Evaluation of Gamma Irradiation Effects on Brazilian Orange Melon (*Cucumis melo L.*)

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***Abstract*—** *Orange flesh melons (Cucumis melo L. var. inodorus) is widely cultivated in Brazil, especially in the Northeast region. The study was carried out to determine the effects of gamma irradiation at doses 0.5 and 1.0 kGy on the carotenoids, sugars, volatile, flavor and sensory characteristics. High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC) and Mass Spectrometry (MS) were used to identify and quantify carotenoids, sugars and volatile compounds. Sensorial analysis was performed by untrained tasters using a just right scale. Were evaluated the suitability of the intensity of firmness. There were no significant changes in the parameters of pH, total titratable acidity, total soluble solids analyzed on samples. Determinations of total carotenoids, volatile compounds, the levels of sugar and flavor and firmness parameters indicated that the best irradiation dose was 0.5 kGy. The orange melon is good source of carotenoids (23,800µg.100g-1), being the major compound β-carotene, can be employed to combat hypovitaminosis A.*

***Keywords*— carotenoids, GC-MS, HPLC, irradiation, orange melon.**

I. INTRODUCTION

The contents of each section may be provided to understand easily about the paper. The melon (*Cucumis melo L.* var. *inodorus*) is one of the oldest crops worldwide. There are many species which differs in fruit size, morphology and organoleptic properties [1]. In Brazil, the melon with a planted area of 12,500 hectares is one of the most cultivated Cucurbitaceae. Currently, the two largest producers are the poles Mossoró-Assu (RN) and Lower Jaguaribe (CE). Among the main types of melons produced in the region, highlight the "honeydew", the "gália", the "cantaloupe", the "charentais", the "yellow" and the "orange flesh". On current data, Brazil exported about 152 million dollars in melon, this fruit is considered the second most consumed fruit "in natura" and that contributed most to the brazilian trade balance [1].

The orange flesh melon, presents an excellent acceptance in domestic and foreign markets, especially due to their high level of soluble solids. Moreover, the β-carotene is its main component and an antioxidant carotenoid pigment. In nature, is a source of vitamin A to obtain through metabolism in the human body. The carotenoid compounds presents a β-ionone in its structure and a side chain with at least two isoprenol units and belong to a large group of compounds collectively known as terpenes and terpenoids. Great discoveries have been made about β-carotene properties. Today we know that it is an antioxidant that benefits the night vision, boosts immunity, gives elasticity to the skin, increases the brightness of the hair and strengthen nails, besides acting in fat metabolism [2,3].

A deficiency of vitamin A was detected in several Brazilian states like Amazonas, Rio Grande do Norte, Paraíba, Pernambuco, Bahia, Minas Gerais, São Paulo and Santa Catarina, and may be recognized as a public health problem [4].

A positive correlation between the consumption of nutritious foods and protection against the development of chronic diseases has been observed. According to the Dietary Reference Intakes (DRI), the Recommended Daily Amount (RDA) of vitamin A for healthy adults is: 900 µg RAE for men and 700 µg RAE for women. Retinol Activity Equivalent (RAE) is used to describe vitamin A activities of carotenoids, 1 µg of RAE is equivalent to vitamin A activity of 12µg of β-carotene in mixed food [3,4,5].

Nowadays, worldwide there is an imbalance between population and food supply, and the problem of food availability has been worsening over the past 20 years. The reduction of post harvest losses that occur annually in different stages of obtaining food from production to consumption is a measure to change the growth pattern of this imbalance. Studies estimate those losses of vegetables occurring in more than 30% at the stages of handling, transport, storage and marketing.3 In this context the use of food irradiation is very promising and very important, through their contribution to conservation, reducing post harvest losses and the possibility of improving the food supply. The process involves exposing food as packaged or in bulk, to a highly penetrating radiation field, for a given time, in order to destroy pathogenic bacteria, parasites, fungi and insects, or inhibit the ripening process of fruits [6,7].

According a joint FAO/IAEA/WHO study group on high-dose irradiation reviewed determining to the toxicological, nutritional, radiation chemical and physical aspects of food irradiated to doses above 10 kGy and concluded that foods treated with doses greater that 10 kGy can be considered safe and nutritionally adequate, when it were produced under established good manufacturing practice (GMP). This study evaluated the gamma irradiation effects in brazilian orange melon by analysis of soluble solids, titratable acidity, pH, total carotenoids, β-carotene, sugar, volatiles compounds and sensory analysis [8].

