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Assessment of the Effect of Gamma Irradiation on Total Carotenoid Content of *Mangifera indica* L. cv. Carabao Puree using Raman Microspectroscopy

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It has been established that the use of gamma radiation is effective in prolonging the shelf life and improving food safety of processed products. However, ionizing radiation poses the risk of affecting the product's quality including its vitamin content. In this research, Raman microspectroscopy was used to assess the effects of gamma radiation on the total carotenoid content of *Mangifera indica* L. cv. Carabao puree. The pulps of Carabao mangoes collected from a local farm in Bataan, Philippines were homogenized and irradiated using various doses ranging from 0.2 to 15kGy at the Cobalt 60 Multi-Purpose Irradiation Facility of the DOST-PNRI. The Raman spectra of the mango puree samples were obtained, showing the characteristic carotenoid peaks at 1156 cm^{-1} and 1519 cm^{-1}. The integrated areas of these peaks were used in quantifying the relative changes in carotenoid levels with increasing dose. Results show that radiation doses of up to 1 kGy have no significant effect on the total carotenoid content of mango purees. However, doses higher than 1 kGy resulted in a significant decrease of the total carotenoid content. There is a good correlation between the gamma radiation dose and the decrease in the total carotenoid content. The results of this study will be useful in the optimization and standardization of the gamma irradiation treatment for mango puree.

Keywords: carotenoid, gamma irradiation, mango, Raman spectroscopy

INTRODUCTION

Mangifera indica L., commonly known as mango, is a dicotyledonous fruit of family Anacardiaceae primarily produced in the developing countries in the tropics. Mangoes have been cultivated in South Asia – especially in Eastern India, Burma, and the Andaman Islands – for thousands of years (Morton 1987).

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Philippine Mango Industry

Mango is the third most significant crop of the Philippines based on export volume and value, next to banana and pineapple. In 1995, the Philippine Carabao mango was listed as the sweetest fruit in the world. Mango industry has been consistently expanding. According to the Department of Agriculture, the country produces more than one million metric tons of mangoes – most being of Carabao variety – contributing 3.5% share in the world's production. Being a seasonal product, mango processing is a crucial part of the industry. Processing ensures

the availability of mangoes throughout the year while providing different choices of mango-based products for the consumers (Briones *et al.* 2013, de la Fuente 2016).

Nutritional Content of Mango

A 165-g serving of mango fruit comprises 25%, 76%, and 9% of Vitamins A, C, and E of the Dietary Reference Intake (DRI), respectively. Significant levels of Vitamin B6 (pyridoxine, 11% DRI); vitamin K (9% DRI); other B vitamins; and essential nutrients such as potassium, copper, and 17 amino acids were also found. Moreover, mango contains other phytonutrients that include carotenoids (mostly β-carotene), polyphenols, and omega-3 and -6 polyunsaturated fatty acids (Fowomola 2010).

Carotenoids

Carotenoids are isoprenoid compounds biosynthesized by the tail to tail connection of two C-20 geranylgeranyl diphosphate molecules, which constructs the parent C-40 carbon skeleton from which all individual variations are made. The carotenoids of fruits are usually fat-soluble and are associated with lipid portions of human tissues, cells, and membranes. During digestion, carotenoids combine with other lipids after being released from associated proteins. It has been reported that the human body absorbs between 5 and 50% of carotenoids, where the absorption efficiency is related to presence or absence of other carotenoids in the diet (Dutta *et al.* 2011).

Nutritional Benefits of Carotenoids

Many biological effects are associated with the presence of carotenoids. It is well-known that carotenoid pigments are effective antioxidants (Olson 1989, Sharma *et al.* 2012). Furthermore, carotenoids have been proven beneficial in protecting against cellular damage, effects of aging, and even some chronic diseases. Research also shows that carotenoids can improve the immune system and reduce the risk of different degenerative diseases such as cancer, cardiovascular disease, and cataract formation (Krinsky 2007, Mathews-Roth 2007, Mercadante *et al.* 1997). Some carotenoids including alpha and beta carotene also act as a precursor of vitamin A. This vitamin is essential to vision, cell differentiation, synthesis of glycoprotein, reproduction, and overall growth and development of bones. The pro-vitamin A activity of α and β carotene in human system are 50 and 100%, respectively, and provides about 60% of dietary vitamin A on a worldwide basis (Dutta *et al.* 2011, Krinsky 2007, Mathews-Roth 2007).

Raman Spectroscopy

Raman spectroscopy – named after its discoverer, Indian physicist Sir Chandrasekhara Venkata Raman – is a spectroscopic technique which can give information on chemical structures and physical forms for substance identification from characteristic spectral patterns ('fingerprinting'), and for quantitative or semi-quantitative determination of the amount of a substance in a sample (Smith and Dent 2005).

Food Irradiation

Food irradiation is the process of treating food by exposing them to ionizing radiation. Gamma irradiation can be used to prolong the shelf life and increase the sanitation of processed products without posing risks of affecting the product's nutritional content at low doses (Morris 2007, Raso and Barbosa-Cánovas 2003).

Although low doses (0–1 kGy) of gamma irradiation pose little to no risk, higher doses (greater than 1 kGy) being used to ensure proper sanitation in various processed products can affect their vitamin content (Dionísio *et al.* 2009, Kilcast 1994). The typical dose of gamma radiation used in inactivating pathogenic organisms, especially in fruit juices, is 1 to 10 kGy (Bhosale *et al.* 2004, Dionísio *et al.* 2009). Some microorganisms such as the *Bacillus cereus*, one of the most important causes of food poisoning in industrialized countries, require higher doses of radiation (3.6kGy for the *Bacillus cereus*) (Ayari *et al.* 2010). Mango fruits are proven to have significant amounts of carotenoids, which are essential to human growth and development (Mercadante *et al.* 1997), thus identifying vitamin losses during the irradiation process is necessary to ensure its quality.

