

ORIGINAL

## Investigation of oxidation resistance of ascorbic acid in gamma-ray-induced solid samples

### Investigación de la resistencia a la oxidación del ácido ascórbico en muestras sólidas inducidas por rayos gamma

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#### ABSTRACT

**Introduction:** this study investigated the effects of gamma irradiation on the antioxidant activity of solid-state ascorbic acid. The goal was to assess how different irradiation doses, ranging from 0 to 5 kGy, altered the molecule's ability to scavenge free radicals.

**Method:** the DPPH radical scavenging method was employed to determine the antioxidant capability of the irradiated samples. The researchers evaluated the reaction rate constant (K1) and the concentration required to achieve 50 % inhibition of the radicals (IC<sub>50</sub>). Furthermore, FT-IR spectroscopy was used to detect structural changes in the ascorbic acid molecule following irradiation.

**Results:** antioxidant activity significantly increased at low irradiation doses (1-2 kGy) compared to the control (0 kGy, IC<sub>50</sub> of 3,39 ± 5,73 µg/mL). The lowest IC<sub>50</sub> values, indicating maximum activity, were observed at 1 kGy (2,67 ± 1,75 µg/mL) and 2 kGy (2,55 ± 1,00 µg/mL). The reaction rate constant peaked at 1 kGy (13,78 M<sup>-1</sup>.s<sup>-1</sup>), confirming the enhanced radical scavenging capacity. However, activity decreased at higher doses (≥ 3kGy). FT-IR analysis indicated structural alterations, specifically in the hydroxyl (-OH) and carbonyl (C=O) groups, following the irradiation process.

**Conclusions:** the results demonstrated that appropriate levels of gamma irradiation can increase the antioxidant effectiveness of ascorbic acid. Conversely, excessive exposure leads to structural changes and a subsequent reduction in its effectiveness.

**Keywords:** DPPH; Kinetics; Antioxidant Activity; Ascorbic Acid; Gamma; Radiation Source.

#### RESUMEN

**Introducción:** este estudio investigó los efectos de la irradiación gamma sobre la actividad antioxidante del ácido ascórbico en estado sólido. El objetivo fue evaluar cómo diferentes dosis de irradiación, de 0 a 5 kGy, alteraban la capacidad de la molécula para neutralizar radicales libres.

**Método:** se empleó el método de neutralización del radical DPPH para determinar la capacidad antioxidante de las muestras irradiadas. Se evaluó la constante de velocidad de reacción (K1) y la concentración necesaria para lograr una inhibición del 50 % de los radicales (IC<sub>50</sub>). Además, se utilizó espectroscopía FT-IR para detectar cambios estructurales en la molécula de ácido ascórbico tras la irradiación.

**Resultados:** la actividad antioxidante aumentó significativamente con dosis bajas de irradiación (1-2 kGy) en comparación con el control (0 kGy, IC<sub>50</sub> de 3,39 ± 5,73 µg/mL). Los valores de IC<sub>50</sub> más bajos, que indican

la máxima actividad, se observaron a 1 kGy ( $2,67 \pm 1,75 \mu\text{g/mL}$ ) y 2 kGy ( $2,55 \pm 1,00 \mu\text{g/mL}$ ). La constante de velocidad de reacción alcanzó su máximo a 1 kGy ( $13,78 \text{ M}^{-1}\cdot\text{s}^{-1}$ ), lo que confirma la mayor capacidad de captación de radicales libres. Sin embargo, la actividad disminuyó a dosis más altas ( $\geq 3 \text{ kGy}$ ). El análisis FT-IR indicó alteraciones estructurales, específicamente en los grupos hidroxilo (-OH) y carbonilo (C=O), tras el proceso de irradiación.

**Conclusiones:** los resultados demostraron que niveles adecuados de irradiación gamma pueden aumentar la eficacia antioxidante del ácido ascórbico. Por el contrario, una exposición excesiva produce cambios estructurales y la consiguiente reducción de su eficacia.

