

## Irradiation Effects on the Chemical Quality of Guavas

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**Abstract:** The aim of this research is to evaluate the effect of radiation treatment on the chemical changes of main components of guavas (*Psidium guajava*, var. media china). The quality of guavas irradiated by Co-60 gamma rays at 150, 200, and 300 Gray as Gy/min were evaluated during storage at room and low temperature. Results indicated that the differences observed are principally associated with maturity stages, temperature, and changes attributed to physiological and metabolic processes. Radiation treatment produced reductions in ascorbic acid and  $\beta$ -carotene. The results suggest that fruit in storage can recover from stress produced by treatment. No other significant changes were observed in any other parameters including sugars, pectin, and citric acid.

**Keywords:** Ascorbic acid,  $\beta$ -carotene, citric acid, guavas, pectin, radiation treatment, sugars

### INTRODUCTION

Research shows that diets rich in fruits and vegetables could be associated with lower risks for chronic diseases (Feng *et al.*, 2006; Sato *et al.*, 2005). Health programs in recent years have encouraged the consumption of fruits and vegetables (Hendy *et al.*, 2005; Klepp *et al.*, 2005; Sandvik *et al.*, 2005; Blanchette and Brug, 2005; Yngve *et al.*, 2005; Devine *et al.*, 2005).

Consumers look for attractive produce with a long shelf life. Maintaining the quality and safety of fresh produce is a challenge. Serious problems associated with fruit are ensuring hygienic quality and assuring the distribution to both national and international markets for retail and wholesale trade.

Guava is an important crop in Mexico. In the last few years, production reached up to 298,000 tons/year, mainly for fresh consumption in local markets or processing. This fruit is very attractive for consumers because of its sensorial and nutritional properties; an exquisite flavor and excellent source of vitamin C (López-Enríquez and Mercado-Silva, 2005; Abu-Bakr *et al.*, 2003). For many years it was impossible to export fresh guava to the United States because the fruit is infested by different quarantine pests, principally fruit flies including, *Anastrepha ludens* and/or *Anastrepha striata* (Toledo *et al.*, 2003).

Today has been demonstrated that irradiation is an adequate quarantine treatment to assure the phytosanitary requirements for guava to be allowed

entry into the United States. Since any other phytosanitary treatment like: hot water treatment, ethylene dibromide, or modified atmosphere, are impractical because they damage the fruit (Hallman, 1998; Gould and Sharp, 1992). The chemical fumigants like, ethylene dibromide is no longer used because it is considered to be a potential cancer causing agent. Methyl bromide production is reduced year by year because it depletes the ozone layer (EPA, 2007).

The USDA, Animal and Plant Health Inspection Service (APHIS), has allowed the use of irradiation to treat papayas with 150 Gy since 1989. APHIS (2006) published a Final Rule "Treatments for Fruits and Vegetables" with the list that contains the minimum radiation dose to treat thirteen different plant pests. The rule requires 150 Gy for fruit flies of the family Tephritidae and 400 Gy as a generic treatment for other arthropod pests. Since 2008, Mexico has been exporting irradiated guavas at 400 Gy as a minimum radiation dose with a distribution dose of 2.3, this means that some part of the fruit reach a radiation dose up to 900 Gy, (Bustos-Griffin *et al.*, 2012)

Higher radiation doses can alter some physiological and biochemical functions of fruit, causing damage in the skin and producing off-flavor, and the level of change depends of: the type of fruit, stage of maturity, crop season, variety, and storage conditions (Castell-Perez *et al.*, 2004; Janave and Sharma, 2005; Cheour and Mahjoub, 2003; Calore and Lopes-Vietes, 2003). It is therefore very important to treat the guavas at lower radiation doses and to evaluate

the quality of irradiated fruit in terms of nutritional, chemical, physiological and sensorial properties. This study presents the results obtained in the evaluation of the effect of the irradiation on the chemical properties of guavas.

## MATERIALS AND METHODS

**Samples:** The research was done with guavas (*Psidium guajava*, var. *media china*) from Calvillo, Aguascalientes, Mexico. The lot for study was export quality and fruits were uniform in size and color.