This research assessed the effects of different doses of gamma radiation on the total carotenoid content of *Mangifera indica* L. cv. Carabao mango puree. The amount of individual carotenoid was not measured. Measurement of microbial load and analysis of other properties of mangoes such as acidity, amount of sugar, and other vitamin content were not done in this study. The results of this study will hopefully contribute to the establishment of an optimum radiation dose treatment for mango (cv. Carabao) puree in the Philippines.

MATERIALS AND METHODS

Homogenate Preparation

Ripe carabao mangoes obtained from a mango farm in Bataan, Philippines were sliced to acquire pulps. Ripeness assessment is based on the Philippine National Standards PNS/BAFPS 13:2004 Fresh fruits – mangoes – specifications (DTI 2004). The mango pulps were homogenized for 3 min using the minimum power of STANDARD SJB-1.5LA blender. The homogenate was divided into 18 equal samples of about 100 g each.

The samples were transferred into clean polyethylene containers and stored in a refrigerator (5 ºC) prior to irradiation and analysis.

Gamma Irradiation

Three of the 18 samples served as the control group and remained non-irradiated. The 15 remaining samples were irradiated at the Cobalt 60 Multipurpose Irradiation Facility of the DOST-PNRI. The doses used were 0.2, 1.0, 6.0, 10, and 15 kGy. These doses are based on the International Atomic Energy Agency Technical Report Series No. 481: Manual of Good Practice in Food Irradiation (IAEA 2015). For each dose, three samples were irradiated.

Raman Microspectroscopy

Three Raman spectra of each sample for each irradiation dose received were obtained 7 d after the irradiation using Renishaw inVia Reflex Raman spectrometer system with 785 nm laser excitation, 250 mW output power, and 1 cm^{-1} spectral resolution achieved with a high-resolution 1200 lines/mm grating. The spectrometer system has a UV and NIR-enhanced Deep-Depletion CCD array detector (576 x 384 pixels). The system was calibrated using an internal Si reference.

Circular polyethylene containers were used as sample holders. Approximately 15–20 mg per sample was used in each trial. The spectrometer beam (spot size ≈ 8 µm) was focused on the surface of the sample using a specially-adapted research-grade Leica DM 2700 microscope with a 5x objective and the scattered light was collected through the same objective before every scan. Rayleigh line rejection is achieved using edge filters set for 785 nm excitation. The acquisition time of the laser took 25 s only to avoid sample heating or burning. Visual inspection of each sample was done after each trial in the spectrometer system to make sure there were no signs of burning. The Raman spectral range recorded was at 800–1800 cm^{-1}.

Data Processing

Data processing was done using Renishaw's Windows-based Raman Environment (WiRE). First, the spectra were pre-processed using intelligent polynomial fitting before cubic spline fitting was applied. Baseline fitting was done to remove the background signal. Then, Savitsky-Golay algorithm (smooth window = 5, polynomial order = 2) was applied using the Renishaw WiRE's smoothing feature to reduce the noise. Lastly, the data sets were merged to obtain the average spectra of the samples irradiated with the same doses.

Data Analysis

Bio-Rad KnowItAll software (trial version) was used to analyze the components of the obtained spectra to ensure that the peaks obtained were due to the presence of carotenoids. The average integrated areas under the highest peaks – 1156 cm^{-1} and 1519 cm^{-1} (labeled as v1 and v2, respectively) – were representative of carotenoid content. Renishaw's Windows-based Raman Environment was used to obtain the integrated areas under these peaks. The change in total carotenoid content of the irradiated sample was quantified by taking the ratio of average integrated areas of the characteristic carotenoid peaks of the irradiated and non-irradiated (control) samples, as shown by the following equation:

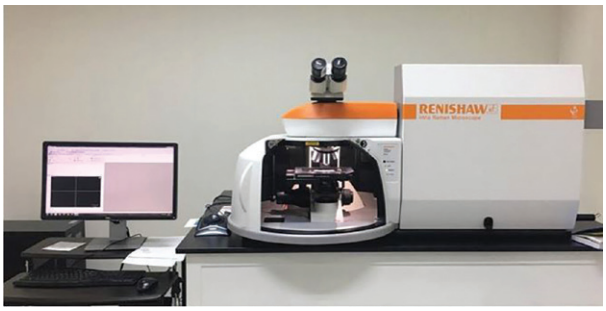


Figure 1. Renishaw Confocal Raman spectrometer located at UP Manila.

$$\text{Ratio} = \frac{\textit{Average integrated area of Raman peak of the irradiated sample}}{\textit{Average integrated area of Raman peak of the controlled group}} \quad (1)$$

T-test was used to assess if gamma irradiation had a significant effect on the total carotenoid content of the samples. Spearman's and Pearson's correlation tests were used to determining if there is a correlation between the total carotenoid content and the gamma radiation dose used. In the correlation tests, each of the individual spectra obtained was used to account for the variances. After establishing the correlation, a regression line was fitted on the relative average carotenoid content for each gamma radiation dose with respect to the control group. The average of the regression lines under the average integrated area at 1156 cm^{-1} and 1519 cm^{-1} peaks was used to obtain an equation that estimates the percent decrease in the total carotenoid content, based on the radiation dose used.