**Palabras clave:** DPPH; Cinética; Actividad Antioxidante; Ácido Ascórbico; Radiación Gamma; Fuente de Radiación.

## INTRODUCTION

Vitamin C also known as Ascorbic acid, is an important compound mentioned in antioxidant studies, and it exhibits a protective effect against radiation induced damage.<sup>(1,2,3,4)</sup> Serves as an important antioxidant in the human body supporting the body to resist stress and oxidation.<sup>(1,5)</sup> Studies have explored the antioxidant potential of Vitamin C as in a kinetic study of the free radical uptake of DPPH when Vitamin C is combined with Zinc.<sup>(6)</sup> Ascorbic acid has also been analyzed for rapid detection along with the total phenolic content and antioxidant activity in dried apples.<sup>(2,4,7)</sup> Furthermore, Vitamin C has been studied for its radiation protection effect and is considered for its radioprotective effect along with Vitamin A and E.<sup>(1,5)</sup> Studies have demonstrated that Vitamin C can reduce the formation of micronuclei caused by low-dose radiation in mouse bone marrow cells.<sup>(8)</sup> The radiation protection of ascorbic acid has also been compared to *EpiGalloCatechin Gallate* (EGCG).<sup>(2,4,7)</sup> In the field of food and pharmaceutical applications, ascorbic acid is evaluated for its pharmacological activities, along with essential oils and flavonoids in *Smyrnium olusatrum L.*<sup>(2,9)</sup> Ascorbic acid is also optimized for loading into halloysite nanotubes and combined with antioxidant-rich natural colorants, in order to develop antioxidant hybrid pigments as a dietary food ingredient. Gamma rays (also known as gamma irradiation or gamma radiation) are a type of radiation that has been studied and applied in many fields, especially in food preservation and product property improvement.<sup>(10,11)</sup> For examples, gamma irradiation has been extensively studied for its effects on the microbial load and quality characteristics of minced camel meat.<sup>(10,11)</sup> Gamma rays have also been applied to prolong the shelf life of carrots, sometimes combined with edible bio-coatings for enhanced effectiveness.<sup>(10,11)</sup> Other studies have looked at the effect of gamma rays on the hygienic and physicochemical qualities of red pepper powder, in which gamma rays are compared to X-rays and electron beams.<sup>(7,13)</sup> In addition, gamma irradiation has also been noted to improve the activity and properties of the product. Specifically, gamma irradiation may enhance the free radical scavenging activity extract of *Cordyceps militaris*.<sup>(14,15)</sup>  $^{60}\text{Co}-\gamma$  radiation has been studied for its effects on the physicochemical properties and antioxidant activity of Pazhu Tibetan medicinal powder.<sup>(14,15)</sup> Furthermore, acute gamma irradiation has also been noted to enhance antioxidant capacity and total phenolic content in leaves *Curcuma alismatifolia*.<sup>(3)</sup> DPPH reaction is an important and popular used method to evaluate the antioxidant activity of compounds and extracts, based on their ability to collect free radicals DPPH (2,2-diphenyl-1-picrylhydrazyl).<sup>(16,17)</sup> The DPPH radical is a stable free radical and has a purple color, when it reacts with antioxidants, it is reduced and fades in color when it stabilizes to yellow, this color change is quantified to determine the antioxidant activity of compounds evaluated by its ability to inhibit 50 % of the activity or  $IC_{50}$ .<sup>(16,17)</sup> In a kinetic study, the DPPH reaction was used to analyze the ability to collect free radicals in the presence of a mixture of Zinc and Vitamin C as an antioxidant, showing that DPPH helps quantify the free radical scavenging efficiency of a substance.<sup>(6)</sup> The free radical scavenging activity of *Cordyceps militaris* extract was enhanced through gamma irradiation, and this result was evaluated by the DPPH method.<sup>(14,15)</sup> Studies have also examined the effects of reaction time and DPPH concentration on antioxidant activity and kinetic parameters of bioactive molecules and plant extracts when reacting with DPPH free radicals, emphasizing the importance of optimizing experimental conditions to obtain accurate results.<sup>(16,17)</sup> The DPPH reaction is also used in quantitative kinetic analyses of the hydrogen transfer reaction from dietary polyphenols to DPPH free radicals, providing insight into the mechanism of action of antioxidants.<sup>(16,17)</sup> In the food sector, the antioxidant activity of dried apples has been rapidly detected along with the total phenolic and ascorbic acid content, of which the DPPH reaction is a potential method used to evaluate antioxidant activity.<sup>(2,4,7,13)</sup> Based on previous studies, only gamma radiation has been determined to affect liquid samples, in order to expand the scope of this research work in the direction of evaluating the antioxidant activity of Vitamin C according to gamma-ray irradiation doses in the form of solid samples.

## METHOD

### Chemicals and instruments

Ascorbic acid (Sigma MCLS), Ethanol 99,99 % (Sigma-Aldrich Quimica) , Micropipette, Falcon Tubing, DPPH (1,1-Diphenyl-2-picrylhydrazyl) (Sigma-Aldrich Quimica), Deionized Water, AOelab UV-VIS Spectrophotometer, Irradiation Device, Nicolet Avatar 370 FTIR Infrared Spectrophotometer with DTGS Probe.

### Preparation of irradiation samples

3 g vitamin C (ascorbic acid) was packed in a glass vial and sealed for preventing from sunlight degradation. The vials were then irradiated with the dose of 0,5, 1,0, 2,0, 3,0, 5 and 5 kGy at the same dose rate of 2,0 kGy per hour under gamma Co-60 source at Hanoi Irradiation Center. B3 dosimeter was used to control the absorbed dose.

