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EFFECT OF GAMMA RADIATION ON THE TITRABLE ACIDITY AND VITAMIN C CONTENT OF CITRUS FRUITS

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Abstract

The study was carried out to assess effect of gamma radiation on the acidity and vitamin C content of the *Citrus macroptera* (Satkora) and *Citrus assamensis* (Ginger lime). Irradiation with doses 0.5, 1.0, 2.0, 3.0 kGy were applied to assess the effect on the titrable acidity and vitamin C contents every one week interval for total five weeks. Both titrable acidity and vitamin C content of *C. macroptera,* and *C. assamensis* are sensitive to both gamma radiation and storage time; have decreased with increase of radiation does as well as storage time and this changes of vitamin C and titrable acidity content with gamma radiation and increasing storage period have found statistically significant.

Keywords: *Citrus macroptera* (Satkora), *Citrus assamensis* (Ginger lime), Titratable Acidity, Vitamin C and Gamma Radiation

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Introduction

Vitamin C is one of the most important nutritional benefits of citrus fruit. Ascorbic acid (Vitamin C) is the only vitamin present in citrus fruit in amounts of major nutritional significance; one orange has 50 mg of vitamin C, which is nearly the double of the recommended daily intake.The concentration of ascorbic acid has been reported to decrease with maturity or remain constant until late in the season & then decline (Baldwin, 1993). Only 25% of ascorbic acid in the fruit is in the juice, the remainder is found in the peel, especially in the flavedo (Kefford, 1959).

Maturity standards for citrus are often based on chemical indicators of fruit flavor, including sweetness and acidity and the ratio between these components. Therefore, acidity levels have a major impact on internal fruit quality and consequently affect the time when the fruit reaches minimum market standards (Marsh *et al.,* 2000). Organic acids significantly contribute to juice acidity, citric acid being primary organic acid (70-90% of total). Citric acid is the major acid in juice, malate predominates in the flavedo and oxalate is the most abundant in albedo. Other minor organic acids found in citrus fruits are acetate, pyruvate, glutarate, formate, succinate and α-ketoglutarate (Yamaki, 1989).

Applying gamma radiation to citrus fruits at the pasteurization dose level could be the means of extending the shelf life of these commodities. The problems are related to physiological response of the citrus fruits being irradiated and the dose requirement to achieve their disinfection. The dose level for disinfection can be intolerable to some citrus fruits and can result in disorders. The feasibility of the treatments depends upon the sensibility of the host tissues. Temple mandarin has been found to be less susceptible to irradiation damage than Pineapple and Valencia oranges (Dennison *et al.,* 1966)

Ionizing radiation can safely and effectively eliminate the pathogenic bacteria from the food (Crawford and Ruff, 1996; Evangelista, 2000; Loaharanu, 1994; Sommers *et al.,* 2004), disinfest the fruits and vegetables (Moy and Wong, 2002; Fan and Mattheis, 2001; Patil *et al.* 2004; Pellegrini *et al.,* 2000), extend the shelf life of many products through ripening delay (Kilcast, 1994), inhibit the sprouting of bulbs and tubers (Aziz *et al.,* 2006; Rios and Penteado, 2003; Curzio *et al.,* 1986), and reduce or totally eliminate the parasitic microorganisms (Crawford and Ruff, 1996; Kilcast, 1994).

The present research work was conducted on *Citrus assamensis* (Ginger limes) and *Citrus macroptera,* (Satkora), two indogenous species of citrus fruits and available in Sylhet region of

Bangladesh. The objectives of the present work are to observe the effect of gamma radiation on the Titrable acidity and Vitamin C content of these two citrus fruits.

Materials and Methods

Citrus assamensis (Ginger limes) and *Citrus macroptera* (Satkora) of fresh, fully mature, large size, juicy type and free from any type of injury or deterioration were procured from the "Bandar Bazar" of Sylhet city and were taken to the Food Technology Laboratory of Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Bangladesh Atomic Energy Commission, Savar, Dhaka under ambient conditions during March.