RESULTS

The mango puree samples showed 3 carotenoid peaks at 1005.72 cm^{-1}, 1156.18 cm^{-1}, and 1518.99 cm^{-1}. However, the peak at 1005.72 cm^{-1} was too weak. Therefore, the peaks at 1156.18 cm^{-1} and 1518.99 cm^{-1} were chosen for the analysis of carotenoid content. These peaks were labeled as v1 and v2, respectively. The Raman spectra obtained from the experiment matched the spectra of carotenes from the Raman spectral database of Bio-Rad

KnowItAll software in terms of spectral locations and amplitudes of the peaks (Figures 2 and 3).

The total carotenoid content was quantified using the ratio of average integrated areas in the peaks v1 and v2 using Equation 1. Results showed that mango purees irradiated using low doses (0.2 and 1 kGy) of radiation had no significant effect ($p > 0.01$) on the total carotenoid content. However, higher doses (6, 10, 15 kGy) of radiation yield a significant decrease ($p < 0.01$) on the total carotenoid content.

Results also showed that there is a high negative correlation between the gamma radiation dose applied and the total carotenoid content of mango purees. Spearman's rank-order correlation between the gamma radiation dose and the average integrated areas under v1 and v2 yielded correlation coefficients of –0.759 and –0.780, respectively. Pearson's correlation between the gamma radiation dose and the average integrated areas under the peaks also yielded significant negative correlation ($p < 0.01$) of –0.759 and –0.799 for v1 and v2, respectively.

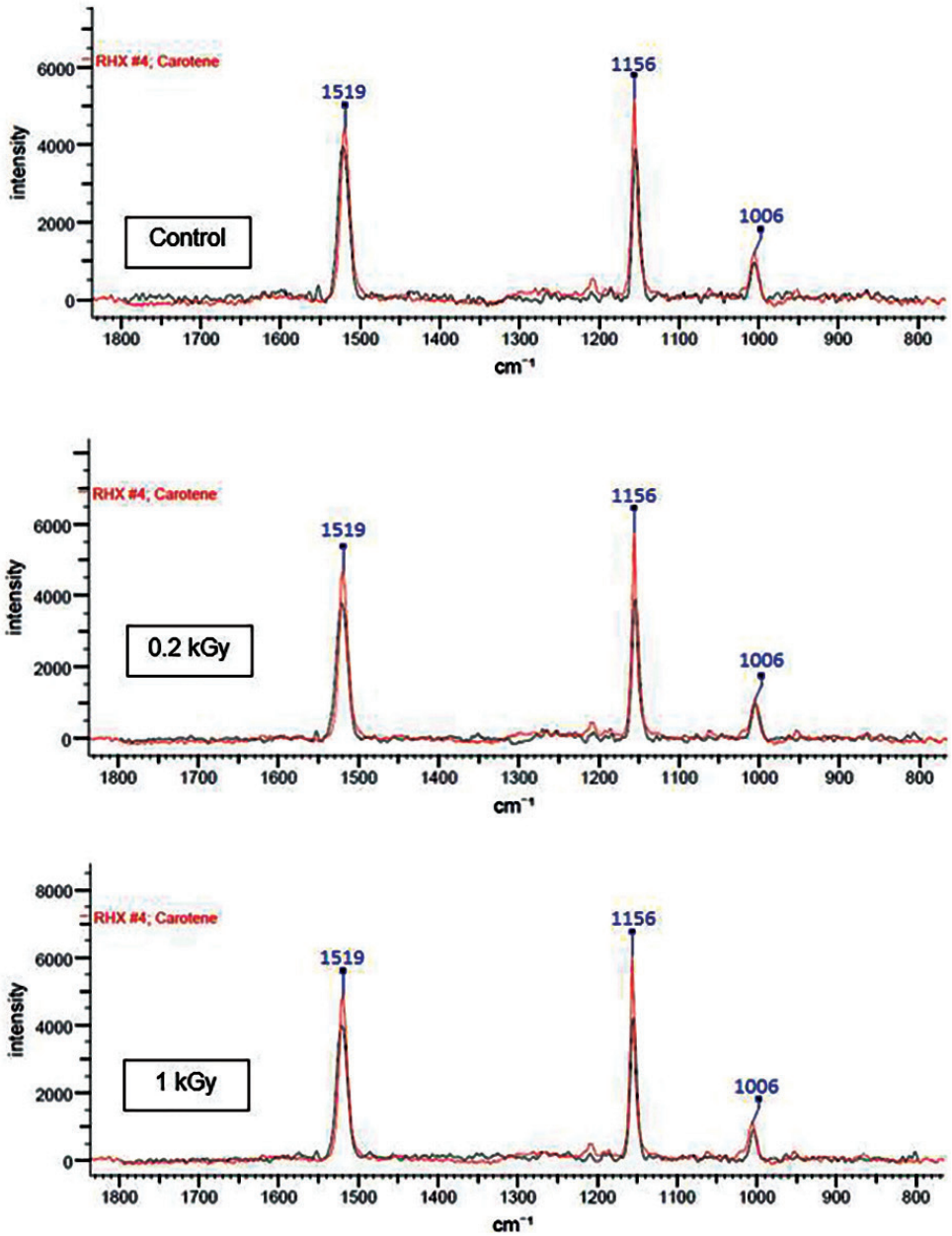


Figure 2. Comparison of the average spectrum of the samples irradiated at different low doses (black) and spectrum of carotene from Bio-Rad Laboratories Informatics Division's Raman spectral database (red).

The regression lines fitted on the ratio of average integrated areas under v1 and v2 plotted versus the radiation doses used, yielded r squares of 0.9656 and 0.9554, respectively. These are shown in Figure 4.