**Irradiation treatment:** The irradiation treatment was carried out in the research loop of the Co-60 gamma irradiation facility, model JS6500 located at the Instituto Nacional de Investigaciones Nucleares (ININ) in Salazar, Edo de Mexico. Fricke dosimeters were used to calibrate the irradiation system and to determine the minimum radiation absorbed dose at 150, 200 and 300 Gray at a rate of 15.94 Gray/min, and with a distribution dose of 1.34. Eighteen guavas were treated at each dose and the same number for the control. Two lots of fruit were irradiated, the first lot was ripe guavas; the second lot was green (immature) guavas.

**Storage conditions:** Once the irradiation treatment was completed, samples (irradiated and controls) were stored at Laboratory of Food and Biotechnology Department of Faculty of Chemistry of National University of Mexico (UNAM). The first lot was stored at room temperature (25°C) for 11 days. The second lot was stored at 5°C for 18 days.

**Sample preparation:** Three guavas from each lot were blended with 100 mL distilled water for 5 min. The homogenized sample was used to evaluate chemical properties. Vitamin assays were carried out immediately following homogenization of the sample.

### Chemical test:

**Ascorbic acid:** The extraction of ascorbic acid was with acetic acid (2%). Readings were done in a High Performance Liquid Chromatography (HPLC), Beckman System Gold 126, coupled to an UV detector, ( $\lambda$  261 nm), Waters Symmetry Shield RP 18 5  $\mu$ m 3.9 x 150 mm column. Mobile phase Methanol: water (5:95) with 5 mM of Cetrimide (hexadecyl trimethyl ammonium bromide, Sigma Chemicals) and 50 mM of monobasic potassium phosphate. The mobile phase was filtered through a 0.45  $\mu$ m membrane (GH poLypro, Gelman laboratory) and degasified in an ultrasonicator (Cavitor, Mettler Electronic Corp) for 30 min. Twenty  $\mu$ L were injected in the column at a rate of 0.8 mL/min (Zapata and Dufour, 1992). A calibration curve was obtained in concentrations ranging from 10 to 250  $\mu$ g/mL of ascorbic acid.

**$\beta$ -carotene (provitamin A):** The extraction was done with hexane-acetone following (Philip and Chen, 1988). Readings were done by HPLC, Beckman Model 110A. The operation condition: Nucleosil 5  $\mu$ m, 100°C, C18 150\*4 mm Column, UV/VIS Detector,  $\lambda$  at 445 nm, phase: Methanol: Hexane (95:5), rate 1 mL/min, Loop: 20  $\mu$ L. The results are expressed in mg  $\beta$ -carotene/100 g and measured using a standard plot in the concentration range from 15 to 50  $\mu$ g/mL.

**Sugars:** Fructose, glucose and sucrose were analyzed by HPLC (Nollet, 1996), Beckman Chromatograph Gold 126 coupled to a Refraction Index Detector (Perkin-Elmer LC-30, and a high performance carbohydrate column (4  $\mu$ m, 4.6x250 mm, Waters). Mobile Phase: Acetonitrile: water (80:20), rate: 1.4 mL/min, Loop: 20  $\mu$ L. The results were expressed in g Carbohydrates/100 g of guava and measured using calibration curves obtained using fructose (Analytical reagent, Técnica Química), glucose (Analytical reagent, Técnica Química), and sucrose (Analytical reagent, Caledon) ranging 1-10 mg/mL.

**Pectins:** This extraction was with 80% ethanol. Pectins were quantified as uronic acids by spectrophotometric technique (Scott, 1979). This is an indirect method to assay the pectins.

**Citric acid (titratable acidity):** Titratable acidity was determined using the AOAC Method 942.15 and is expressed conventionally in citric acid (g)/100 g guava (Cunnif, 1995).

**Statistical analysis:** Analysis of Variance (ANOVA) were conducted with Statgraphics Centurion XV for Windows Version 15.02.06, (1982-2007) with a mean study using the Duncan method at  $p < 0.05$ .