II. MATERIAL AND METHOD

2.1 Melon samples

Orange melons (*Cucumis melo L.* var. *inodorus*) were purchased from Mossoró – RN, Brazil. The melons were purchased packed in corrugated cardboard boxes of 10 kg, type 9. The fruits in optimal ripeness for consumption had a spherical shape, smooth skin yellowish-white and orange pulp. The units are divided into three groups each of nine melons. The purpose of using different samples of fruit for application of the same dose is that the results obtained can be ripeness independent or specific conditions but still shows what is observed in the group. The fruits were stored refrigerated (7-10 °C) for 24 hours before treatment with gamma irradiation, relative humidity 80-85%.

2.2 Irradiation

The samples irradiation occurred at Army Technological Center (CTEx), at Guaratiba, Rio de Janeiro, RJ (Brazil) with a research irradiator with cesium-137 source, at doses 0.5 and 1.0 kGy. The irradiator apparatus is a shielded cavity and has the useful volume of the order 100 L. The irradiator was designed and built at Brookhaven National Laboratory (USA) in 1969. The current activity of the source is approximately 45 kCi with a dose rate 1.6 kGy h^{-1} with good uniformity, irradiation temperature of 28-30°C. The exposure time was calculated using a program developed especially for the radiator, based on a dosimetric mapping, which takes into account the current activity of the source, desired mean dose (Gy), diameter or height, density and geometry of the sample, high- attenuation factor and the build-up [9].

2.3 Physical and chemical analysis

After treatments, the melons samples were evaluated using the following parameters:- pH, directly determined in pH-meters; - total titratable acidity, determined by titration (results expressed as % citric acid); - total soluble solids, determined by direct reading refractometer, portable refractometer Quimis model Q767-4, the obtained results were expressed as °Brix, corrected for the room temperature [10].

2.4 Carotenoids analysis

The total carotenoids were obtained by UV spectrophotometry, the β-carotene and its isomers by high performance liquid chromatography (HPLC). The carotenoids were extracted with acetone and then extracted with petroleum ether, after which were concentrated by flow under nitrogen gas and dissolved in 1µL of acetone. The mobile phase was transferred to a volumetric flask (100 ml) with anhydrous sodium sulfate and quantified by UV spectrophotometry at 450 nm. The chromatographic analysis was performed using a Shimadzu chromatograph with a chromatographic column C30 3 µm 141 4.6 x 250 mm - Waters YMC carotenoid, with the mobile phase gradient of methanol / methyl t-butyl ether - 80:20 to 10:90 in 28 minutes, with flow 0.8 mL min^{-1} and 30 °C [9,11].

2.5 Volatile compounds analysis

For volatile compounds was used gas chromatography (GC) coupled with mass spectrometry (MS). The aroma determination of the fruit was held in the headspace of vials of 60 mL for solid phase microextraction (SPME) using a fiber polydimethylsiloxane (PDMS) of 100 µm thickness and bath temperature of 60°C for 20 minutes. The chromatographic separation was performed on a column of 30 m x 0.32 mm and 0.2 mm thick, with 5% diphenyl 95% dimethylpolysiloxane as stationary phase. The chromatographic conditions were: linear heating from 30 to 240°C with a rate of 10°C . min^{-1}; gun "split-splitless" rate "split" of 1/100, and flow rate of 1 mL min^{-1}. SCAN mode was used for m z^{-1} 15 to 300, mobile phase helium, manual injection and flow rate of 1.00 mL . min^{-1}. The heating was linear from 40 °C to 300 °C with a rate of 10 °C . min^{-1} [12].

2.6 Sugar analysis

High Performance Liquid Chromatography (HPLC) quantified the main sugars. These compounds were extracted with water and acetonitrile. Analyses were performed on a Shimadzu liquid chromatography, using an amino column 30 cm x 4.6 mm, 1.4 mL min^{-1} flow, 75% acetonitrile as mobile phase and a detector - IR 2410 [13].