Using the average of the regression lines from v1 and v2, the decrease in the total carotenoid content due to the gamma irradiation treatment can be approximated as:

$$\mathbf{P\ (\%) = 3.25\ D} \qquad (2)$$

Where:
P = total decrease on the total carotenoid content in %
D = gamma radiation dose in kGy

DISCUSSION

No significant effects on the total carotenoid were observed in the samples irradiated with low doses of radiation. This is in agreement with the results of Mitchell *et al.* (1990), and Reyes and Cisneros-Zevalos (2007). However, it was shown that the use of high dose of radiation can significantly decrease the total carotenoid content of mango purees and this decrease is linearly dependent on the radiation dose.

In 1956, Lukton and Mackiney claimed that carotenoids in plant tissues, such as fresh fruits like mango, are more resistant to destruction by radiation compared to

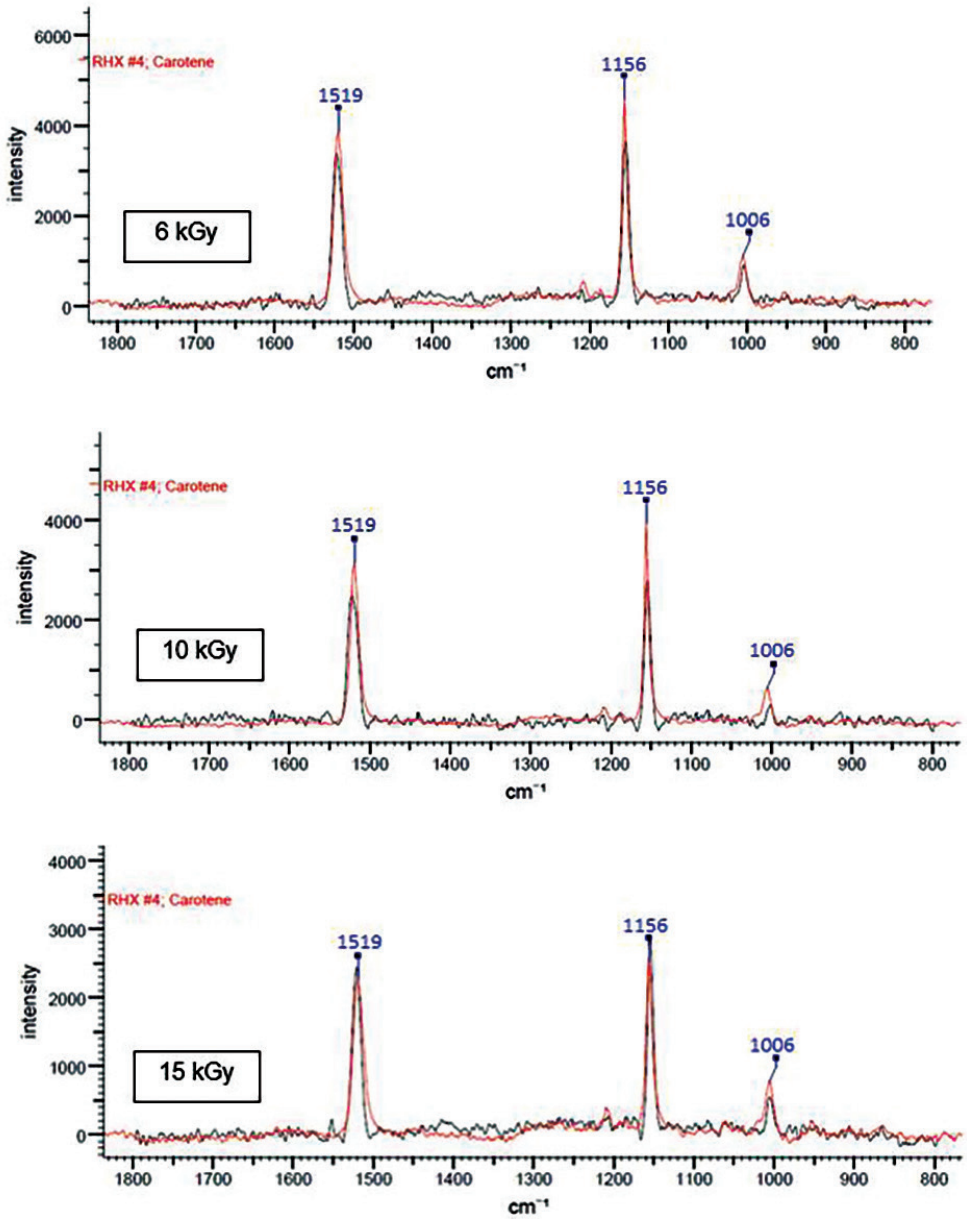
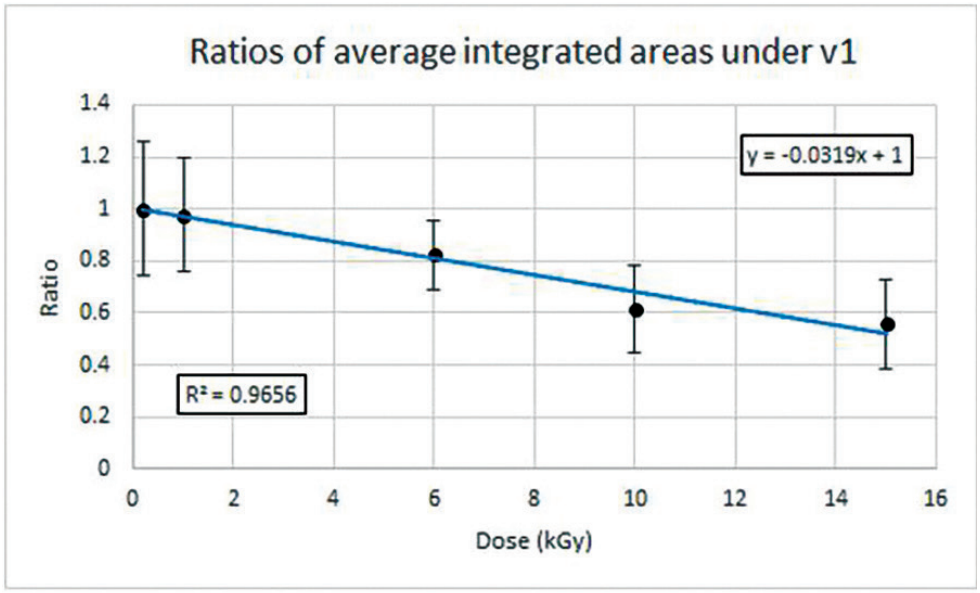


