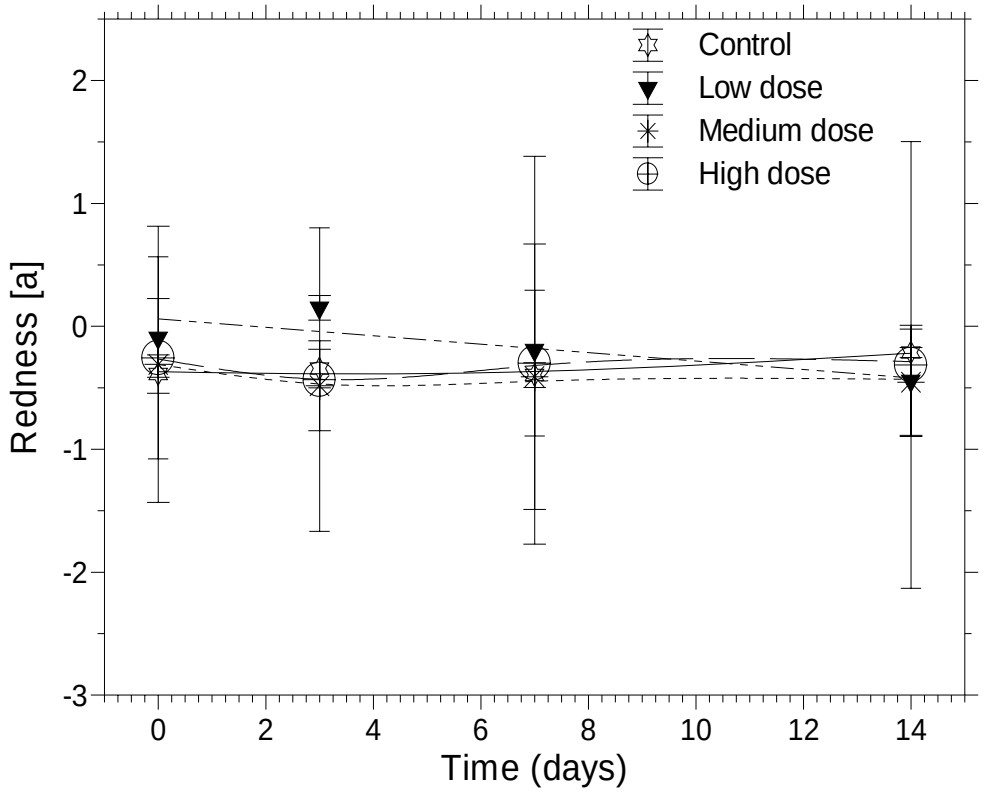

Figure 4-48. Changes in redness (*a* values) of blueberries stored up to 14 days at 5°C. (Control =non-irradiated, low dose =1.1 kGy, medium dose =1.6 kGy, high dose =3.2 kGy).

The changes in redness were described by the following equations:

a (control) $= (-0.369)+(-0.010)*t + 0.0015t^2$, $R^2= 0.82$ (4-54)

a (1.1 kGy) $= (-0.021)+(0.013)*t + (-0.0032)t^2$, $R^2= 0.76$ (4-55)

a (1.6 kGy) $= (-0.91) *(t^{(0.170)}) + (-0.31)$, $R^2= 0.88$ (4-56)

a (3.2 kGy) $= (-0.72) *(t^{(-1.353)}) + (-0.26)$, $R^2= 0.93$ (4-57)

From the above equations the *a* values of the berries would be increased as the storage time is extended.

4.3.1.1.2. Yellowness

Throughout the storage time, different trends were observed in the yellowness of the non-irradiated and irradiated blueberries. Control samples had a significant ($P>0.05$) increase in *b* values on day fourteen indicating the ripening of the fruits (Figure 4-49).

The samples treated with a low dose (1.1 kGy) showed an increase in *b* values during storage, while the samples exposed to medium dose (1.6 kGy) had decreased *b* values. The decrease in *b* values could be associated with the reduction of carotenoids. In some fruits these pigments are masked by more intense pigments such as anthocyanins. These samples also had a reduction of phenolics within time. The changes in color (yellowness) were described by different equations as:

b (control) $= (-2.88) + (-0.209)*t + 0.018t^2$, $R^2= 0.90$ (4-58)

b (1.1 kGy) $= (-0.307)*\exp^{(0.1387*t)} + (-1.69)$, $R^2=0.97$ (4-59)

b (1.6 kGy) $= (-0.53) *(t^{(0.319)}) + (-2.63)$, $R^2= 0.99$ (4-60)

b (3.2 kGy) $= (-3.01) *(t^{(-1.16)}) + (-2.73)$, $R^2= 0.83$ (4-61)

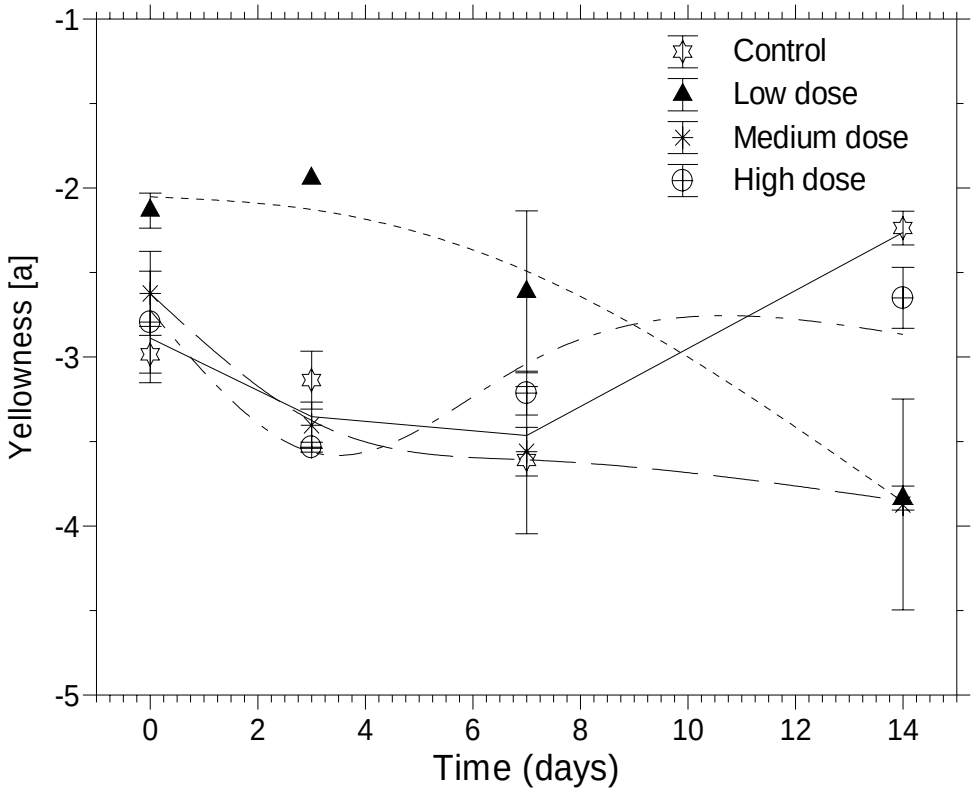

Figure 4-49. Changes in yellowness (*b* values) of blueberries stored up to 14 days at 5°C. (Control =non-irradiated, low dose =1.1 kGy, medium dose =1.6 kGy, high dose =3.2 kGy).

4.3.1.1.3. Lightness

Different trends were observed for lightness of both control and irradiated samples. For instance, a significant (P>0.05) decrease (21.33%) in *L* values (darker) of the control samples was observed on day fourteen, while samples exposed to low dose (1.1 kGy) showed a slight reduction half way the storage time (day 7), but then the samples became significantly lighter (higher *L* values) at the end of the storage. Samples treated with medium (1.6 kGy) dose became significantly lighter throughout the storage time. The color (*L* values) of the samples treated with high (3.2 kGy) dose remained constant throughout storage (Figure 4-50). The variation of lightness values is associated with the changes in *a* and *b* values, and also with the activity of the enzymes responsible of pigment synthesis. According to Seymour et al. (1993) there is a correlation between anthocyanins and PAL activity which increase during the ripening.

The changes in lightness were described by the following equations:

$$L \text{ (control)} = 17.89 + (0.512)*t + (-0.05)*t^2,\ R^2 = 0.81 \quad (4\text{-}62)$$

$$L \text{ (1.1 kGy)} = (0.05)*(t^{(1.705)}) + (15.68),\ R^2 = 0.93 \quad (4\text{-}63)$$

$$L \text{ (1.6 kGy)} = (3.82)*(t^{(-0.215)}) + (16.87),\ R^2 = 0.99 \quad (4\text{-}64)$$

$$L \text{ (3.2 kGy)} = (-0.53)*(t^{(0.112)}) + (18.28),\ R^2 = 0.95 \quad (4\text{-}65)$$

The results indicated the variation between the changes in lightness for each treatment. For instance, for the control and for the fruits treated at medium dose *L* values increases at extended storage times while for the samples exposed to low and high doses these values are decreased.