2.7 Sensorial analysis

Sensorial analysis was performed by 40 untrained tasters using a just right scale. They were evaluated the suitability of the intensity of firmness with the follow scale: from –4 (extremely hard) to +4 (extremely soft), and ideal treatment received a score of 0. The results were evaluated by linear regression [9].

2.8 Statistical analysis

For statistical analysis was used the Student’s t Test, where assumed that the samples came from normal populations with equal variances. The level of significance was 5% [14].

III. RESULTS AND DISCUSSION

3.1 Physical and chemical analysis

The short shelf-life of orange flesh melons, hinders their commercialization, and producers need to use post-harvest techniques to enhance your life. The evaluation of physical and chemical parameters of the fruits contributes to determining the shelf life. In this study there was no variation between the different treatments, and that average values obtained for pH 6.15, total titratable acidity 0.01% v m^{-1} and the total soluble solids 8.0 °Brix, corroborate with reported in literature data for melons [15]. In studies where the chilling was applied as a technique for increasing the shef-life post-harvest, was observed that factors such as firmness, weight loss of the fruit and changes in pH with greater intensity [16]. The process of irradiation at doses of 0.5 and 1.0 kGy was favorable in the maintenance of pH, titratable acidity and soluble solids.

3.2 Carotenoids

The mean values obtained by spectrophotometry for total carotenoids of sample were 23.800 µg . 100 g^{-1}, 21.900 µg . 100 g^{-1} and 20.500 µg . 100 g^{-1} in control fruits, in irradiated fruits 0.5 and 1 kGy, respectively. Carotenoids content in plants may change due to oxidation caused by increased temperature, exposure to light, water activity, the presence of metals, acidity and irradiation [17].

The β-carotene and its isomers were quantified by High Performance Liquid Chromatography (HPLC) (Fig. 1, Table 1) with UV detector (Fig. 2). The 9-cis-β-carotene and 13-cis-β-carotene isomers did not change their concentrations after treatment of gamma radiation. The β-carotene has been reported to be the main carotenoid found in melons, such as hereditary factor and furthermore, it is also correlated with the color of fruit mesocarp [19].

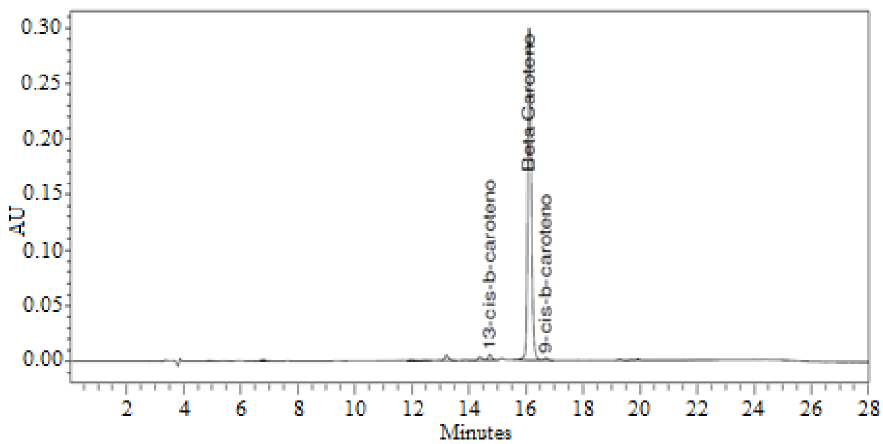

FIGURE 1. HPLC ANALYSIS OF β-CAROTENE AND ITS ISOMERS (13-cis-β-carotene, β-carotene and 9-cis-β-carotene) to the control

TABLE 1
CONTENTS OF CAROTENOIDS (µg.100g-1) IN ORANGE MELON

Carotenoids			
Melon	**β-carotene**	**9-cis- β-carotene**	**13-cis- β-carotene**
Control	9298 ± 238c	8.50 ±0.18b	15.3 ±0.7b
0.5 kGy	9243 ± 165b	8.60 ±-0.09b	14.7 ±0.5b
1.0 kGy	9222 ± 156b	8.70 ±0.32b	16.1 ±0.8b

aMeans +- SD (n=3)
bNo significant difference to a level of 5%
cSignificant difference to a level of 5%