Figure 3. Comparison of the average spectrum of the samples irradiated at different high doses (black) and spectrum of carotene from Bio-Rad Laboratories Informatics Division's Raman spectral database (red).



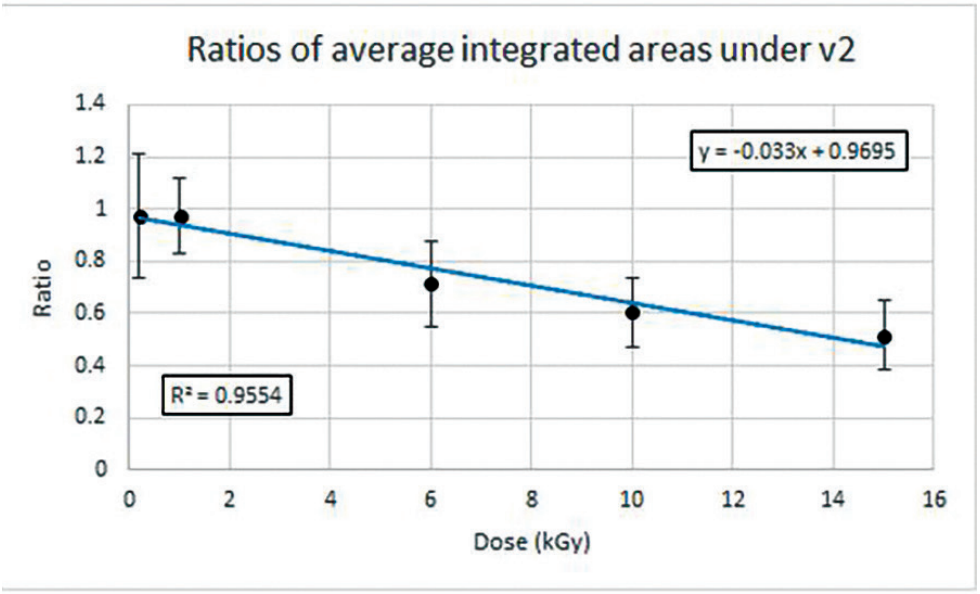


Figure 4. Ratio of the average integrated area under v1 and v2 for each radiation dose (kGy) used.

the carotenoids in solutions due to the presence of other compounds in the tissue. These compounds can protect the carotenoids against radiation-induced free radicals (Lukton and Mackinney 1956). However, since purees were used in this study, our findings show that the use of a high dose of radiation on mango purees can significantly decrease the total carotenoid content of mango purees and this decrease is linearly dependent on the radiation dose. This difference may be due to multiple factors such as composition, density, and processing conditions such as temperature and humidity (Kilcast 1994).

The decrease in the total carotenoid content due to gamma irradiation was approximated using the average of the regression lines from v1 and v2, as seen in Equation 2.

CONCLUSION

Gamma irradiation is already proven to increase the shelf life and improve the food safety of products like fresh fruits. However, the sensitivity of essential vitamins to ionizing radiation such as carotenoids found in processed food may contribute to affecting their qualities when undergoing gamma irradiation. The effects of varying doses of gamma radiation on the carotenoid content of mango purees were assessed using Raman microspectroscopy. The total carotenoid content was represented by the average integrated areas of the highest carotenoid peaks in the Raman spectra. It was found that mango purees can be irradiated at recommended doses (1–2.5 kGy) defined in Philippine National Standards PNS/

BAFS 151:2015 ICS 67.020 Code of Hygienic Practice for Radiation Processing of Food, which increases the shelf life and safety of the product while having minimal effects on the total carotenoid content. For higher doses however a significant decrease in the total carotenoid content was observed. The decrease on total carotenoid content of mango purees due to gamma irradiation treatment can be approximated as P = 3.25D, where P is the total decrease in percentage (%) and D is the gamma radiation dose in kGy. The strong correlation between the gamma radiation dose and the decrease in the total carotenoid content will be beneficial in the optimization of the mango puree irradiation treatment. The results of this study can contribute to the standardization of irradiation protocol for mango purees in the Philippines.

ACKNOWLEDGMENTS

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NOTE ON APPENDICES

The full Appendices section of this article can be found at http://philjournalsci.dost.gov.ph.