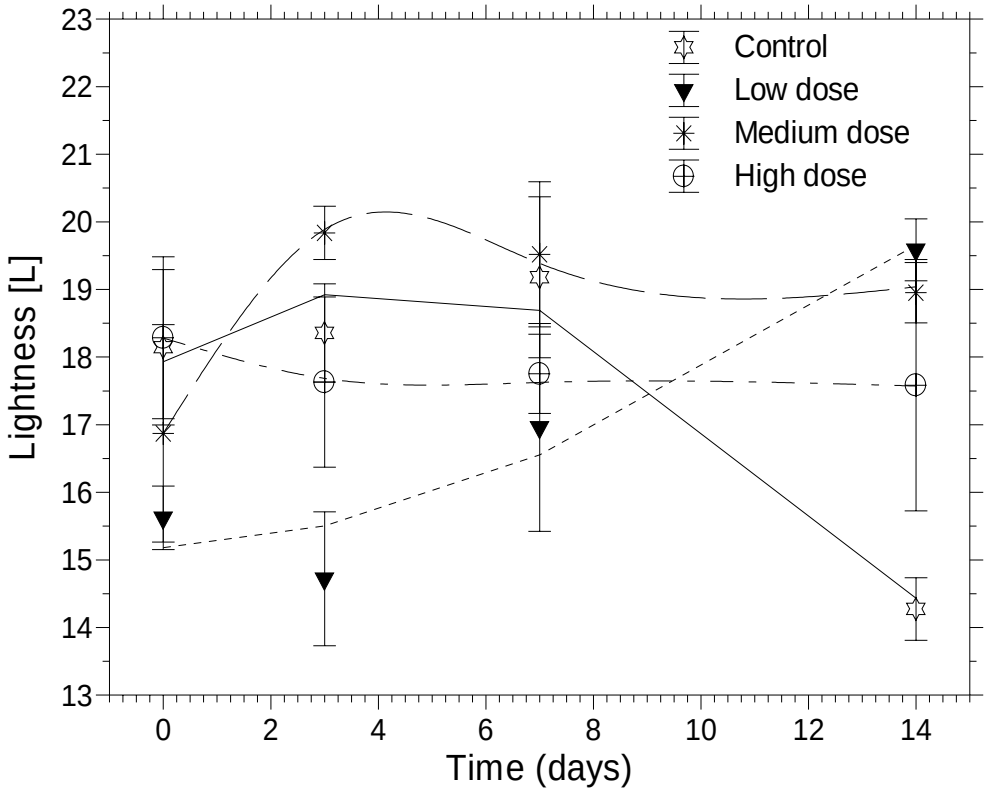

Figure 4-50. Changes in lightness (*L*) of blueberries stored up to 14 days at 5°C. (Control =non-irradiated, low dose =1.1 kGy, medium dose =1.6 kGy, high dose =3.2 kGy).

4.3.2.2 Texture changes

Softening of the fruit has been one of the most frequent phenomena observed in the application of irradiation treatment. Zero, first order and fractional conversion kinetics models were used to evaluate the reaction order of the changes in texture of blueberries. Low correlation was obtained therefore, the kinetics describing changes in firmness (shear force) and toughness with the time of blueberries was not determined.

4.3.2.2.1. Firmness

No changes in the shear force (SF) of the samples treated with low and medium dose were observed during time. The control samples had a significant (P>0.05) increase in the shear force values on days three and seven with a subsequent decrease on day fourteen. The samples exposed to high dose had a significant decrease in firmness on day seven (Figure 4-51). These differences may be related to different maturity levels between the fruits and the changes in cell wall structure by the degradation of the cell wall constituents and the presence of depression on the skin of the fruits.

The changes in firmness (shear force) were described by the following equations:

$$SF \text{ (Control)} = 166.5 + (13.03)*t + (-0.977)*t^2,\ R^2 = 0.96 \quad (4\text{-}66)$$

$$SF \text{ (1.1 kGy)} = 87.67 + (-2.89)*t + (0.219)*t^2,\ R^2 = 0.38 \quad (4\text{-}67)$$

$$SF \text{ (1.6 kGy)} = 112.1 + (0.84)*t + (-0.030)*t^2,\ R^2 = 0.34 \quad (4\text{-}68)$$

$$SF \text{ (3.2 kGy)} = 19.72 + (2.24)*t + (-0.95)*t^2,\ R^2 = 0.99 \quad (4\text{-}69)$$

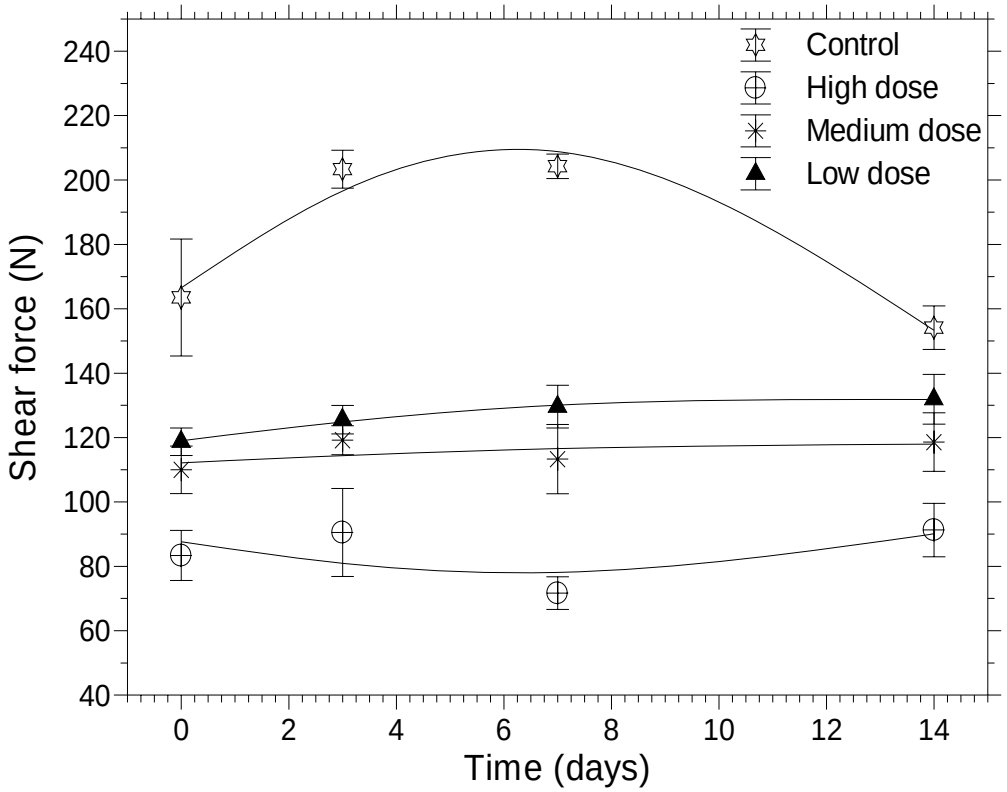

Figure 4-51. Changes in texture- shear force (SF) of blueberries during storage up to 14 days at 5°C (control =non-irradiated, low dose =1.1 kGy, medium dose =1.6 kGy, high dose =3.2 kGy).

4.3.2.2.2. Toughness

Throughout storage, no differences were found in the values of toughness for samples treated with low and medium doses. However, for the control fruit had a significant (P>0.05) increase on day seven with a subsequent decrease by the end of the storage. The toughness of the fruits exposed to high dose (3.2 kGy) increased on days three (17.1%) and fourteen (11.8%). This increase corresponded to higher values of shear force for these samples in these days (Figure 4-52). Softening increases as the ripening occurs therefore, the differences in toughness with time are related to the variation in maturity levels of the fruits.