## RESULTS AND DISCUSSION

**Ascorbic acid:** The initial concentration of ascorbic acid was similar in unripe and ripe guavas and was lower than McCook-Russell *et al.* (2012) reported. No differences were found for different stages of maturity. A decrease of ascorbic was observed in both lots after the irradiation (Fig. 1 and 2). The loss of ascorbic acid increased with radiation dose. Ripe guavas irradiated at 150 Gy lost close to 19%, and approximately 30% at 300 Gy. The loss of ascorbic acid in storage was less at higher doses than lower doses, such that, after eleven days of storage at room temperature, the loss of ascorbic acid is very similar at any radiation dose. This indicates that irradiated fruit try to recover normal physiological processes after the damage produced by irradiation. The loss of ascorbic acid in unirradiated fruit was 24.71% during the storage time.

The loss of ascorbic acid in unripe guavas one day after the irradiation treatment was only 1.83% at 150 Gy, 11.81% at 200 Gy, and 20% at 300 Gy. After

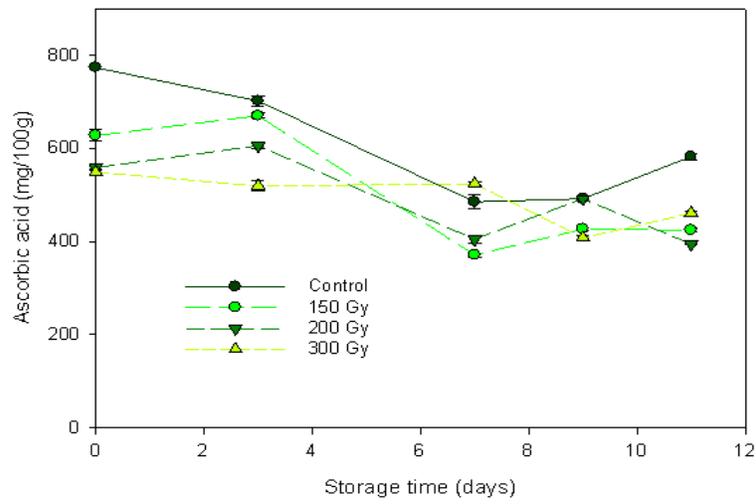


Fig. 1: Ascorbic acid content in irradiated guavas stored at room temperature (25°C)