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APPENDIX I

Average Spectra

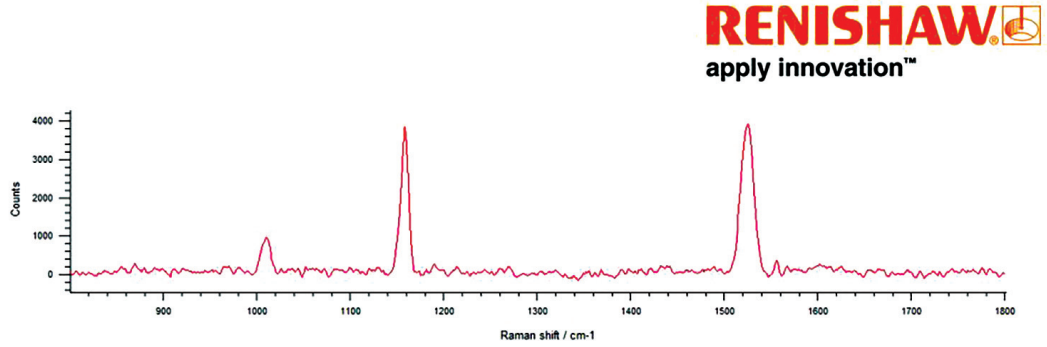

Figure I.1. Average spectrum, control group.

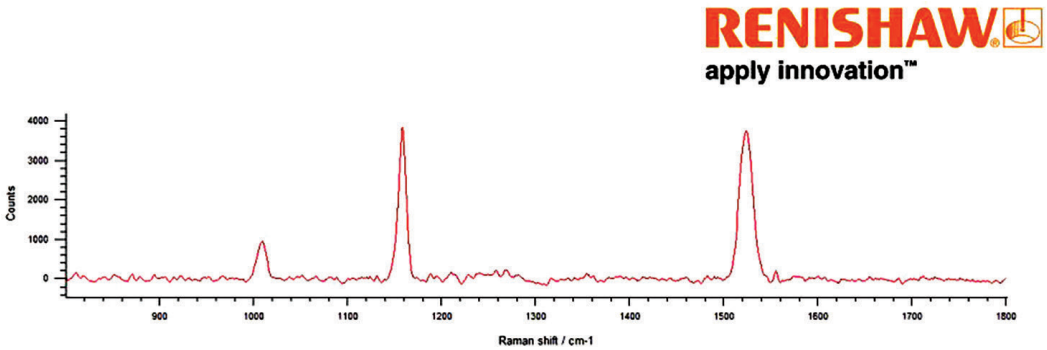

Figure I.2. Average spectrum, 0.2 kGy.

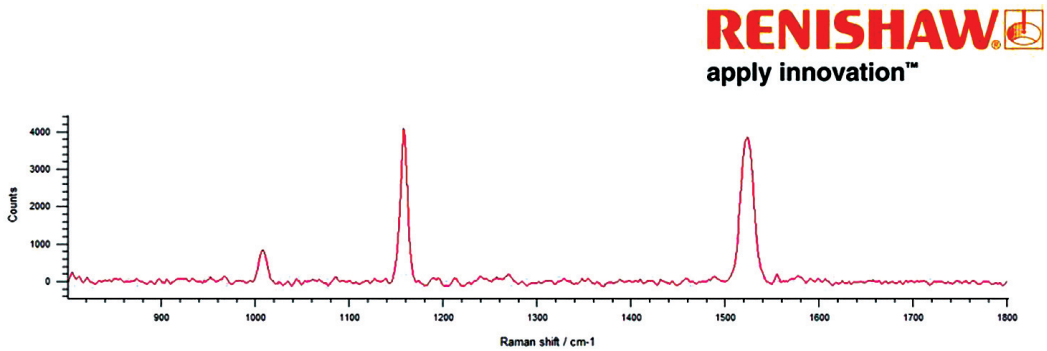

Figure I.3. Average spectrum, 1 kGy.

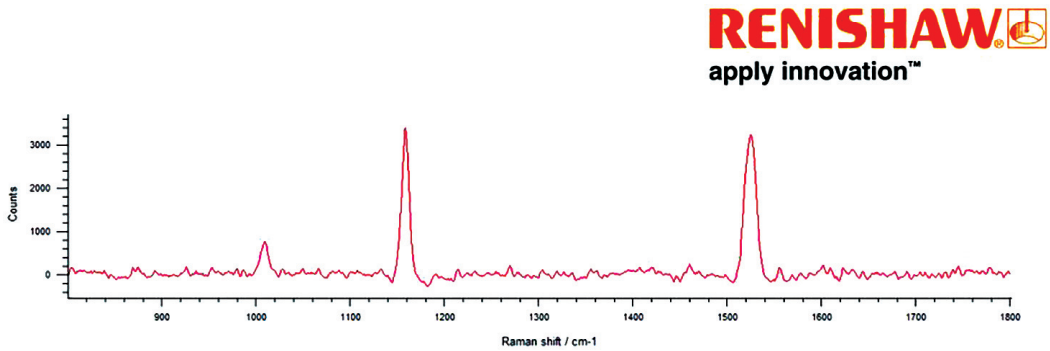

Figure I.4. Average spectrum, 6 kGy.

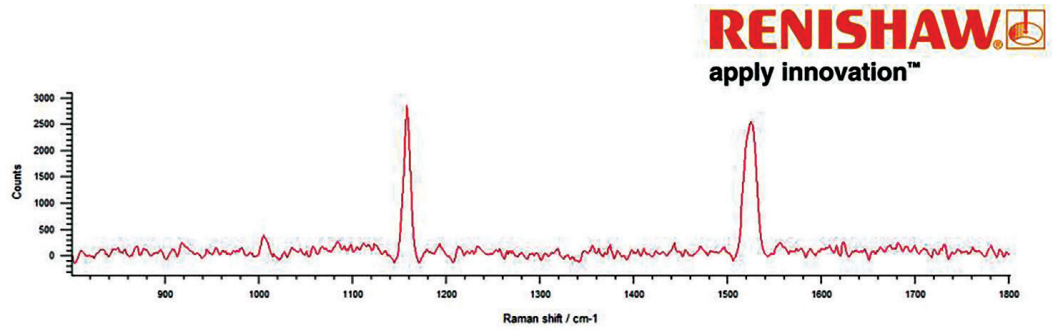

Figure I.5. Average spectrum, 10 kGy.