The changes in toughness are described by the following equations,

$$T(\text{Control}) = 4.20 + (0.29)*t + (-0.024)*t^2,\ R^2 = 0.46 \quad (4\text{-}70)$$

$$T(1.1\ \text{kGy}) = (0.5\ 4)*(t^{(-0.163)}) + (2.97),\ R^2 = 0.97 \quad (4\text{-}71)$$

$$T(1.6\ \text{kGy}) = (0.17)*(t^{(0.112)}) + (2.75),\ R^2 = 0.79 \quad (4\text{-}72)$$

$$T(3.2\ \text{kGy}) = 480.7*(t^{(-0.665)}) + (2.16),\ R^2 = 0.51 \quad (4\text{-}73)$$

4.3.2.3 Degradation of ascorbic acid

Control blueberries had a significantly (P<0.05) lower (29.0%) of ascorbic acid contents after days seven and fourteen (figure 4-53). In addition, samples treated with low (1.1 kGy) dose had a decrease of 32.13% on the seventh day of storage. The samples exposed to medium (1.6 kGy) dose had a 27.90% increase while those samples exposed to the higher dose had a reduction of 2.15% over the storage time. These changes in ascorbic acid concentration may be associated with the formation of radiolytic products

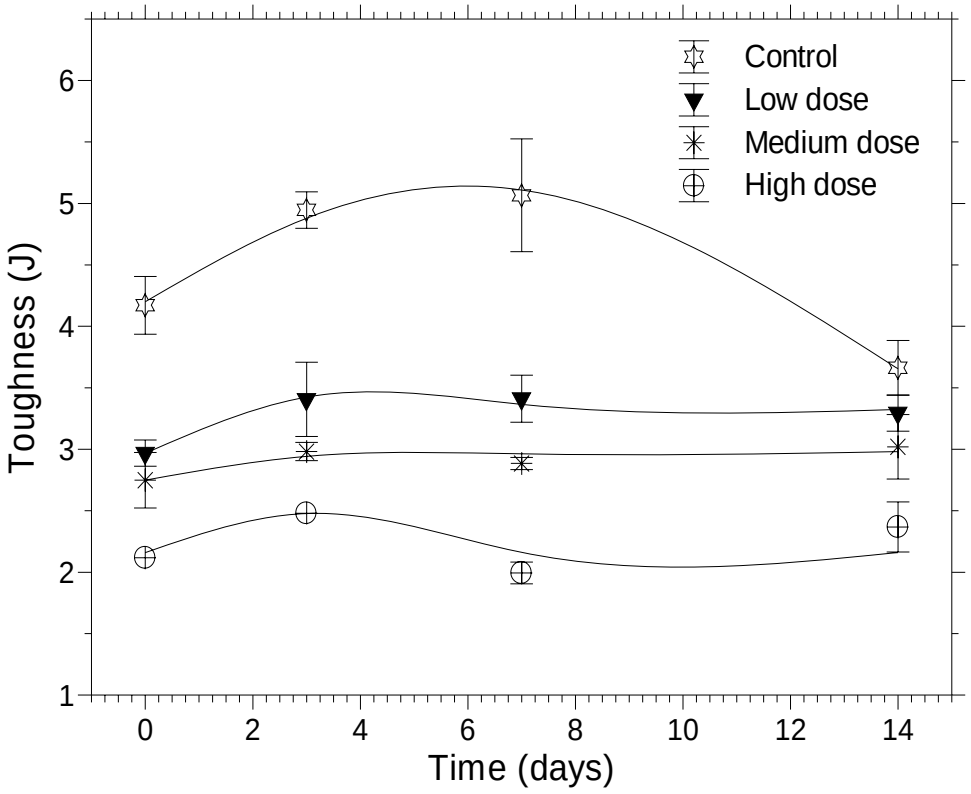

Figure 4-52. Changes in texture- toughness (J) of blueberries during storage up to 14 days at 5°C. (Control =non-irradiated, low dose =1.1 kGy, medium dose =1.6 kGy, high dose =3.2 kGy).

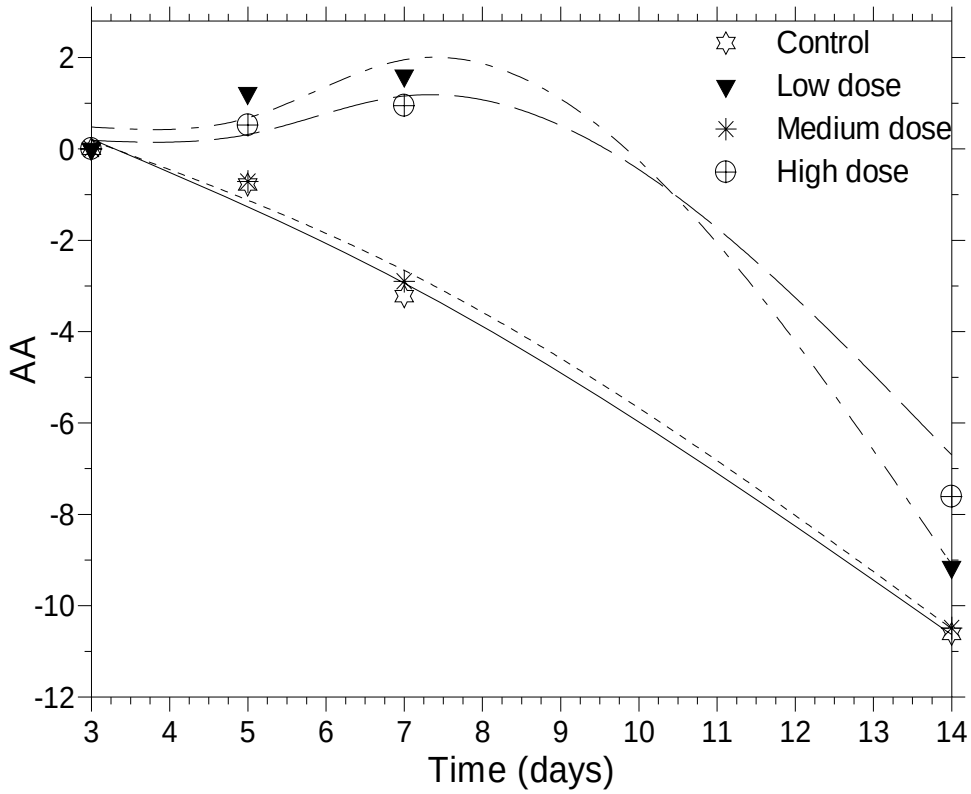

Figure 4-53. Effect of time of storage on ascorbic acid (AA) concentration of blueberries stored at 5°C. (Control =non-irradiated, low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.2 kGy).

such as dehydroascorbic acid which increases at maximum at some dosages but it decreases at other irradiation levels (Rosenthal, 1992).

Zero, first order and fractional conversion kinetics models were used to evaluate the reaction order of the changes in ascorbic acid content of blueberries. The best correlation describing changes in ascorbic acid with the time was obtained with the fractional conversion model (Eqn 4-13) (see Figure 4-53) where ascorbic acid concentration is described as $(AA) = \frac{C - C_e}{C_o - C_e} = \text{Aexp}^{(-kt)}$

The reaction rate constants (k) and the initial concentration of ascorbic acid were calculated from the $ln\ (C\text{-}C_e/C_o\text{-}C_e)$ versus time plots (Figure 4-54) and presented in Table 4-27.

Table 4-27 Rate constant (k) for degradation of ascorbic acid in blueberries stored up to 14 days at 5°C

Treatment	***k* (days^{-1})**	**(EMS)***	**R^2**
0.0 kGy	1.01	0.409	0.99
1.1 kGy	0.94	8.244	0.79
1.6 kGy	1.00	0.514	0.99
3.2 kGy	0.77	4.571	0.82

*Mean square error

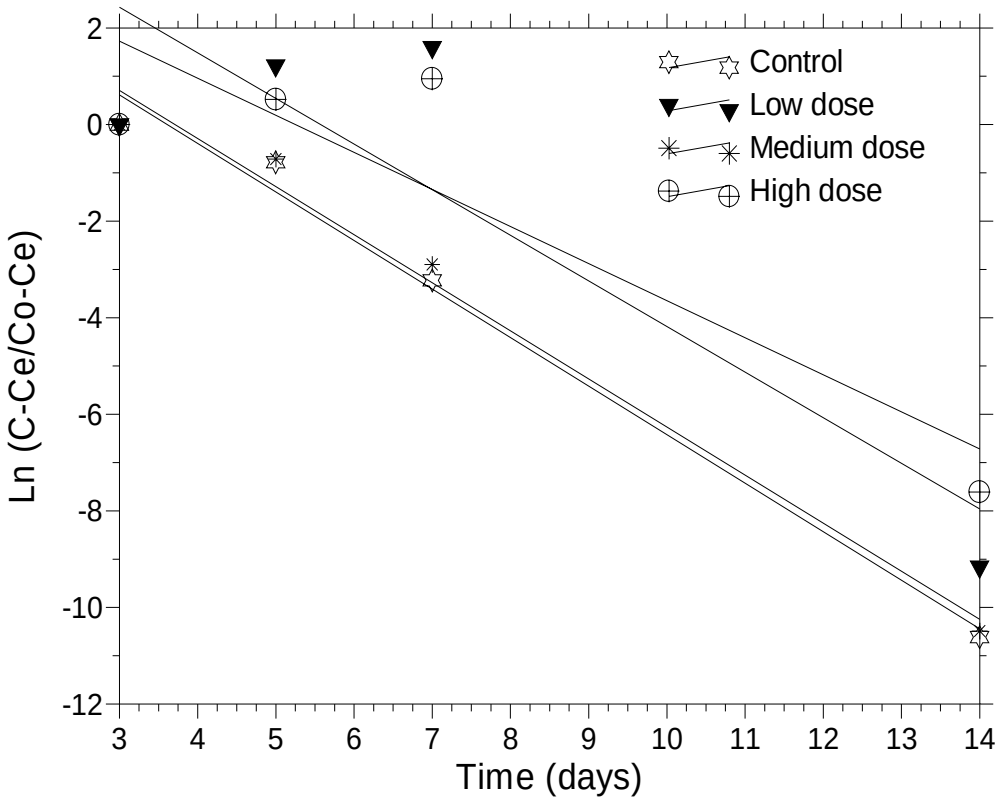

Figure 4-54. Relationship between ascorbic acid content and time of storage at different irradiation doses applied on blueberries stored at 5°C. (Control =non-irradiated, low dose =1.1 kGy, medium dose =1.6 kGy, high dose =3.2 kGy).