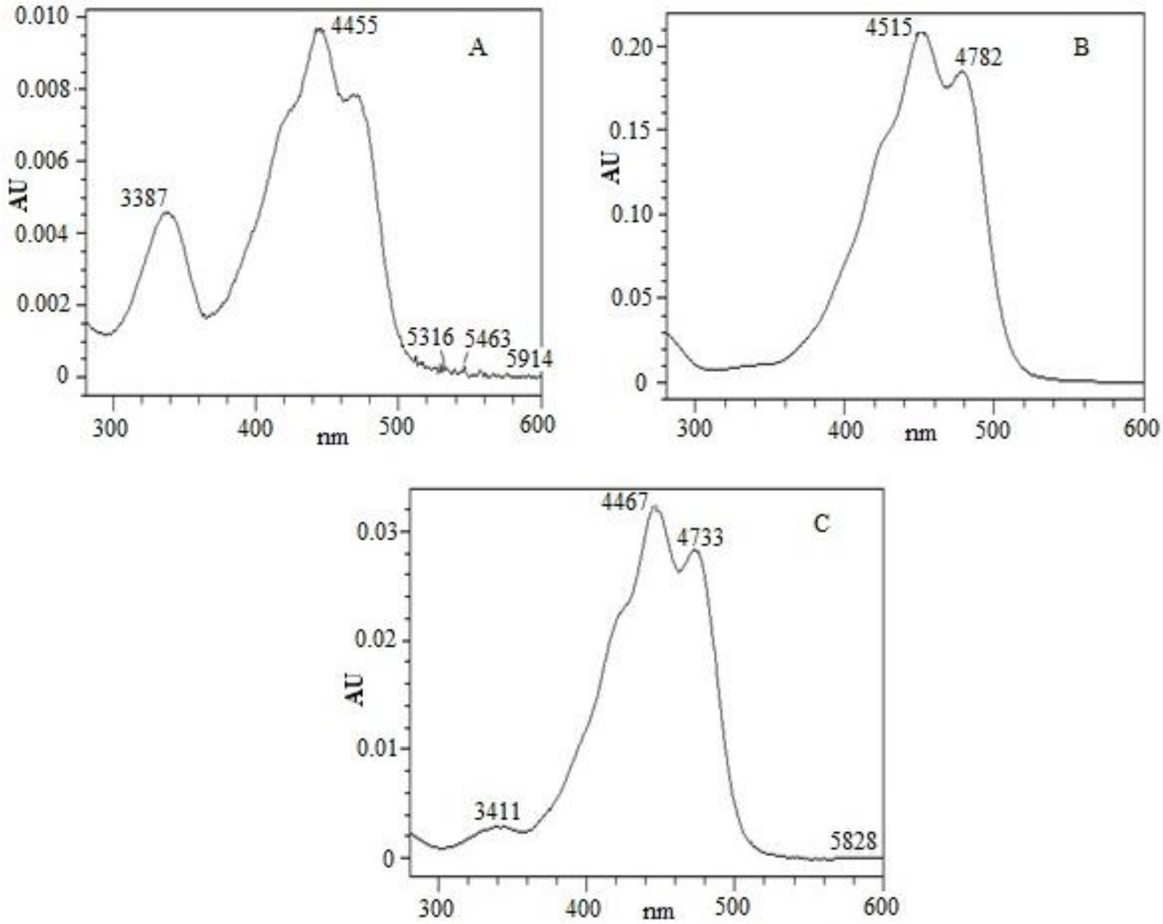

FIGURE 2. ORANGE MELON UV/V IS SPECTRA OF: (A) 9-cis-β-carotene, (B) β-carotene, (C) 13-cis-β-carotene

In 9298 µg . 100 g^{-1} the fruit was found control of β-carotene corresponding to 775 µg RAE, a very close value to the recommended daily intake of provitamin. The irradiation caused a significant reduction in β-carotene levels in fruits compared to control, but still, the irradiated fruits showed high content of β-carotene (770.2 µg RAE and 768.5 µg RAE to 0.5 and 1.0 kGy respectively). The results to β-carotene indicate that the melon orange nutritional has a greater value [2,19].

3.3 Sugar

The sugar were quantified by HPLC (Fig. 3, Table 2). Were identified in melons fruit samples, fructose, sucrose and glucose as the main sugar, and sucrose showed the highest concentration.