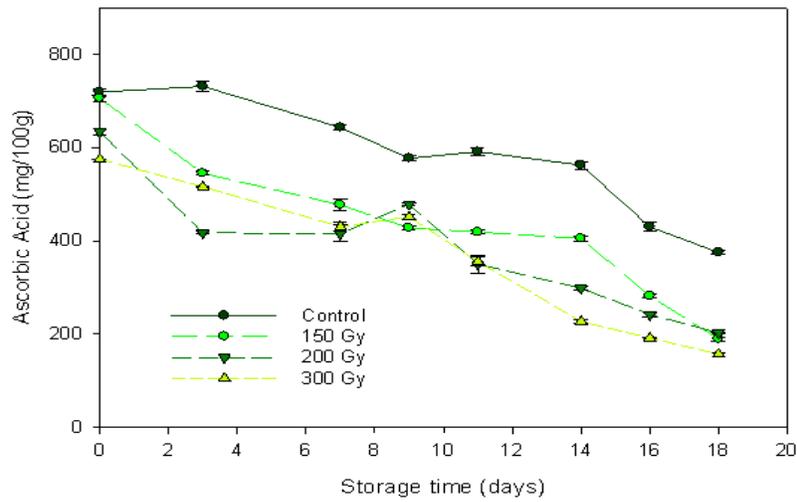


Fig. 2: Ascorbic acid content in irradiated unripe guavas stored at 5°C

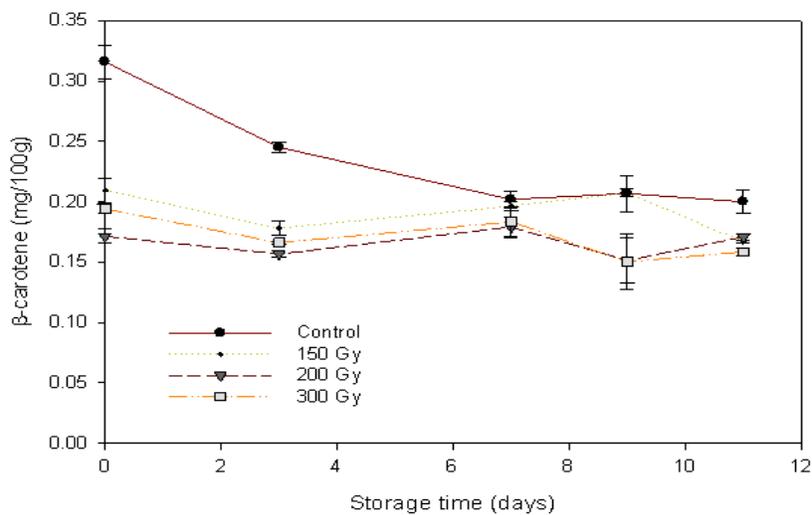


Fig. 3: beta-carotene content in irradiated ripe guavas stored at room temperature (25°C)

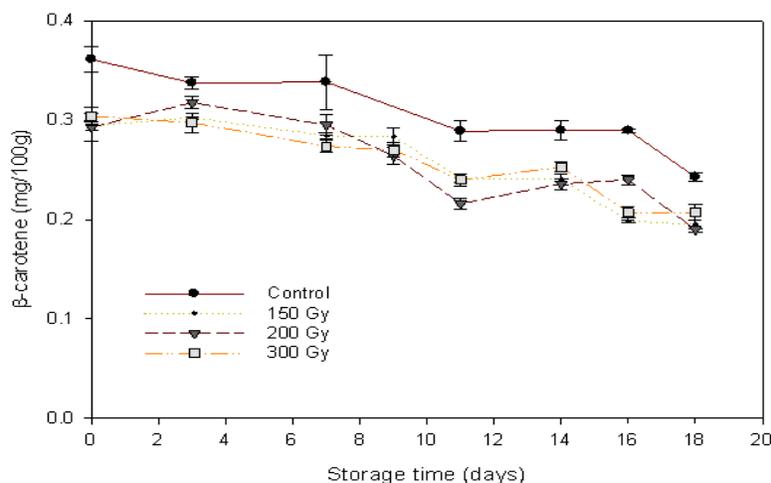


Fig. 4:  $\beta$  carotene content in irradiated unripe guava stored at cold temperature (5°C)

eleven days of storage at 5°C, the loss of ascorbic acid in unirradiated guavas was approximately 20%, and 40±5% in irradiated guavas. The results were similar to guavas stored eleven days at room temperature, but in the assay done 18 days later, the unirradiated guavas had lost 48% while the irradiated guavas 70±3%.

In Fig. 2, the synergistic effect produced by irradiation and temperature is clearly observed. The loss of ascorbic acid is steady during storage at 5°C. This temperature is not appropriate for storing green guavas; 8°C is better temperature (Kader, 2006).

Ascorbic acid is a very sensitive molecule. The loss is produced not only by irradiation but also by temperature and ripening (Bashir *et al.*, 2003). The result agrees with previous research reporting that irradiation of fruits has a significant effect ( $p < 0.05$ ) on the content of vitamin C- (Xuetong *et al.*, 2005; Calucci *et al.*, 2003; Youssef *et al.*, 2002; Lima *et al.*, 2001; Bustos *et al.*, 1992).

**$\beta$ -carotene (provitamin A):**  $\beta$ -carotene also decreased after irradiation. The loss was 40±5% in irradiated ripe guavas within the doses range 150-300 Gy. The concentration of  $\beta$  carotene at these radiation doses did not change significantly during storage ( $p > 0.05$ ). The level of  $\beta$  carotene in unirradiated guavas decreased 35% after 14 days stored at room temperature (Fig. 3).