The reaction rate constants (*k)* obtained indicated that the degradation of ascorbic acid over time occurred faster in the control samples and in the fruits exposed to medium dose than in the other treatments. In this study the ascorbic acid degraded at a slower rate in the fruits treated at high dose (3.2 kGy). These samples had the lowest reduction of ascorbic acid (2.5%) among the time.

The changes in ascorbic acid concentrations (*AA*) were defined as:

$$AA \text{ (control)} = -3.63\exp^{(-1.01\,t)},\ R^2 = 0.99 \quad (4\text{-}74)$$

$$AA \text{ (1.1 kGy)} = 5.26\exp^{(-0.95\,t)},\ R^2 = 0.79 \quad (4\text{-}75)$$

$$AA \text{ (1.6 kGy)} = 3.69\exp^{(-0.99\,t)},\ R^2 = 0.99 \quad (4\text{-}76)$$

$$AA \text{ (3.2 kGy)} = 4.03\exp^{(-0.76\,t)},\ R^2 = 0.82 \quad (4\text{-}77)$$

The influence of dose on the reaction rate constant (*k)* was assumed to follow an Arrhenius type relationship (Eqn 4-6) but not correlation was found between the data (R^2= 0.02) therefore, the activation energy was not calculated.

4.3.2.4. Changes in phenolic compounds

A decreasing trend on the phenolics content of all fruits was obtained with time. This reduction was 10.67% for control, 8.15% for low dose, 20.93% for medium dose, and 29.29% for high dose. Zero, first order and fractional conversion kinetics models were used to evaluate the reaction order of the changes in phenolics content of blueberries. The best correlation describing the changes in phenolics with time was obtained with the fractional conversion model (Eqn 4-13) (Figure 4-55).

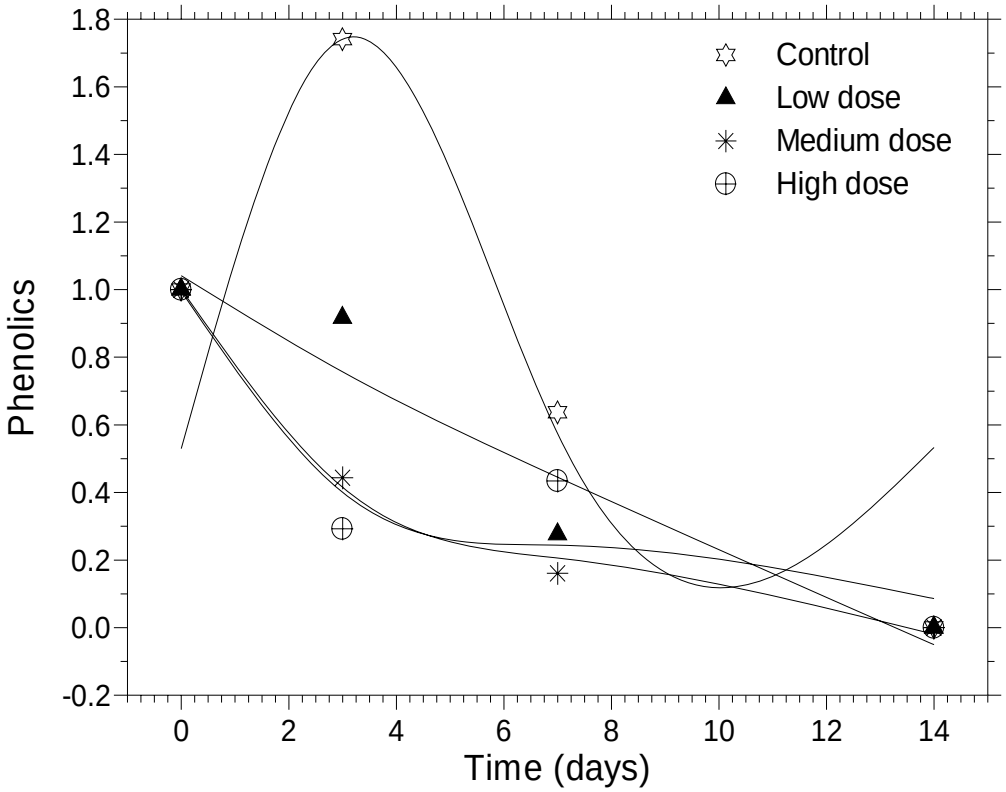

Figure 4-55. Effect of time of storage on phenolics content of blueberries stored up to 14 days at 5°C. (Control =non-irradiated, low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.2 kGy).

The reaction rate constants (k) for phenolics concentration were calculated using Eqn. 4-13 from the ln $(C-C_e/C_o-C_e)$ versus time plots (Figure 4-56) and presented in Table 4-28.

Table 4-28
Rate constant (k) and R^2 for changes in phenolics of blueberries stored up to 14 days at 5°C

Treatment	k (days^{-1})	(EMS)*	R^2
0.0 kGy	0.86	9.9	0.80``
1.1 kGy	0.88	7.6	0.85
1.6 kGy	0.57	1.55	0.92
3.2 kGy	0.93	11.2	0.81

*Mean square error

The reaction rate constants indicate that in this study, the changes in phenolic compounds occurred faster in samples treated at high dose. The phenolics content decreased at a slower rate in the 1.6 kGy treated samples. This variation may be associated with the ripening of the fruits and therefore, differences in phenolics content. The concentration of phenolics decreases as the fruit matures so; it is possible that the fruits treated with 1.6 kGy had been more mature than those treated with the other doses.

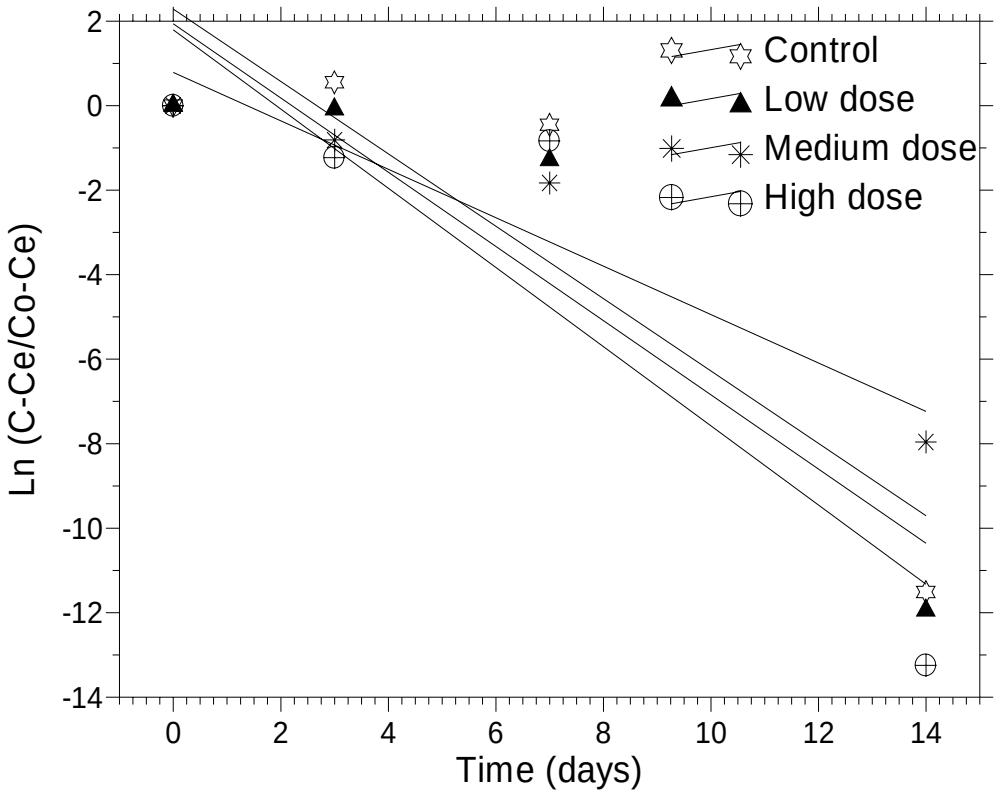

Figure 4-56. Relationship between phenolics content and time of storage at different irradiation doses applied on blueberries stored up to 14 days at 5°C. (Control=non-irradiated, low dose=1.1 kGy, medium dose=1.6 kGy, high dose=3.2 kGy).