Each sample was packed into sterilized (by 15 kGy gamma radiation) polythene paper and then sealed. The packets were well ventilated by making small holes at 0.5% area of the bag was punched with small circular holes then exposed to a Co^{60} gamma Irradiator with doses 0.5, 1.0, 2.0, 3.0 kGy. Among 25 samples of each type 5 samples were exposed to each dose and 5 samples were left as control sample. Then both irradiated and control samples were kept at room temperature (at about 22-25°C) on a clean and open surface. The acidity and ascorbic acid contents were assessed every one week interval for total five weeks duration.

Titrable acidity was determined by the method of AOAC (1990) and the estimation of ascorbic acid content were carried out by the titration result of the sample extract with 2,6-Dichlorophenol-Indophenol (Dye) (Johnson, 1948).

Mean, Standard deviation and T-test of the change of titrable acidity and vitamin C content and Analysis of variance (ANOVA) of the overall changes of titrable acidity and vitamin C content due to application of radiation doses were measured using SPSS (v.16) program.

Results and Discussion

Citurs macroptera (Satkora)

The quantity of titrable acidity of *C. macroptera* (Satkora) irradiated with different radiation doses is presented in the table 1.

Table 1. Amount of titrable acidity of satkora irradiated with different radiation doses

Weeks Doses	1st week TA (%)	2nd week TA (%)	3rd week TA (%)	4th week TA (%)	5th week TA (%)	Mean	Std. Deviation
Control (0 kGy)	0.8956	0.6307	0.5150	0.3580	N/d	0.479860	0.3323939
0.5 kGy	0.8563	0.5842	0.5087	0.4075	0.3063	0.532600	0.2090916
1.0 kGy	0.5794	0.5494	0.4363	0.3532	N/d	0.383660	0.2327432
2.0 kGy	0.5582	0.4075	0.3256	0.2568	N/d	0.309620	0.2063264
3.0 kGy	0.5150	0.3865	0.3045	0.2405	0.2321	0.335720	0.1177907

TA: Titrable Acidity,
N/d: Not done

It is found that all the values of titrable acidity obtained in the samples from 1st week to 5th week radiated with various doses are less than the titrable acidity value of fresh, non-radiated sample (0.8956% titrable acidity). And the values decrease with increase of radiation dose and storage period. So, the obtained results of present study about the change of titrable acidity of *C. macroptera* (Satkora) irradiated with different radiation doses shows a gradual decrease of titrable acidity content with the increase of radiation dose and storage time.

The table 1 shows a gradual increase of titrable acidity change with increase of radiation doses as well as with increase of storage period. So, quantity of titrable acidity of *C. macroptera (*Satkora) is inversely proportional to radiation dose and storage period. Again, the decreasing rate of titrable acidity is directly proportional to the radiation dose as well as to the storage period, i.e. the changing rate increases with increase of radiation dose and storage period.

Ladaniya *et al.* (2003) found that, doses up to 1.5 kGy cause decrease of titrable acidity of 'Nagpur' mandarin (*Citrus reticulata* Blanco), 'Mosambi' sweet orange (*Citrus sinensis* Osbeck) and 'Kagzi' acid lime (*Citrus aurantifolia* Swingle) which has similarity with our present findings.

The correlation and significance values of obtained data are measured by "Pearson Correlation (2-tailed)" method. We have taken 5% level of acceptance for measuring significance. At 5% level of acceptance, the significance values (p) below 0.05 are considered as "Significant", while above 0.05 is considered as "Insignificant". The effects of radiation doses are negatively correlated with storage time in respect of titrable acidity of *C. macroptera* and the calculated correlation co-efficient are -0.982, -0.965, -0.921, -0.971 and -0.955 for radiation doses 0, 0.5, 1.0, 2.0 and 3.0 kGy, respectively. The negative correlation is obtained due to the decrease of titrable acidity with increase of radiation doses. And all the changes of titrable acidity have found statistically significant (obtained "p" values are 0.003, 0.008, 0.027, 0.006 and 0.011 for radiation doses 0, 0.5, 1.0, 2.0 and 3.0 kGy, respectively. On the other hand, the effects of storage time are also negatively correlated with radiation doses in respect of titrable acidity of *C. macroptera* except for 5th week change and the calculated correlation co-efficient are -0.873, -0.973, -0.007, -0.896 and 0.207 for 1st, 2nd, 3rd, 4th and 5th week samples respectively. Here, the cause of negative correlation is decrease of titrable acidity with increase of storage period. The change of titrable acidity in 2nd, 3rd and 4th week have found statically significant (calculated "p" values are 0.005, 0.007 and 0.04, respectively) while changes are insignificant in 1st and 5th week (calculated "p" values are 0.054 and 0.738, respectively).