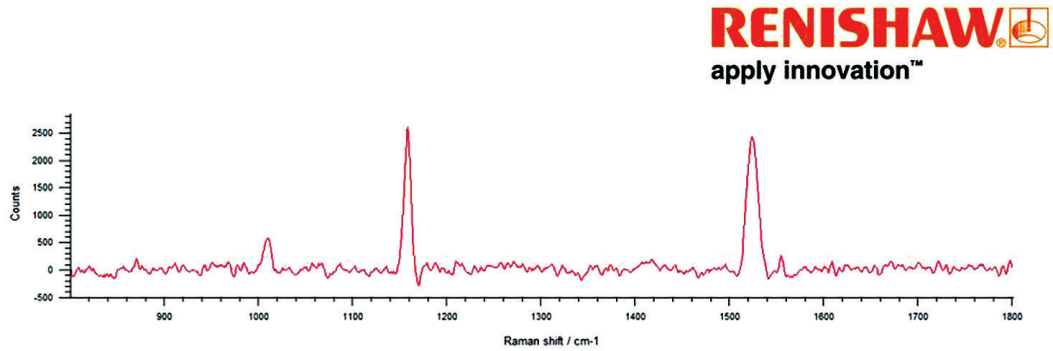

Figure I.6. Average spectrum, 15 kGy.

APPENDIX II
Integrated Area Under the Peak (Units: counts x cm^{-1})

Table II.1. Integrated areas under the v1 and v2 peak of the spectra of the control group.

Control group			
Sample	Trial	v1 (1156 cm^{-1})	v2 (1519 cm^{-1})
A	1	43388.790	62445.194
	2	53459.127	64510.305
	3	36697.780	76475.087
B	1	47840.479	61009.477
	2	42111.105	66975.322
	3	38997.238	73120.290
C	1	39900.680	59951.372
	2	42181.962	71585.019
	3	37403.501	55570.231
	Average	**42000**	**66000**
	Std. dev.	**5000**	**6000**

Table II.2. Integrated areas under the v1 and v2 peak of the spectra of the spectra of samples irradiated at 0.2 kGy.

1.0 kGy			
Sample	Trial	v1 (1156 cm^{-1})	v2 (1519 cm^{-1})
A	1	34518.212	52774.643
	2	47430.401	51792.137
	3	57216.015	78056.466
B	1	45423.452	66787.068
	2	40443.464	67187.406
	3	38848.344	76446.816
C	1	34838.675	66902.563
	2	33050.373	56219.092
	3	40917.375	59656.689
	Average	**41000**	**64000**
	Std. dev.	**7000**	**9000**

Table II.3. Integrated areas under the v1 and v2 peak of the spectra of samples irradiated at 1 kGy.

1.0 kGy			
Sample	Trial	v1 (1156 cm^{-1})	v2 (1519 cm^{-1})
A	1	34518.212	52774.643
	2	47430.401	51792.137
	3	57216.015	78056.466
B	1	45423.452	66787.068
	2	40443.464	67187.406
	3	38848.344	76446.816
C	1	34838.675	66902.563
	2	33050.373	56219.092
	3	40917.375	59656.689
	Average	**41000**	**64000**
	Std. dev.	**7000**	**9000**

Table II.4. Integrated areas under the v1 and v2 peak of the spectra of samples irradiated at 6 kGy.

6.0 kGy			
Sample	Trial	v1 (1156 cm^{-1})	v2 (1519 cm^{-1})
A	1	37478.787	61136.457
	2	38441.872	47907.070
	3	35207.465	55686.571
B	1	35184.489	54450.862
	2	38731.623	30188.342
	3	29175.963	46807.696
C	1	29259.782	44092.403
	2	35057.260	37510.960
	3	35783.593	44406.485
	Average	**35000**	**47000**
	Std. dev.	**3000**	**9000**

Table II.5. Integrated areas under the v1 and v2 peak of the spectra of samples irradiated at 10 kGy.

10 kGy			
Sample	Trial	v1 (1156 cm^{-1})	v2 (1519 cm^{-1})
A	1	13723.935	32833.035
	2	21347.489	39228.184
	3	23663.991	36919.472
B	1	27038.718	56978.442
	2	33351.823	45490.766
	3	29225.581	34845.220
C	1	26412.214	31706.123
	2	34655.560	37778.378
	3	26741.801	41966.327
	Average	**26000**	**40000**
	Std. dev.	**6000**	**7000**

Table II.6. Integrated areas under the v1 and v2 peak of the spectra of samples irradiated at 15 kGy.