The changes in phenolics were described by $(P) = \frac{C - C_e}{C_o - C_e} = A exp^{(-kt)}$ as,

$$P(\text{control}) = 2.28\exp^{(-0.86t)},\ R^2 = 0.80 \quad (4\text{-}78)$$

$$P(1.1\ \text{kGy}) = 1.94\exp^{(-0.88\ t)},\ R^2 = 0.85 \quad (4\text{-}79)$$

$$P(1.6\ \text{kGy}) = 0.79\exp^{(-0.57\ t)},\ R^2 = 0.92 \quad (4\text{-}80)$$

$$P(3.2\ \text{kGy}) = 1.80\exp^{(-0.93\ t)},\ R^2 = 0.81 \quad (4\text{-}81)$$

The above equations suggest a decreasing trend on phenolics content with time independent of the treatment.

4.3.2.5 *Changes in respiration rate*

The changes in respiration rate indicated a rapid reduction of this parameter in all samples from day zero until day three. From day three until the end of the storage the respiration rate was almost constant for all berries (Figure 4-57).

The rate constant *k* was calculated from the slope (dCO_2/dt) of each of the decreasing trend from day zero until day three. The results (Table 4-29) indicate that the respiration rate decreased faster in samples exposed to high dose (3.2 kGy) than in the other treatments. This behavior could be associated with the structural changes induced by irradiation which induce changes in the respiration's mechanism. In addition, the decrease in respiration is associated with the variation of the maturity levels of the samples. In non-climacteric fruits such us blueberries there is not a raise in the respiration during ripening.

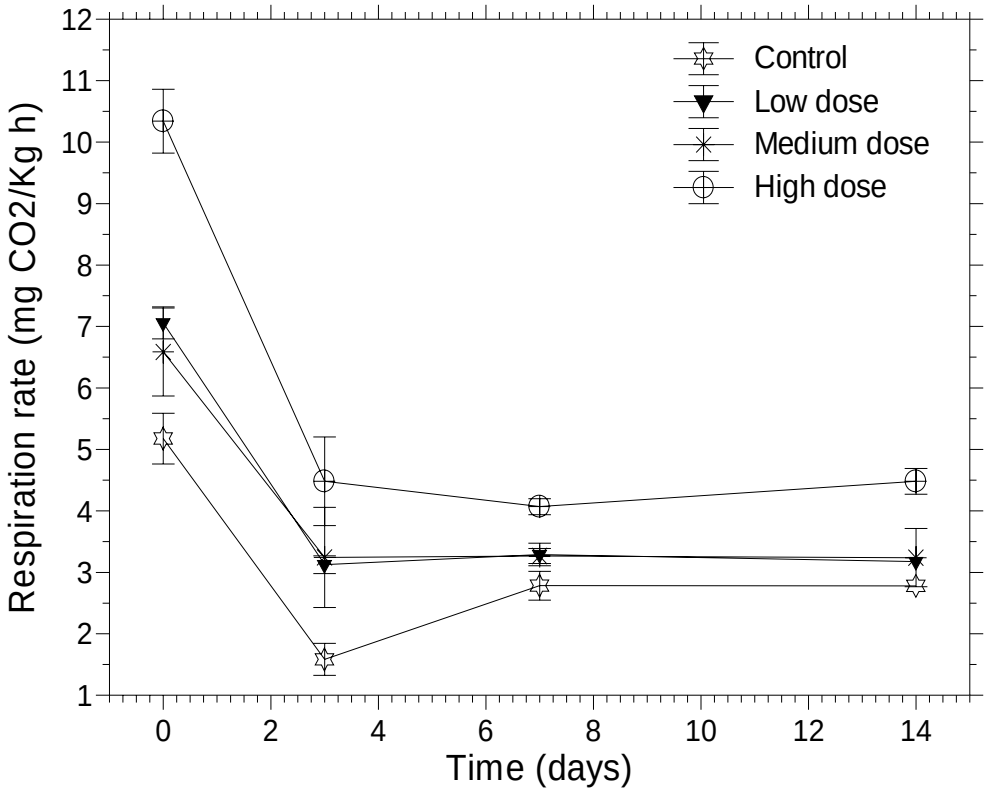

Figure 4-57. Effect of time of storage on respiration rate (mg CO_2/Kg h) of blueberries stored up to 14 days at 5°C. (Control =non-irradiated, low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.2 kGy).

Table 4-29
Rate constant (k) and R^2 for changes in respiration rate of blueberries stored up to 14 days at 5°C

Treatment	***k* (days^{-1})**	**R^2**
0.0 kGy	1.16	0.87
1.1 kGy	1.27	0.97
1.6 kGy	1.03	0.84
3.2 kGy	2.01	0.96

CHAPTER V

CONCLUSIONS

This study was focused on determining the effect of electron beam irradiation on the quality attributes of mango and blueberries and the following conclusions were reached:

For the application of electron beam irradiation on mangoes at low (1.0 kGy), medium (1.5 kGy) and high (3.1 kGy) doses:

1. Lightness (*L*) increased significantly (P>0.05) by the end of storage time in all irradiated samples while redness (*a*) and yellowness (*b*) values increased significantly (P>0.05) in all treatments at the fifth day of storage. Possible changes in carotene content could be related to the color changes. Values of redness (*a*) of mangoes decreased in samples irradiated at high dose, meaning they had a greener color. These results might be associated with the delay in ripening due to irradiation at the higher dose. Yellowness (*b*) values were larger for the control than the irradiated samples by the end of the storage, giving an indication of ripening. Chroma (*C*) and hue (*H*) parameters increased significantly (P>0.05) with time being significant for all the irradiated samples. The total color difference (ΔE)) values were not affected significantly (P>0.05) by irradiation. The low dose (1.0 kGy) was the best irradiation dose to maintain fruit color quality attributes.

2. Irradiated mango samples were significantly (P>0.05) less firm than the control immediately after the irradiation treatment and throughout the storage time. Samples irradiated at high dose (3.1 kGy) were significantly (P>0.05) softer, requiring 82.7% less force to rupture than the control samples. Both the control and the samples irradiated with 1.0 kGy showed more resistance to rupture. The softness of the samples was associated with the changes in the structural cell such as cracks and depressions on the surface and the breakdown of the cells and its components. Irradiated samples were less tough and less stiff than the control fruit. In summary, irradiation of mango fruits with doses higher than 1.0 kGy has a detrimental effect on the fruit's texture quality.
3. The exposure of mangoes to irradiation increased the respiration rate. Doses up to 1.5 kGy keep respiration at a normal level and extend the fruit's shelf life by approximately 3 days.
4. Irradiation did not affect the specific gravity of mangoes.
5. No significant (P>0.05) effect of irradiation on moisture and juiciness of mangoes was detected at the dose levels this study.
6. Differences in pH values were reported during storage time but the exposure of mangoes to irradiation levels up to 1.5 kGy does not affect the pH and the fruit has an acceptable acidity level.
7. Overall, mango samples had increased (approximately 7.0%) acidity during the storage time indicating the unripe stage of the samples. However, when the

mangoes were exposed to irradiation levels up to 1.5 kGy the acidity levels were normal.

8. The total soluble solids content of mangoes was reduced (12.3%) by irradiation treatment during the storage time. The exposure of the fruits at 1.0 kGy minimizes the change.
9. The exposure of mangoes to ionizing irradiation has an inconsistent effect in total sugars but it enhances the reducing sugars content of the fruit. Doses up to 1.5 kGy keep the sugars content of mangoes within an acceptable quality.
10. Phenolics compounds in mango significantly ($P>0.05$) increased with the irradiation treatment; concentrations were higher in samples irradiated at medium (27.44%) and high (18.34%) doses. This trend corresponded to the increase in the antioxidant activity index and the stimulation of the synthesis of phenyalalanine ammonia lyase (PAL).
11. Irradiation at all levels caused a significant ($P>0.05$) decrease (50 to 70%) in the ascorbic acid content of mango during storage. However, the treatment at dose levels up to 1.0 kGy minimizes the ascorbic acid reduction.
12. Irradiation of mangoes at dose levels up to 3.1 kGy raised the concentration of the volatile compounds which are important components to the odor and flavor of the fruit.
13. The exposure of mangoes to ionizing irradiation at low (1.0 kGy) and medium (1.5 kGy) doses increased significantly carotenoids content of the fruits.

14. Irradiation of mangoes up to 1.5 kGy does not affect the sensory quality of the fruits.

Overall, the previous results suggest that irradiation treatment of mangoes may lead to a stress condition which depending of the dose, may or may not cause the physicochemical changes that depend on the physiological parameters of the fruit. In general, irradiation of mangoes up to 1.5 kGy treatment maintains the overall quality of mangoes and may increase the shelf-life by three days (from 18 days to 21 days when stored at 12˚) by delaying the ripening.