Table 2. Amount of vitamin C (mg/100g) content of *C. macroptera* (Satkora) irradiated with different doses

Weeks Doses	1st week AA(*)	2nd week AA(*)	3rd week AA(*)	4th week AA(*)	5th week AA(*)	Mean	Std. Deviation
Control (0 kGy)	56.00	41.63	40.61	39.60	N/d	35.568000	20.9819916
0.5 kGy	50.64	33.29	30.41	27.86	25.32	33.504000	10.0261373
1.0 kGy	40.00	31.95	27.52	23.09	N/d	24.512000	15.0591358
2.0 kGy	37.06	30.50	25.09	18.01	N/d	22.132000	14.2164929
3.0 kGy	36.28	26.52	16.76	14.03	13.32	21.382000	9.8584999

AA: Ascorbic Acid or Vitamin C
N/d: Not done

In case of ascorbic acid of *C. macroptera* (Satkora), the study also shows a gradual decrease. All the obtained values of vitamin C for the samples of 1st to 5th week, radiated with different doses (0, 0.5, 1.0, 2.0 & 3.0 kGy) are less than the vitamin C content value of fresh, non-radiated sample (56.00 mg/100g). This can be seen from the Table 2 that the more the radiation dose increases, the less vitamin C content is obtained from the samples.

Here, we have compared all the values of vitamin C content obtained from different samples with the reference value of vitamin C obtained from fresh, non-radiated sample.

In case of vitamin C content, the effects of radiation doses are negatively correlated with storage time, the calculated co-efficient are -0.859, -0.884, -0.933, -0.963 -0.937 for radiation doses 0, 0.5, 1.0, 2.0, and 3.0 kGy, respectively. Except for the control sample (p=0.062), all changes in at other radiation doses are statistically significant (obtained "p" values are 0.046, 0.021, 0.008 and 0.019, respectively for 0.5, 1.0, 2.0 and 3.0kGy dose).

The effects of storage time are negatively correlated with radiation doses in respect of vitamin C content of *C. macroptera* (Satkora) except for 5th week. The calculated co-efficient values are -0.972, -0.893, -0.943, -0.927 and 0.043, respectively for 1st, 2nd, 3rd, 4th and 5th week. The changes of vitamin C content of Satkora are found significant at 5% level of acceptance for 1st to 4th week and insignificant for 5th week. The calculated "p" values for 1st to 5th weeks are 0.006, 0.042, 0.016, 0.024 and 0.945, respectively.

C. assamensis (Ginger lime)

In case of *C. assamensis* (Ginger lime) all the values of titrable acidity obtained in the samples from 1st week to 5th week radiated with various doses (0, 0.5, 1.0, 2.0 & 3.0 kGy) are less than the titrable acidity value of fresh, non-radiated sample (1.18% titrable acidity). The values decrease with increase of radiation dose and storage period. So, the obtained results of present study about the change of titrable acidity of ginger lime irradiated with different radiation doses shows a gradual decrease of titrable acidity content with the increase of radiation dose and storage time. The percentage of titrable acidity change with respect to the reference sample (fresh and non-radiated) is presented in the following table.

Table 3. Effect of gamma radiation on titrable acidity of *C. assamensis* (Ginger lime)

Weeks Doses	1 week TA *(%)	2 week TA (%)	3 week TA (%)	4 week TA (%)	5 week TA (%)	Mean	Std. Deviation
Controlled	1.18	0.9657	N/d*	N/d	0.923	0.613740	0.5686654
0.5 KGy	1.025	0.807	0.773	N/d	0.623	0.645600	0.3884376
1.0 KGy	0.981	0.847	0.81	0.773	N/d	0.682200	0.3893632
2.0 KGy	0.98	0.91	0.88	N/d	N/d	0.554000	0.5070306
3.0 KGy	0.901	0.858	N/d	N/d	N/d	0.351800	0.4819618

Many scientists have observed the effect of irradiation on limes and other citrus. Ladaniya *et al*. (2003) found that, doses up to 1.5 kGy cause decrease of titrable acidity of 'Nagpur' mandarin lime (*Citrus reticulata* Blanco), 'Mosambi' sweet orange (*Citrus sinensis* Osbeck) and 'Kagzi' acid lime (*Citrus aurantifolia* Swingle) which has similarity with our present findings though acidity changes in our study was not statistically significant.