After eleven days of storage at 5°C, loss of  $\beta$ -carotene was approximately 20% in green guavas irradiated in the dose range of 150 to 300 Gy (Fig. 4). The concentration of  $\beta$ -carotene is very similar in all irradiated samples. The level of  $\beta$ -carotene decreased steadily afterward until the end of the storage period. Unirradiated guavas lost 33.33% while the irradiated guavas lost between 59 and 64%. There was a synergistic effect of irradiation and low temperature that produced a rapid decrease in  $\beta$ -carotene. This effect was also observed in ascorbic acid. Despite the decrease of  $\beta$ -carotene in the doses range 150 to 300 Gy the difference was not significant ( $p < 0.05$ ).

The loss of vitamin C and  $\beta$  carotene in irradiated guavas stored at 5°C is attributed more to the temperature effects than irradiation treatment. To avoid damage by low temperature it is better to store green guavas between 8 to 10°C (Kader, 2006).

Changes caused by irradiation treatment in the molecules of ascorbic acid and  $\beta$ -carotene occur as a result of the interaction between radiation and the fruit. Ions and excited molecules released during treatment form free radicals, like Hydroxyl (OH) and hydrogen (H). These free radicals are formed very quickly ( $10^{-17}$  to  $10^{-4}$  sec) and are very reactive. The ascorbic acid molecule can react with both radicals, deriving different ascorbic acid radicals which quickly recombine to form other compounds in the fruit (Romani, 1967; Taub, 1984).

$\beta$ -carotene is an unsaturated compound that is extremely reactive to hydroxyl radicals. The principal reaction is the addition to double bonds, or it can also react with the hydrogen atom to form a saturated compound. Research on this point is variable, mentioning that the irradiation of mangoes and papaya at 250 Gy produced no significant effect on carotenoids (Thorne, 1991) while Lima *et al.* (2004) found that doses 0.25-1 kGy lead to losses of 55% in total carotenoids in carrots.

**Sugars:** The changes observed in the principal sugars; fructose, glucose and sucrose, assayed in ripe guavas and stored at room temperature, were similar.

An elevation in the concentration of any of these sugars was found nine days after the treatment. This is the climateric peak in which the fruit present the maximum respiratory rate and need a higher concentration of sugars (Abu-Bakr *et al.*, 2003). In irradiated fruit, it was possible to observe a slight decrease in any of these sugars, but the decreases were mainly in glucose and sucrose (Table 1). These decreases can be due to the stress in the fruit produced by the irradiation treatment. The metabolism increases,

Table 1: Changes in sugars content (fructose, glucose and sucrose) in irradiated ripe guavas stored at room temperature (25°C)

Storage time (days)	Control	150 gray	200 gray	300 gray
<b>Fructose (g/100 g)</b>				
0	7.62±1.35	8.91±0.74	5.46±0.09	6.33±0.05
3	5.41±0.28	4.84±0.39	4.23±0.25	4.56±0.22
7	6.25±0.68	6.67±0.60	4.54±0.21	5.13±0.42
9	7.41±0.70	9.13±0.14	6.92±0.43	7.22±0.04
11	5.23±0.19	5.64±0.79	6.15±0.20	5.46±0.48
14	6.10±0.51	5.94±0.66	7.18±0.30	6.95±0.27
<b>Glucose (g/100 g)</b>				
0	3.58±0.37	5.71±1.48	3.08±0.07	3.65±0.03
3	3.09±0.38	2.71±0.32	2.57±0.21	2.71±0.13
7	3.95±0.32	3.52±0.19	2.28±0.15	2.66±0.27
9	5.08±0.70	5.88±0.12	4.69±0.44	5.03±0.25
11	3.64±0.44	3.61±0.43	3.58±0.38	3.52±0.14
<b>Sucrose (g/100 g)</b>				
0	8.82±0.72	7.86±0.46	4.96±0.21	7.61±0.49
3	5.79±0.52	4.54±1.17	4.71±0.28	5.41±0.34
7	7.32±0.45	6.73±0.46	3.47±0.69	3.70±0.59
9	5.76±0.35	4.68±0.12	5.30±0.35	3.39±0.39
11	3.17±0.28	3.63±0.45	4.09±0.45	2.58±0.35