For the application of electron beam irradiation of blueberry fruits exposed at low (1.1 kGy), medium (1.6 kGy) and high (3.2 kGy) doses:

1. The irradiation of blueberries at low (1.1 kGy) and medium (1.6 kGy) doses reduced the lightness (L) of the fruits. Redness (a) and yellowness (b) values were higher in samples irradiated at low dose (1.1 kGy). Chroma (C) and total color difference (ΔE) increased significantly ($P>0.05$) in all irradiated samples during storage time. The best dose to preserve the color quality of irradiated blueberries was 1.6 kGy.
2. The shear force of blueberries decreased significantly ($P>0.05$) as the irradiation dose increased. The samples treated with a higher dose (3.2 kGy) required much less force to shear. Irradiation of blueberries with doses as high as 3.2 kGy will yield unacceptable fruits in terms of texture. However, doses up to 1.6 kGy do not cause any detrimental change in texture.

3. Irradiation induces drying of the skin of blueberries with no consistency in the cells shape. This effect is more severe at a high dose producing micro-cracks and bleeding of the surface. Irradiation causes the loss of cell structure and shape of the blueberry epidermis due to tissue shrinkage.
4. Irradiation of blueberries increases the respiration rate of the fruits. However, doses up to 3.1 kGy keeps respiration at a normal level for shelf life preservation.
5. The exposure of blueberries to irradiation at dose levels up to 3.2 kGy does not affect the density and specific gravity of the fruit.
6. The exposure of blueberries at dose levels up to 3.2 kGy would preserve the moisture content of the fruit.
7. Irradiation of blueberries at doses up to 3.2 kGy does not affect the water activity of the fruits and may increase their shelf-life.
8. The pH of blueberries is not affected by irradiation doses up to 3.2 kGy.
9. The irradiation of blueberries at doses up to 3.2 kGy maintains the acidity levels of the fruit.
10. Irradiation decreases the total soluble solids (°Brix) content of blueberries, but the exposure of the fruits at 1.1 kGy minimizes the decrease.
11. The exposure of blueberries to irradiation doses up to 3.2 kGy decreases the total and reducing sugars of the fruit. However, the exposure at dose levels up to 1.6 kGy better maintains the sugar content of the fruit.

12. The exposure of blueberries at dose levels up to 1.6 kGy may enhances the phenolics content of the fruit and maintains their nutritional and flavor properties.
13. The exposure of blueberries up to dose levels of 3.2 kGy decreases (~28.15%) the ascorbic acid concentration but at doses up to 1.6 kGy the reduction is minimized.
14. The exposure of blueberries to irradiation levels up to 3.2 kGy enhances the production of the volatile compounds that characterize the aroma of the fruits.
15. Irradiation of blueberries at doses levels up to 3.2 kGy enhances the tannins content of the fruits.
16. Irradiation of blueberries up to 1.6 kGy does not affect the sensory quality of the fruits.

These results support the applicability of electron beam irradiation at doses up to 1.6 kGy to ensure and preserve the shelf-life of blueberries up to 14 days while maintaining the quality characteristics of the fruits. A potential benefit is the increased phenolics and tannins content of fruit irradiated at 1.1 kGy.

The second part of this study focused on quantifying the main quality changes by using kinetics principles. According to the results, the main effect of irradiation on physical and chemical properties was observed in color, texture and ascorbic acid content. Therefore, these parameters were considered for kinetics evaluation. The following conclusions were reached:

1. For both fruits, mango and blueberry, the changes in color parameters found in the present study could not be described by any simple kinetics model.
2. Radiation softening of mango was described by a first order rate process. At the conditions used in this study, non- irradiated fruits had less texture degradation than the irradiated samples.
3. The changes in firmness followed a fractional conversional model with a faster reaction rate in samples treated with a high dose (3.1 kGy).
4. Ascorbic acid degradation of irradiated mango followed a first order reaction kinetics. In the present study, ascorbic acid degraded faster in samples irradiated with a medium dose than the other treatments.
5. Due to the limited availability of data, no single model was found to define the kinetics of softening in blueberries.
6. The degradation of ascorbic acid over time in blueberries occurred faster in the control samples than in all the other treatments. Thus, irradiation dose slowed down this reaction.
7. The changes in the phenolics compounds of blueberries occurred at a faster rate in the samples treated with a high dose of irradiation.
8. The respiration rate of blueberries decreased faster in samples exposed to high dose (3.2 kGy) than in the other treatments.

CHAPTER VI

RECOMMENDATIONS FOR FURTHER STUDY

The following are recommendations for future research in the application of electron beam irradiation to fruits and vegetables:

- Evaluate different fruit maturity levels and the combined effect of the treatment on produce quality and safety.
- Pre-treat the fruits by using salts and then evaluate the combined effect with irradiation on fruit firmness.
- Develop an experimental design that gives the feasibility of the kinetic analyses.
- Perform kinetics studies of the fruits evaluating different types of irradiation.
- Determine phenolic concentrations of irradiated fruits and their antifugal activity.
- Evaluate the starch content in the irradiated fruits.
- Determine the ethylene production of the irradiated fruits.
- Perform the electron scanning microscopy of the chloroplast in the tissues of the irradiated samples.

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APPENDIX A

APPENDIX A-A.

ACCEPTANCE TEST

Date: / / 2004 Product code: _____

1. Please, evaluate the **overall quality** of this sample. Place a mark in the box which you feel best describes how you like the sample. **Don't eat the sample**. An honest expression of your personal feelings will help us.
Thank you.

☐	☐	☐	☐	☐
Like extremely	Like slightly	Neither like nor dislike	Dislike slightly	Dislike extremely

2. Indicate by placing a mark how you feel the sample rate in each category below:
Color

☐	☐	☐	☐	☐
Like extremely	Like slightly	Neither like nor dislike	Dislike slightly	Dislike extremely

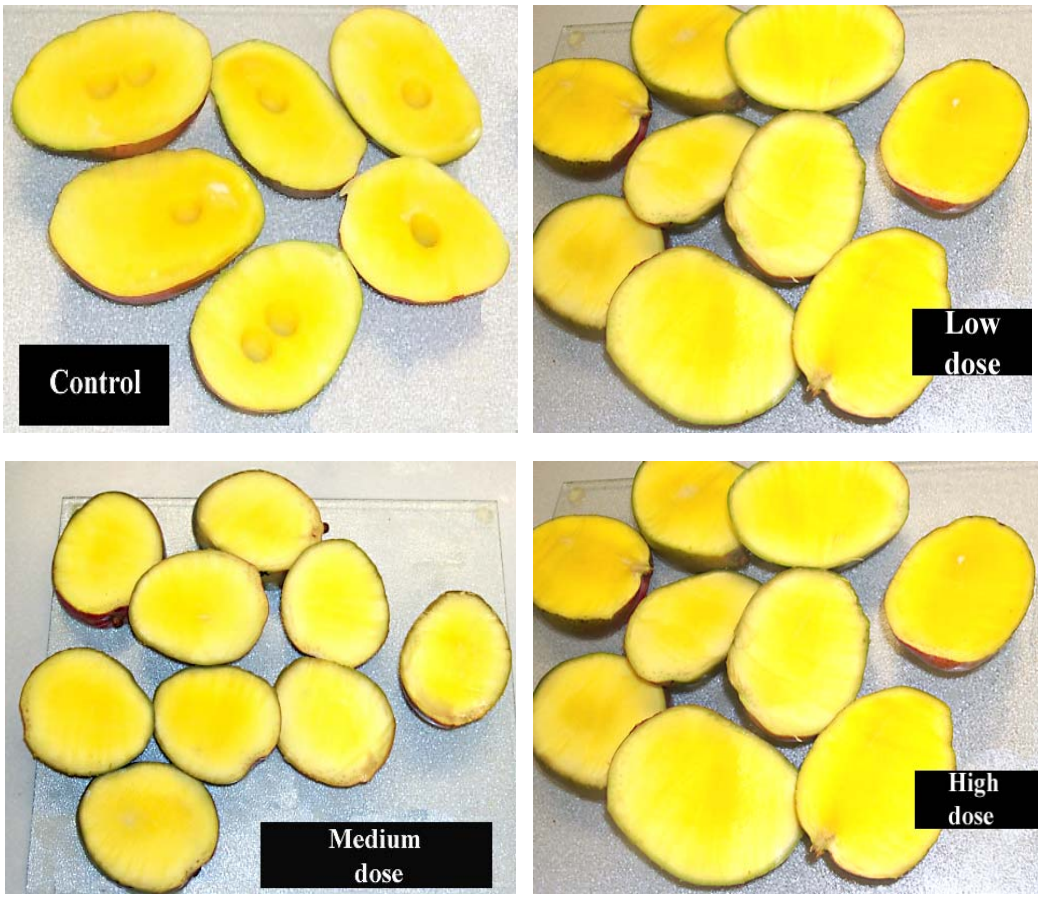
Texture

☐	☐	☐	☐	☐
Firm	Slightly firm	Somewhat firm-soft	Slightly soft	Soft

Aroma (mango/ blueberry)

☐	☐	☐	☐	☐
Strong	Moderate strong	Moderate	Slightly	None

Comments:……
……..