The main sugar present in melon are glucose and fructose, which contribute nearly 100% of total soluble sugar in the early stage of fruit development, and sucrose, which can reach 50% of the soluble sugars in the final stages of maturation, with ratio of approximately 25% to glucose and 25% to fructose. The sugar identified show that the fruits were ripe.20 Comparing the results between control and irradiated fruit melons, there was a slight decrease in the concentration of sugars (glucose, fructose and sucrose) on fruits treated with doses of 0.5 and 1 kGy. The decrease sugar contents due to possible breakage of glycosidic bonds and chemical changes [17].

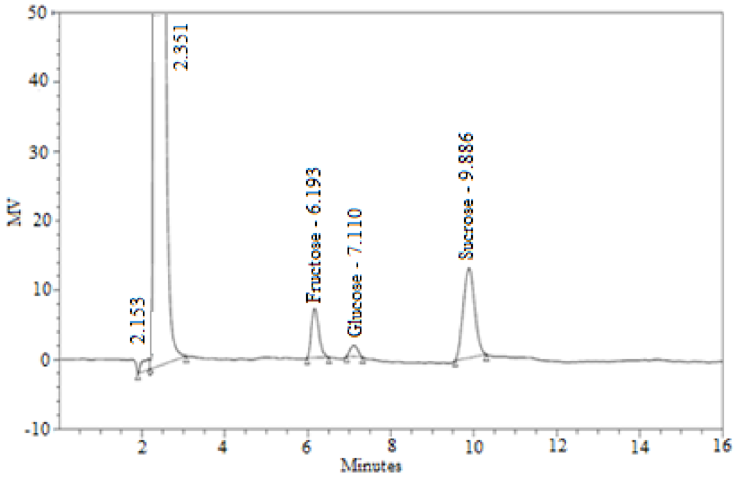

FIGURE 3. HPLC ANALYSIS OF FRUCTOSE, GLUCOSE AND SUCROSE TO THE SAMPLE IRRADIATED WITH 0.5 kGy.

TABLE 2
CONTENTS OF SUGAR (µg . 100 g-1) IN ORANGE MELON

	Sugar		
Melon	**glucose**	**fructose**	**Sucrose**
Control	10.53±0.55c	20.25±0.64b	60.18±0.86c
0.5 kGy	8.96±0.37b	19.20±0.57b	55.40±0.75c
1.0 kGy	8.53±-0.27b	15.84±0.42c	45.44±0.68c

aMeans +- SD (n=3)
bNo significant difference to a level of 5%
cSignificant difference to a level of 5%

3.4 Volatile compounds

For analysis of volatile compounds, this study used Gas Chromatography (GC) coupled with Mass (MS) detector, in this way it could be identify with relative accuracy the volatile compounds presents in the analyzed products (Fig. 4). The volatile compounds extracted by the technique of solid phase microextraction are shown in Table 3: (3Z)-3-decenyl acetate (28.6%), n-nonyl ethanoate (11.66%) and hexyl acetate (10.76%) were the major esters found in control and (3Z) -3-decenyl acetate (29.2%), 2-methylbutyl acetate (18.4%) and hexyl acetate (12.75%) in irradiated fruits. The identification of these compounds was made by comparison with the NIST library through the similarity of the mass spectrum found at the points with largest area in the chromatograms. Volatile compounds are the main determinants of quality of melon noted by consumers, whose acceptance of the melon is driven mostly by acidity, sweetness, and also by a bouquet of aromas caused the presence of volatile compounds [21,22].