Values are shown as mean±S.D. of three determinations

Table 2: Changes in sugars content (fructose, glucose and sucrose) in irradiated ripe guavas stored at cold temperature (5°C)

Storage time (days)	Control	100 gray	150 gray	300 gray
<b>Fructose (g/100 g)</b>				
0	3.77±0.32	2.91±0.12	6.32±0.08	6.13±0.55
3	6.75±0.58	5.44±0.43	5.72±0.14	6.07±0.62
7	6.83±0.71	5.93±0.39	8.16±0.71	7.76±1.00
9	7.26±0.18	7.85±0.39	7.26±0.18	9.74±0.73
11	6.64±0.09	6.58±0.39	9.46±0.53	7.22±0.49
14	5.49±0.89	5.72±0.14	6.27±0.50	6.81±0.89
16	5.82±0.89	6.58±0.51	8.06±1.39	8.14±0.67
18	8.55±0.26	7.62±0.29	7.78±0.39	6.57±1.09
<b>Glucose (g/100 g)</b>				
0	3.39±0.25	3.18±0.17	2.59±0.02	6.74±0.58
3	7.72±0.66	5.24±0.35	6.57±0.67	6.28±0.36
7	6.32±1.03	5.69±0.16	6.28±0.35	5.91±0.62
9	7.62±0.10	6.49±0.35	5.22±0.88	5.35±0.38
11	5.13±0.51	3.81±0.58	5.95±0.36	5.85±0.09
14	4.02±0.13	3.77±0.12	3.37±1.54	3.87±0.40
16	4.03±0.19	3.94±0.24	4.33±0.83	4.31±0.20
18	4.10±0.36	2.85±0.19	2.54±0.06	2.52±0.40
<b>Sucrose (g/100 g)</b>				
0	6.48±0.59	4.62±0.14	6.17±0.33	9.96±0.49
3	5.19±3.76	7.28±0.30	7.68±0.29	6.34±0.12
7	8.48±0.54	8.08±0.61	8.43±1.05	8.95±0.64
9	8.52±0.72	6.40±0.29	7.03±0.62	7.77±0.62
11	7.79±0.66	6.23±0.28	8.60±0.76	8.03±0.52
14	4.92±0.94	4.07±0.49	4.53±0.41	5.75±0.54
16	4.51±0.41	5.16±0.22	5.40±0.91	5.66±0.65
18	5.69±0.08	5.98±1.00	6.56±0.25	4.93±0.52

Values are shown as mean±S.D. of three determinations

and with it, the increase in the respiration rate that requires the consumption of additional sugars. The statistical analysis showed that the differences found in the sugars of ripe guavas irradiated up to 300 Gy and stored at room temperature for 14 days, were not significant ( $p \leq 0.05$ ).

The initial concentration of fructose, glucose and sucrose in green guavas was higher than the concentration found in ripe guavas. This is because the maturity process was just beginning and had not been completed. Ripe guavas already used sugars for biochemical and physiological processes. The climacteric

peak is also observed between seven to nine days after the treatment. After this, the concentration of glucose and sucrose start to decrease. It is clear that this effect is due to the ripening process and not associated with the treatment. Irradiated green guavas stored at low temperature did not demonstrate the slight decrease in the concentration of sugars in comparison to unirradiated fruit. This can be explained by the effect of low temperature that reduces the metabolic processes so the respiration rate is less and the consumption of sugars is reduced. Table 2 demonstrates how the concentration of glucose changed in guavas stored at low temperature.