Appendix A-B. Effect of irradiation on the flesh of mango stored at 12°C for 21 days. (Control =non-irradiated, low dose =1.0 kGy, medium dose =1.5 kGy, high dose =3.1 kGy).

Table A-1. Effect of irradiation dose on texture attributes of mangoes stored up to 21 days at 12°C

	Dose/Day	0	5	10	21
Force to rupture (N)	Control* (0 kGy)	177.94 (54.48) ax	121.41 (108.91) ax	119.27 (96.67) ax	140.99 (41.06) ax
	Low (1.0 kGy)	73.50 (53.04) ay	47.54 (9.78) ay	82.87 (68.44) axy	102.87 (30.49) ay
	Medium (1.5 kGy)	66.90 (23.93) ay	16.21 (848) by	57.78 (31.21) axy	82.65 (15.05) ay
	High (3.1 kGy)	24.29 (9.24) aby	17.13 (0.29) ay	28.70 (7.66) by	31.30 (4.78) bz
	Dose/Day	0	5	10	21
Toughness(J)	Control* (0 kGy)	0.42 (0.15) ax	0.37 (0.44) ax	0.30 (0.27) ax	0.35 (0.11) ax
	Low (1.0 kGy)	0.14 (0.11) ay	0.06 (0.04) ay	0.13 (0.20) ay	0.11 (0.07) ay
	Medium (1.5 kGy)	0.12 (0.35) ay	0.02 (0.01) by	0.06 (0.03) cy	0.08 (0.01) cyz
	High (3.1 kGy)	0.05 (0.14) ay	0.03 (0.02) by	0.04 (0.01) aby	0.04 (0.01) abz
	Dose/Day	0	5	10	21
Stiffness (Young 's Modulus, MPa)	Control* (0 kGy)	0.78 (0.24) ax	0.53 (0.48) ax	0.53 (0.43) ax	0.62 (0.18) aw
	Low (1.1 kGy)	0.32 (0.23) ay	0.21 (0.18) ay	0.37 (0.30) axy	0.45 (0.13) ayz
	Medium (1.6 kGy)	0.29 (0.11) ay	0.07 (0.04) by	0.25 (0.14) axy	0.36 (0.07) ayz
	High (3.2 kGy)	0.11 (0.04) aby	0.08 (0.05) ay	0.13 (0.03) by	0.14 (0.02) bx

*Control, non-irradiated samples. All analyses were made is SPSS, SNK procedure 95%confidence
Values in parenthesis are the standard deviations.
[a-c]Means within a column which are not followed by a common superscript letter are significantly different (P<0.05).
[x-z]Means within a row which are not followed by a common superscript letter are significantly different (P<0.05).

Table A-2. Effect of irradiation dose on specific gravity of mangoes stored up to 21 days at 12°C

Dose	Days			
	0	**5**	**10**	**21**
Control* (0 kGy)	1.165 [ax] (0.426)	1.172 [ax] (0.200)	1.161 [ax] (0.143)	0.987 [ax] (0.010)
Low (1.0k Gy)	1.018 [ax] (0.016)	1.025 [ax] (0.046)	1.037 [ax] (0.055)	1.027 [ax] (0.047)
Medium (1.5 kGy)	1.042 [ax] (0.032)	1.015 [ax] (0.004)	1.034 [ax] (0.029)	1.016 [ax] (0.021)
High (3.1 kGy)	1.032 [ax] (0.018)	1.031 [ax] (0.031)	1.084 [ax] (0.092)	1.025 [ax] (0.014)

*Control, non-irradiated samples. All analyses were made is SPSS, SNK procedure 95%confidence

Values in parenthesis are the standard deviations.

[a]Means within a column which are not followed by a common superscript letter are significantly different (P<0.05).

[x]Means within a row which are not followed by a common superscript letter are significantly different (P<0.05).

Table A-3. Effect of irradiation dose on water activity of mangoes stored up to 21 days at 12°C

Day/Dose	Control* (0 kGy)	Low (1.0 kGy)	Medium (1.5 kGy)	High (3.1 kGy)
0	0.89^{ax}(0.01)	0.88^{ax}(0.02)	0.89^{ax}(0.01)	0.89^{ax}(0.00)
5	0.90^{ax}(0.00)	0.90^{bx}(0.00)	0.91^{ax}(0.00)	0.90^{ax}(0.01)
10	0.86^{bx}(0.02)	0.91^{by}(0.00)	0.90^{ay}(0.00)	0.90^{ay}(0.01)
21	0.92^{cx}(0.00)	0.91^{by}(0.00)	0.92^{bx}(0.00)	0.91^{bxy}(0.01)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.

Numbers in parenthesis are the standard deviation.

[a-c]Means within a row which are not followed by a common superscript letter are significantly different (P<0.05).

[x-y]Means within a column which are not followed by a common superscript letter are significantly different (P<0.05).

Table A-4. Effect of irradiation dose on pH of mangoes stored up to 21 days at 12°C

Dose	Days			
	0	**5**	**10**	**21**
Control* (0 kGy)	3.14 [ax] (0.020)	3.27 [bx] (0.015)	3.33 [bx] (0.061)	3.19 [aw] (0.010)
Low (1.0 kGy)	3.39 [ay] (0.015)	3.29 [bx] (0.017)	3.19 [cy] (0.036)	3.11 [dx] (0.006)
Medium (1.5 kGy)	3.26 [az] (0.006)	3.44 [by] (0.021)	3.23 [cy] (0.010)	3.15 [dy] (0.000)
High (3.1 kGy)	3.24 [az] (0.015)	3.21 [bz] (0.010)	3.23 [aby] (0.006)	3.28 [cz] (0.010)

*Control, non-irradiated samples. All analyses were made is SPSS, SNK procedure 95%confidence
Values in parenthesis are the standard deviations.
[a-d]Means within a column which are not followed by a common superscript letter are significantly different (P<0.05).
[w-z]Means within a row which are not followed by a common superscript letter are significantly different (P<0.05).

Table A-5 Ratio sugar/acid of irradiated and non-irradiated mangoes stored up to 21 days at 12°C

Dose/Day	Control* (0.0 kGy)	Low (1.0 kGy)	Medium (1.5 kGy)	High (3.1 kGy)
0	9.53	11.76	10.95	11.62
5	10.54	9.50	11.44	8.75
10	12.69	8.29	9.29	9.45
21	9.83	8.96	8.97	10.74

*Control, non-irradiated samples.

Table A-6. Effect of irradiation dose on total phenolics (mg Gallic acid/100g w.b.) and antioxidant activity index (% of reduction DPPH/g w.b) of mangoes stored up to 21 days at 12°C

Total phenolics (mg Gallic acid/100g w.b.)	**Dose/Day**	**0**	**5**	**10**	**21**
	Control*	13.732 ^{ax}	24.703 ^{bw}	21.895 ^{cw}	18.824 ^{dx}
	(0 kGy)	(0.372)	(0.689)	(0.392)	(0.433)
	Low	21.275 ^{ay}	18.523 ^{bx}	17.583 ^{bx}	20.495 ^{ay}
	(1.0 kGy)	(0.853)	(1.520)	(0.592)	(0.355)
	Medium	17.681 ^{az}	22.167 ^{by}	20.747 ^{by}	24.370 ^{cz}
	(1.5 kGy)	(0.532)	(1.783)	(0.421)	(2.590)
	High	17.725 ^{az}	20.119 ^{bz}	22.664 ^{cz}	21.707 ^{dy}
	(3.1 kGy)	(0.282)	(0.523)	(0.795)	(0.351)
Antioxidant index (% /g w.b.)	**Dose/Day**	**0**	**5**	**10**	**21**
	Control*	4.527 ^{ax}	5.066 ^{ax}	5.956 ^{bx}	4.298 ^{ax}
	(0 kGy)	(0.486)	(0.042)	(0.608)	(0.487)
	Low	4.811 ^{ax}	4.319 ^{by}	3.969 ^{cy}	4.459 ^{bx}
	(1.0 kGy)	(0.262)	(0.001)	(0.159)	(0.021)
	Medium	4.465 ^{ax}	5.315 ^{bx}	4.549 ^{ay}	4.981 ^{by}
	(1.5 kGy)	(0.211)	(0.077)	(0.017)	(0.187)
	High	4.308 ^{ax}	4.470 ^{ay}	5.306 ^{bz}	5.118 ^{by}
	(3.1 kGy)	(0.233)	(0.287)	(0.192)	(0.057)

*Control, non-irradiated samples. All analyses were made is SPSS, SNK procedure 95%confidence

Values in parenthesis are the standard deviations.

$^{a-d}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

$^{w-z}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).