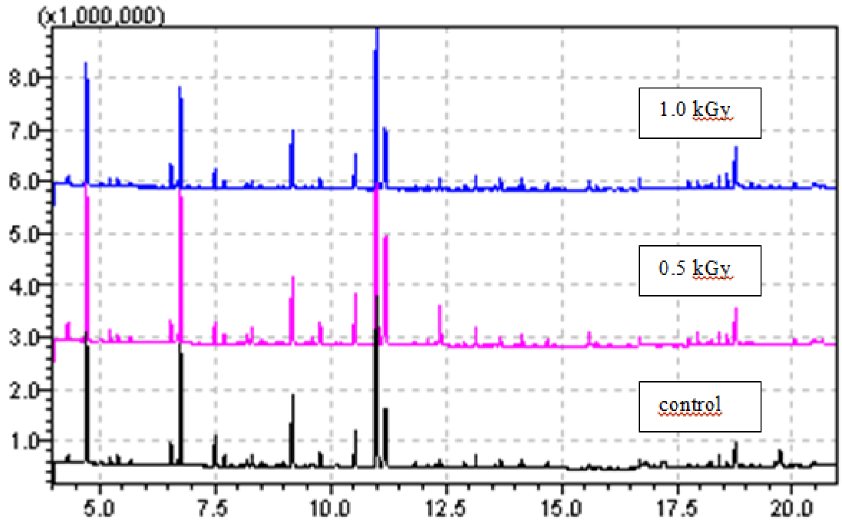

FIGURE 4. COMPARISON OF TOTAL ION CHROMATOGRAMS (TIC)

TABLE 3
VOLATILES COMPOUNDS IN ORANGE MELON

Compounds	RT (min)	Control (%)	0.5 kGy (%)	1.0 kGy (%)
2-Methylbutyl acetate	4.8	7.54	18.39	18.55
Ethyl hexanoate	6.6	7.04	2.4	2.74
Hexyl acetate	6.8	10.76	13.64	11.87
2,3-Butanedioldiacetate	7.5	-	3.35	2.43
Benzyl acetate	9.2	3.03	8.52	7.53
Ethyl octanoate	9.6	3.95	-	-
Octyl acetate	9.8	3.71	1.15	-
β.-Phenethyl acetate	10.6	6.16	4.2	4.46
(3Z)-3-Decenyl acetate	11.0	28.6	29.25	29.24
1-Decanol acetate	11.2	6.3	11.59	13.2
n-Nonyl ethanoate	11.2	11.66	-	-
Ethyl caprinate	12.4	3.17	-	-
cis-Geranylacetone	13.1	-	-	1.57
Palmitic acid, methyl ester	18.4	-	1.46	1.6
(+)-Ascorbic acid 2,6-dihexadecanoate	18.9	-	2.95	4.88

1. Retention Time (RT)

In a previous study [23], the composition of volatiles in cantaloupe, galia and honeydew melons and found the 2-methylbutyl acetate and hexyl acetate esters as the major esters in the three varieties of melon, results corroborate the data presented in this paper. It is observed that after the process of gamma irradiation higher esters such as n-nonyl ethanoate undergo a reduction or total elimination, which causes a consequent increase in chain lower esters such as 2-methylbutyl acetate.

3.5 Sensorial

After evaluating the results from the linear regression analysis, the dose of 0.75 kGy was considered ideal for the firmness attribute and a dose of 0.2 kGy for the flavor attribute. Under the conditions of analysis, the doses were sufficient to maintain the sensory quality of orange melons. The results obtained in the sensory analysis and sugar content show that the fruit must be irradiated at low doses to preserve the flavor. Note also that the major compounds esters collaborate with flavor, as determined by analysis of volatiles.

IV. CONCLUSION

Analyses using spectrophotometry and HPLC indicated that the Brazilian orange melon is a good source of carotenoids (23.800 μg . 100 g^{-1}) and total β-carotene and can be used in combat Vitamin A deficiency was highly prevalent in the population Brazilian.

A dose of 1.0 kGy caused a reduction in the concentration of total carotenoids of the orange melon , yet remains a good source of provitamin A because 100g pulp may contribute approximately 768.5μg the daily requirement of vitamin A.

The volatile compounds extracted by the technique of solid phase microextraction are: (3Z)-3-decenyl acetate (28.6%), n-nonyl ethanoate (11.66%) and hexyl acetate (10.76%) were the major esters found in control and (3Z) -3-decenyl acetate (29.2%), 2-methylbutyl acetate (18.4%) and hexyl acetate (12.75%) in irradiated fruits.

There were no significant changes in the parameters of pH, total titratable acidity, total soluble solids analyzed on samples. Determinations of total carotenoids, volatile compounds, the levels of sugar and flavor and firmness parameters indicated that the best irradiation dose was 0.5 kGy.

The data obtained indicate irradiation as a method great potential for food conservation , since that chemical changes do not differ from those in other methods as changes in nutritional value of the orange melon (*Cucumis melo L.* var. *inodorus*).

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