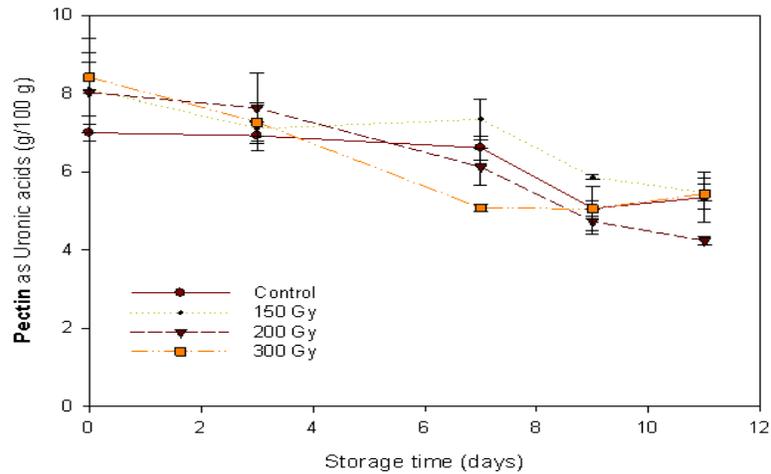


Fig. 5: Pectin as uronic acid in irradiated ripe guavas stored at room temperature (25°C)

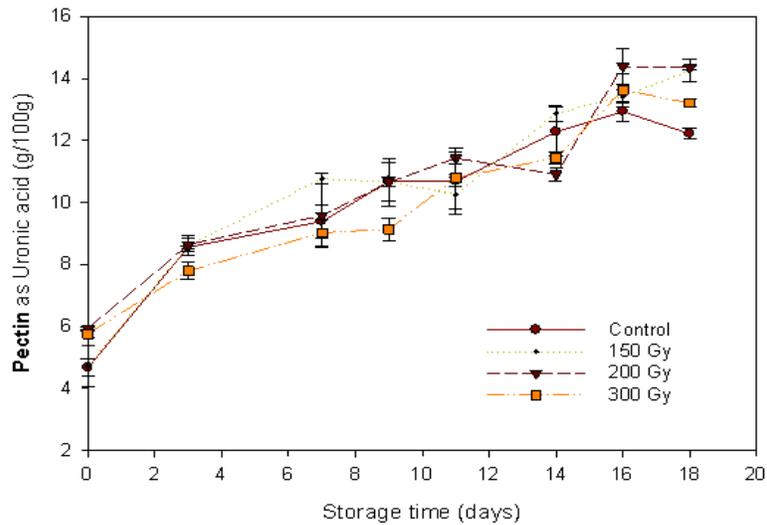


Fig. 6: Pectin as uronic acid in irradiated unripe guavas and stored at cold temperature (5°C)

The statistical differences were not significant ( $p \leq 5\%$ ) in guavas irradiated up to 300 Gray and stored at low temperature.

Yusof and Mohamed (1987) found that fructose, sucrose, and glucose were the three predominant sugars present in guava. According to Youssef *et al.* (2002) no significant differences were found in total and reducing sugars between irradiated and non-irradiated mango pulp at zero time and all storage periods afterward. Castell-Perez *et al.* (2004), confirmed that irradiation treatment had no significant effect on the sugar content in cantaloupes, and O'Mahony *et al.* (1985), did not find significant differences in soluble solids between irradiated and control fruit of Navel oranges after 6 weeks of storage.

**Pectins:** Ripe guavas presented the highest concentration of pectins after treatment and the concentration was similar in all the samples;

unirradiated and irradiated. A decrease in concentration was found at the end of storage at room temperature (Fig. 5). In ripe guavas it is normal to find this effect since all the protopectin found in the cell wall is transformed to soluble pectin.

Green guavas showed a steady increase during storage at 5°C. The highest concentration was found at the end of the storage time, and were very similar in the unirradiated and irradiated samples (Fig. 6) indicating there are no differences due to the radiation treatment ( $p \leq 5\%$ ). The maturity process was the only effect observed. Unripe guava have more protopectin to transform to soluble pectin, therefore the quantity of pectin increased during the storage time. Pectins are structural substances for the cellular wall. The results show that irradiation at these doses do not break the molecular links of the structural substances like as pectins.