Table A-7. Effect of irradiation dose on texture attributes of blueberries stored up to 14 days at 5°C

	Dose/Day	0	3	7	14
Shear Force (N)	Control* (0 kGy)	163.475 [ax] (18.161)	203.359 [bx] (5.879)	204.251 [bw] (3.797)	154.147 [ax] (6.758)
	Low (1.1 kGy)	118.692 [ay] (7.793)	125.523 [ay] (13.646)	129.586 [ax] (5.064)	131.893 [ay] (8.276)
	Medium (1.6 kGy)	109.991 [ay] (7.372)	119.189 [ay] (4.524)	113.298 [ay] (10.739)	118.568 [ay] (9.083)
	High (3.2 kGy)	83.357 [az] (4.266)	90.542 [az] (4.430)	71.666 [az] (6.617)	91.270 [az] (7.761)
	Dose/Day	0	3	7	14
Toughness (J)	Control* (0 kGy)	4.171 [ax] (0.235)	4.946 [bw] (0.149)	5.065 [bw] (0.459)	3.663 [ax] (0.223)
	Low (1.1 kGy)	2.968 [ay] (0.107)	3.406 [ax] (0.301)	3.412 [ax] (0.192)	3.295 [axy] (0.148)
	Medium (1.6 kGy)	2.785 [ay] (0.226)	2.981 [ay] (0.074)	2.884 [ay] (0.051)	3.020 [ay] (0.262)
	High (3.2 kGy)	2.117 [az] (0.028)	2.480 [bz] (0.000)	1.994 [az] (0.089)	2.369 [bz] (0.204)

*Control, non-irradiated samples. All analyses were made is SPSS, SNK procedure 95%confidence

Values in parenthesis are the standard deviations.

[a-b]Means within a column which are not followed by a common superscript letter are significantly different (P<0.05).

[w-z]Means within a row which are not followed by a common superscript letter are significantly different (P<0.05).

Table A-8. Effect of irradiation dose on specific gravity of blueberries stored up to 14 days of at 5°C

Dose	Day			
	0	**3**	**7**	**14**
Control* (0 kGy)	0.978 [ax] (0.017)	0.975 [ax] (0.003)	0.914 [bx] (0.010)	0.979 [ax] (0.029)
Low (1.1 kGy)	0.962 [ax] (0.041)	0.982 [abx] (0.016)	0.986 [aby] (0.026)	1.025 [by] (0.014)
Medium (1.6 kGy)	0.972 [ax] (0.022)	0.980 [ax] (0.004)	0.953 [ay] (0.011)	0.980 [ax] (0.029)
High (3.2 kGy)	0.962 [ax] (0.252)	0.973 [ax] (0.004)	0.985 [ay] (0.004)	0.982 [ax] (0.024)

*Control, non-irradiated samples. All analyses were made is SPSS, SNK procedure 95%confidence

Values in parenthesis are the standard deviations.

[a-b]Means within a column which are not followed by a common superscript letter are significantly different (P<0.05).

[x-y]Means within a row which are not followed by a common superscript letter are significantly different (P<0.05).

Table A-9. Effect of irradiation on moisture content (%w.b.) of blueberry up to 14 days of storage at 5°C

Dose	Day			
	0	**3**	**7**	**14**
Control* (0 kGy)	79.58 [ax] (0.28)	80.81 [bw] (0.21)	80.49 [bx] (0.21)	79.58 [ax] (0.21)
Low (1.1 kGy)	80.36 [axy] (0.38)	80.40 [ax] (0.07)	80.63 [ax] (0.10)	81.00 [ay] (0.41)
Medium (1.6 kGy)	80.66 [ay] (0.42)	81.47 [aby] (0.29)	81.85 [by] (0.20)	80.75 [ay] (0.58)
High (3.2 kGy)	81.87 [az] (0.93)	81.87 [az] (0.18)	81.55 [ay] (0.11)	81.83 [az] (0.90)

*Control, non-irradiated samples. All analyses were made is SPSS, SNK procedure 95%confidence

Values in parenthesis are the standard deviations.

[a-b]Means within a column which are not followed by a common superscript letter are significantly different (P<0.05).

[w-z]Means within a row which are not followed by a common superscript letter are significantly different (P<0.05).

Table A-10. Effect of irradiation dose on water activity of blueberries stored up to 14 days at 5°C

Day/Dose	Control* (0 kGy)	Low (1.1 kGy)	Medium (1.6 kGy)	High (3.2 kGy)
0	0.89^{ax}(0.01)	0.92^{ax}(0.00)	0.87^{ax}(0.01)	0.87^{ax}(0.01)
3	0.89^{ax}(0.00)	0.89^{bx}(0.00)	0.89^{bx}(0.00)	0.89^{bx}(0.00)
7	0.86^{ax}(0.01)	0.89^{by}(0.00)	0.89^{by}(0.00)	0.89^{by}(0.00)
14	0.89^{ax}(0.00)	0.90^{ay}(0.00)	0.91^{by}(0.02)	0.91^{by}(0.00)

*Control, non-irradiated samples. All analyses were made in SPSS, SNK procedure 95% confidence.
Numbers in parenthesis are the standard deviation.
[a-b]Means within a row which are not followed by a common superscript letter are significantly different (P<0.05).
[w-y]Means within a column which are not followed by a common superscript letter are significantly different (P<0.05).

Table A-11. Effect of irradiation dose on pH of blueberries stored up to 14 days at 5°C

Dose	Day			
	0	3	7	14
Control* (0 kGy)	3.05 (0.010) ax	3.14 (0.012) bx	3.17 (0.010) bcx	3.18 (0.020) cx
Low (1.1 kGy)	2.99 (0.021) ay	3.17 (0.010) bxy	3.14 (0.021) bx	3.22 (0.030) cx
Medium (1.6 kGy)	3.04 (0.000) ax	3.17 (0.012) bxy	3.17 (0.006) bx	3.21 (0.020) cx
High (3.2 kGy)	2.96 (0.010) az	3.19 (0.012) bcy	3.17 (0.015) bx	3.21 (0.025) cx

*Control, non-irradiated samples. All analyses were made is SPSS, SNK procedure 95%confidence
Values in parenthesis are the standard deviations.
[a-d]Means within a column which are not followed by a common superscript letter are significantly different (P<0.05).
[w-z]Means within a row which are not followed by a common superscript letter are significantly different (P<0.05).

Table A-12 Ratio sugar/acid of irradiated and non-irradiated blueberries stored up to 14 days at 5°C

Dose/Day	Control* (0.0 kGy)	Low (1.1 kGy)	Medium (1.6 kGy)	High (3.2 kGy)
0	18.99	17.86	19.30	17.10
3	18.69	20.24	18.94	24.06
7	22.21	21.49	20.49	24.98
14	21.53	21.45	22.53	21.55

*Control, non-irradiated samples.

Table A-13. Effect of irradiation dose on total phenolics (mg Gallic acid/100g w.b.) and antioxidant activity index (% of reduction DPPH/g w.b) of blueberries stored up to 14 days at 5°C

Total phenolics (mg Gallic acid/100g w.b.)	**Dose/Day**	**0**	**3**	**7**	**14**
	Control*	187.117 ^{ax}	132.401 ^{bx}	154.433 ^{cx}	167.153 ^{dx}
	(0 kGy)	(5.073)	(3.216)	(7.440)	(8.758)
	Low	189.541 ^{ax}	159.921 ^{by}	178.359 ^{cy}	174.085 ^{cx}
	(1.1 kGy)	(4.899)	(3.450)	(3.197)	(2.165)
	Medium	199.393 ^{ay}	139.171 ^{bx}	150.975 ^{bcx}	157.655 ^{cx}
	(1.6 kGy)	(10.578)	(5.907)	(4.515)	(12.40)
	High	193.133 ^{axy}	120.024 ^{bz}	161.105 ^{cz}	136.563 ^{dy}
	(3.2 kGy)	(4.095)	(17.399)	(5.104)	(6.503)
Antioxidant index (% /g w.b.)	**Dose/Day**	**0**	**3**	**7**	**14**
	Control*	13.819 ^{ax}	11.415 ^{bx}	11.967 ^{bx}	13.552 ^{ax}
	(0 kGy)	(0.686)	(0.782)	(0.665)	(0.345)
	Low	13.481 ^{ax}	12.702 ^{ax}	12.471 ^{ax}	12.576 ^{axy}
	(1.1 kGy)	(0.523)	(0.512)	(0.699)	(1.423)
	Medium	15.250 ^{ay}	10.655 ^{bx}	11.214 ^{bx}	11.860 ^{bxy}
	(1.6 kGy)	(0.746)	(0.813)	(0.867)	(4.673)
	High	13.335 ^{ax}	10.200 ^{bx}	12.336 ^{abx}	10.457 ^{by}
	(3.2 kGy)	(0.655)	(1.778)	(0.371)	(0.656)

*Control, non-irradiated samples. All analyses were made is SPSS, SNK procedure 95%confidence

Values in parenthesis are the standard deviations.

$^{a-d}$Means within a column which are not followed by a common superscript letter are significantly different ($P<0.05$).

$^{x-z}$Means within a row which are not followed by a common superscript letter are significantly different ($P<0.05$).

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