Here, we have taken the titrable acidity value of fresh, non-radiated *C. assamensis* as reference and its value is 1.18%. We have compared all the obtained values of titrable acidity with this reference value. It is observed from the table4 that, the values are gradually increasing in a row from left to right as well as from top to bottom in a column. This means, the decreasing rate of titrable acidity of radiated samples, stored for different period is increasing with increase of radiation dose and storage period (as radiation doses are gradually increasing from top to bottom and storage period is increasing from left to right in a row of the table 3.

Similarly, the quantity of vitamin C of *C. assamensis* irradiated with different radiation doses is presented in the following table.

Table 4. Effect of gamma radiation on vitamin C of *Citrus assamensis* (Ginger lime)

Weeks / Doses	1 week AA* (mg/100g)	2 week AA (mg/100g)	3 week AA (mg/100g)	4 week AA (mg/100g)	5 week AA (mg/100g)	Mean	Std. Deviation
Controlled	31.7135	19.80	N/d*	N/d	16.8	13.662700	13.6627159
0.5 KGy	28.66	20.00	20.00	N/d	13.34	16.400000	10.6601032
1.0 KGy	27.58	22.44	21.459	17.325	N/d	17.760800	10.5785659
2.0 KGy	27.79	21.64	12.38	N/d	N/d	12.362000	12.5473392
3.0 KGy	24.73	14.03	N/d	N/d	N/d	7.752000	11.2688318

TA*=Titrable Acidity
N/d*=Note done

The study revealed that vitamin C also changes with changes in radiation dose and storage time like titrable acidity at the same manner, that is, a gradual decrease of vitamin C content is occurred with the increase of radiation dose and storage time. The percentage of vitamin C change with respect to the reference sample (fresh and non-radiated, 31.72 mg/100g vitamin C) is presented in the table 4.

In this study, the effect of irradiation on titrable acidity of ginger lime with time is negatively correlated but the changes are not statistically significant. In this study, it is observed that changes in vitamin C content of ginger lime in the doses of 1KGy, 2KGy and 3KGy are statistically significant. Ladaniya *et al*., (2003) found that, doses up to 1.5 kGy caused decrease of vitamin C content of 'Nagpur' mandarin lime (*Citrus reticulata* Blanco), 'Mosambi' sweet orange (*Citrus sinensis* Osbeck) and 'Kagzi' acid lime (*Citrus aurantifolia* Swingle).

The results of the study of five weeks have been statistically analyzed to do a comment that either the changes of titrable acidy or vitamin C are significant or not. The correlation and significance values of obtained data are measured by "Pearson Correlation (2-tailed)" method. We have taken 5% level of acceptance for measuring significance. At 5% level of acceptance, the significance values (p) below 0.05 are considered as "Significant", while above 0.05 is considered as "Insignificant".

The effects of radiation doses are negatively correlated with storage time in respect of titrable acidity of *C. assamensis* and the calculated correlation co-efficient are -0.408, -0.656, -0.827, -0.895, -0.873 and -0.955 for radiation doses 0, 0.5, 1.0, 2.0 and 3.0kGy, respectively. The negative correlation is obtained due to the decrease of titrable acidity with increase of radiation doses. And all the changes of titrable acidity have found statistically insignificant except for 2.0KGy (obtained "p" values are 0.496, 0.230, 0.084, 0.040 and 0.054 for radiation doses 0, 0.5, 1.0, 2.0 and 3.0 kGy, respectively). On the other hand, the effects of storage time are also negatively correlated with radiation doses in respect of titrable acidity of *C. assamensis an*d the calculated correlation co-efficient are -0.873, -0.201, -0.113, -0.138 and -0.808 for 1st, 2nd, 3rd, 4th and 5th week samples, respectively. Here, the cause of negative correlation is due to decrease of titrable acidity with increase of storage period. All the changes of titrable acidity have found statistically insignificant (obtained "p" values are 0.053, 0.746, 0.857, 0.823 and 0.098 for 1st, 2nd, 3rd, 4th, and 5th week, respectively).