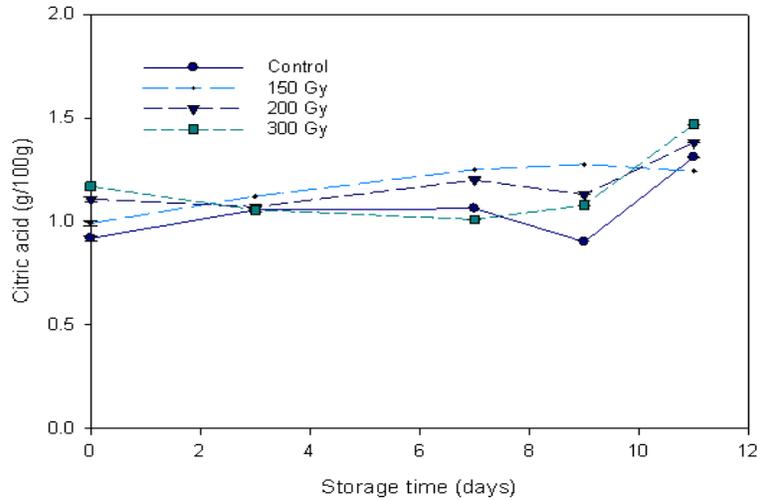


Fig. 7: Change in citric acid content in irradiated ripe guavas stored at room temperature (25°C)

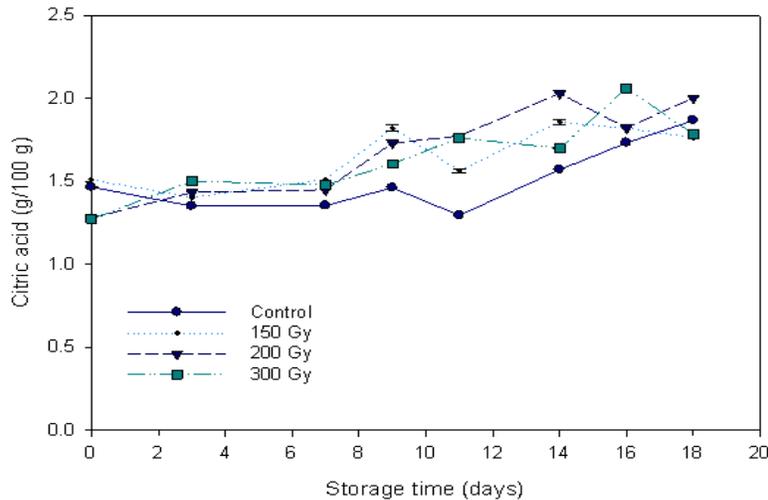


Fig. 8: Change in citric acid content in irradiated unripe guavas stored at cold temperature (5°C)

**Citric acid (titratable acidity):** Results of acidity values (g citric/100 g guava) of irradiated and unirradiated guavas stored at room temperature and refrigerated are presented in Fig. 7 and 8, respectively. The concentration of organic acids in both lots of guavas increases steadily during storage, however the concentration in irradiated fruit was higher.

The increase of acidity in the irradiated samples could be the result of changes in physiological functions (Bashir *et al.*, 2003) such as the activation of enzymes that increase the concentration of phenol or ethanol, thereby increasing the acidity of the irradiated fruit. (Arevalo *et al.*, 2002)

Results reported are variable. Some authors report that irradiation has no effect (Nagai and Moy, 1985; O'Mahony *et al.*, 1985; Mahrouz *et al.*, 2002). Other authors report that total acidity can decrease because the irradiation causes decarboxilation (Guerrero *et al.*, 1967).

## CONCLUSION

The results of this evaluation of chemical parameters associated with guavas irradiated up to 300 Gy indicate that irradiation is useful as a quarantine treatment. The most significant changes are in ascorbic acid and  $\beta$ -carotene, but these changes are normal as there are present in any other method of food preservation. No significant differences are found in any of the other parameters studied, including sugars, pectins, and acidity. There are more differences associated with maturity and temperature than the irradiation treatment.

It is important to propose to APHIS accepted lower radiation dose to irradiated guavas, since they are tropical fruit, are sensitive and lower radiation dose produce less damages in the fruit and also the cost of the treatment is less.

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