15 kGy			
Sample	Trial	v1 (1156 cm^{-1})	v2 (1519 cm^{-1})
A	1	17667.163	30447.055
	2	20779.270	42240.783
	3	18374.822	38690.060
B	1	29129.332	37323.157
	2	23037.511	24830.729
	3	28875.542	35911.523
C	1	25293.758	45830.081
	2	14477.779	27498.672
	3	35625.148	23219.331
	Average	**24000**	**34000**
	Std. Dev.	**6000**	**7000**

APPENDIX III
Data Processing

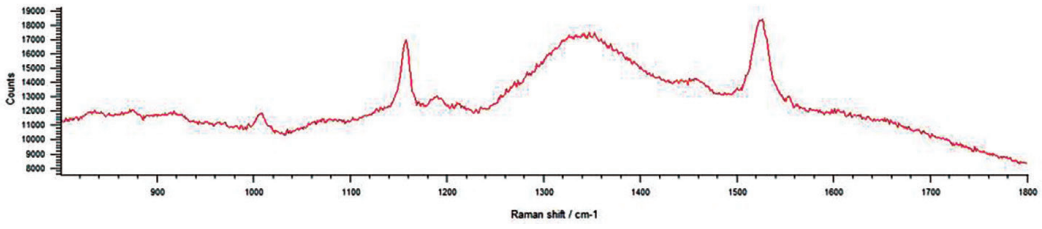

Figure III.1. Raw spectrum of the sample.

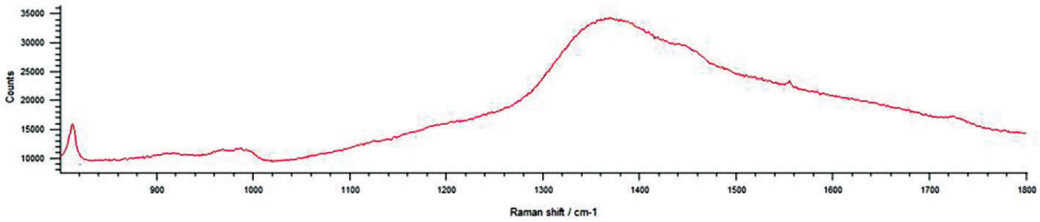

Figure III.2. Background spectrum.

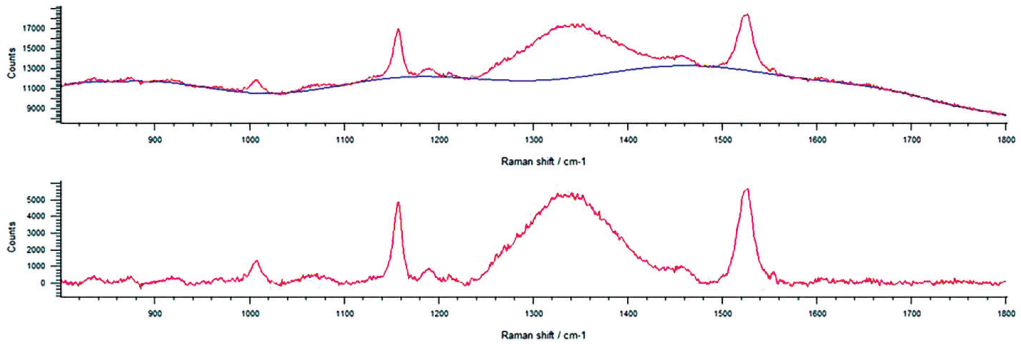

Figure III.3. Intelligent polynomial fitting.

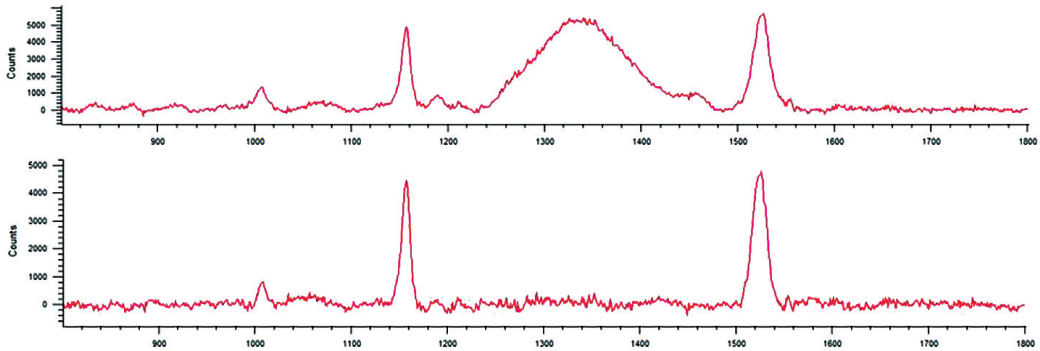

Figure III.4. Cubic spline fitting.

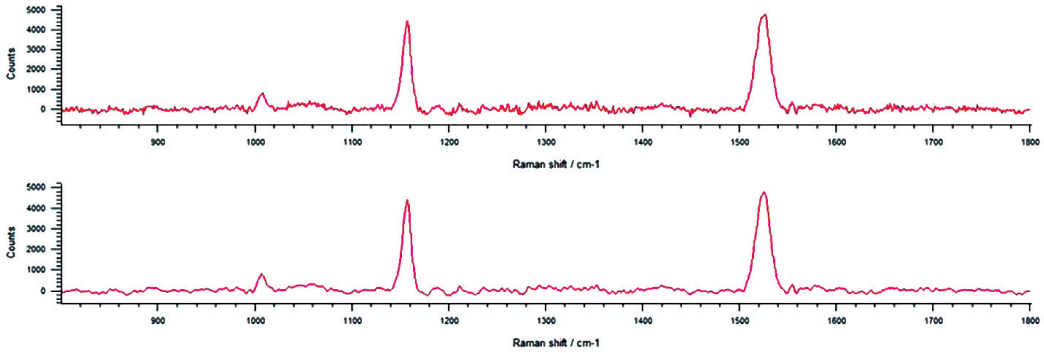

Figure III.5. Smoothing.