In case of vitamin C content, the effects of radiation doses are negatively correlated with storage time and the calculated co-efficient are -0.574, -0.143, -0.90p1, -0.973 and -0.891 for radiation doses 0, 0.5, 1.0, 2.0 and 3.0 kGy, respectively. Except for the control and 0.5 KGy samples (p=0.311and 0.143 respectively), all changes in at other radiation doses are statistically significant (obtained “p” values are 0.037, 0.005 and 0.043, respectively for 1.0, 2.0 and 3.0kGy dose).

The effects of storage time are negatively correlated with radiation doses in respect of vitamin C content of *C. assamensis*. The calculated co-efficient values are -0.906, -0.597, -0.274, -0.139 and -0.809, respectively for 1st, 2nd, 3rd, 4th and 5th week. The changes of vitamin C content of Ada lebu are found significant at 5% level of acceptance for 1st week only and it is significant for all other weeks. The calculated “p” values for 1st to 5th weeks are 0.034, 0.288, 0.655, 0.823 and 0097, respectively.

In this study, it is observed that changes in vitamin C content of ginger lime in the doses of 1 KGy, 2 KGy and 3 KGy are statistically significant. Ladaniya *et al.* (2003) found that, doses up to 1.5 kGy cause decrease of vitamin C content of ‘Nagpur’ mandarin lime (*Citrus reticulata* Blanco), ‘Mosambi’ sweet orange (*Citrus sinensis* Osbeck) and ‘Kagzi’ acid lime (*Citrus aurantifolia* Swingle).

Rao (1962) observed that ascorbic acid is rapidly destroyed by gamma radiation in dilute solutions. Zhang *et al.,* (2006) have found that, the loss of vitamin C of fresh-cut lettuce irradiated with 1.0 kGy was significantly (a = 0.05) lower than that of non-irradiated.

Hussain and Maxie (1974) have registered 70.2 % vitamin C losses for orange juice irradiated with 2.5 to 10 kGy. So it can be said that, the loss rate of vitamin C content increased with increase of radiation dose. This is very similar to our present finding. Benkeblia and Khali (1999) has found that gamma irradiation at 0.10, 0.15 and 0.30 kGy causes losses of 10, 13 and 20% of ascorbic acid content respectively in onion bulbs. Another study using higher radiation doses (3.0 and 4.0 kGy), resulted in 62 and 81% losses of ascorbic acid, respectively (Clark, 1959)

Increasing the radiation dosage gradually decreased the fruits vitamin C concentration of Lycium (Wen *et al*., 2006). Evangelista (2000) has found alterations in vitamin C contents in potatoes are proportional to the irradiation dosage.

Lacroix *et al.,* (1990) have found that, the content of vitamin C of papaya and mango irradiated at 0.5 to 0.95 kGy were not significantly affected by the irradiation. In another study of the ascorbic acid content of oranges, Nagai and Moy (1985) have found no significant differences between irradiated and control fruit at dose levels up to 1 kGy. Fan and Mattheis (2001) commented that, adequate doses for insect disinfestations (normally 1-2 kGy) showed non-significant effects in vitamin C contents of citric fruits. Maxie *et al.* (1964) have found that, strawberries (*Fragaria* sp.) presented minute, non-significant decrease in vitamin C levels when submitted to 1.0-2.0 kGy doses, during 11 days of storage at 5ºC. Similar observations were reported by Lopez *et al.* (1967). In our study we have found that in control and 0.5 KGy dose of radiation the changes in vitamin C is not statistically significant but in 1 KGy, 2 KGy and 3 KGy the changes are statistically significant. From the above discussion, we see that in most of the limes and citrus vitamin C content decreases with increase of doses of radiation, at the lower doses the changes may be insignificant but at the higher doses the changes are statistically significant which is too much similar to our study for ginger lime.

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