### FT-IR Spectroscopy

Grind 1-2mg of Ascorbic acid with 300-400 mg of potassium bromide (KBr) in fine powder. Carefully grind the mixture and spread it evenly into a suitable mold. Apply sufficient pressure to the mold containing the test mixture to form the pellet form. Samples are prepared under laboratory conditions with a temperature of 22-24°C and a humidity of about 40-50 %. Measurements were taken using the Nicolet Avatar 370 FTIR infrared spectrometer with a DTGS probe. The infrared spectrum is measured in pass-through mode with a wavelength range of 4000 - 400 cm<sup>-1</sup> and a resolution of 4 cm<sup>-1</sup>. The sample was analyzed 5 times to determine the repeatability and reliability of the results. Spectrum data is collected using OMNIC software installed on the computer connected to the device.

### Antioxidant activity in DPPH reactions

39,4mg DPPH is weighted and transferred into a 10,00mL volumetric flask to the point where the DPPH solution is obtained, the solution is stored at 0-2°C, sealed in foil away from light to minimize oxidation. Dilute the solution to 1 mM by aspirating 1000µL and quoting with 9000µL of Ethanol then further dilute to 0,1mM by analogy obtaining a working DPPH solution at a concentration of 0,1mM.

Prepare samples of Ascorbic acid solids, weigh 50,00mg accurately, transfer them to a 50,00 mL rated flask, reach the line with Ethanol solution, obtained 6 bottles of 1000ppm, respectively, arranged in the order of irradiation doses: 0 KGy, 0,5 KGy, 1 KGy, 2 KGy, 3 KGy, 5 KGy. Constructing a concentration range to determine IC<sub>50</sub> through a reduction in optical absorption at 517nm, taking 2mL of 0,1mM DPPH in response to 1mL of specimens all performed in the dark. The DPPH free radical neutralization ability of the test solution is evaluated through the inhibition percentage value (I%) and calculated according to the formula:

$$I\% = \frac{A_0 - A_1}{A_0} * 100$$

In which:

I%: Percentage of inhibition.

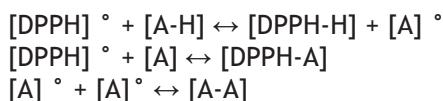
A<sub>0</sub>: The optical absorption value of DPPH.

A<sub>1</sub>: The optical absorption value of the test specimen.

Representing the test sample concentrations according to the value of I%, the linear regression line  $y=ax+b$  instead of  $y=50$  to the upper regression line obtains the IC<sub>50</sub> value with the number of experiment repetitions n=3.

### Kinetics of the DPPH reaction

The kinetics of the reaction with DPPH free radicals in the Ethanol environment is characterized by secondary reactions but occurs at a limited rate.<sup>(17,18)</sup>



The kinematics of the reaction depend on the concentration of the agents involved in the reaction, during the survey [DPPH] is 0,1mM and the concentration of [AH] is 0,1mM establishing the equilibrium state [DPPH]=[AH] and following the 2nd order reaction kinetics. Putting 1,5mL of 0,1mM DPPH solution into the cuvette reacts with 1,5mL of 0,1mM Vitamin C solution that occurs in the dark, setting the kinetic scanning parameters at 517nm wavelength with each measurement being 0,1s performed in 50s with a repeat measurement of 3 times obtained an average value that reduces the error in the experiment to a lower level.

The reaction velocity expression of the 2nd order kinematic equation is of the form:  $R= K^* [DPPH] \times [A-H]$

The reaction occurs in a ratio of [DPPH]:[AH] of 1:1, so over time there are the following consequences:  
The 2nd order kinematic equation after integral is:<sup>(12,17)</sup>

$$\frac{1}{[DPPH^{\circ}]t} = \frac{1}{[DPPH^{\circ}]_0} + K_1 t \quad (1)$$

Equation (1) will take the form of a straight line  $y=K_1 t+b$ , the slope of the graph is  $K_1$  with:

$$y: \frac{1}{[DPPH^{\circ}]t} \text{ (L.mol}^{-1}\text{s}^{-1})$$

t: Time reactions (s)

$K_1$ : Reaction rate constant (L.mol<sup>-1</sup>.s<sup>-1</sup>)

After the representation, the linear regression line will be calibrated using the solver function present in Excel to obtain the most accurate  $K_1$  value.

## Data processing methods

The metrics are represented as averages  $\pm$  relative standard deviations ( $X \pm \%RSD$ ). Microsoft Excel 2019 and Originlab 2025 software are used to calculate metrics and graph.

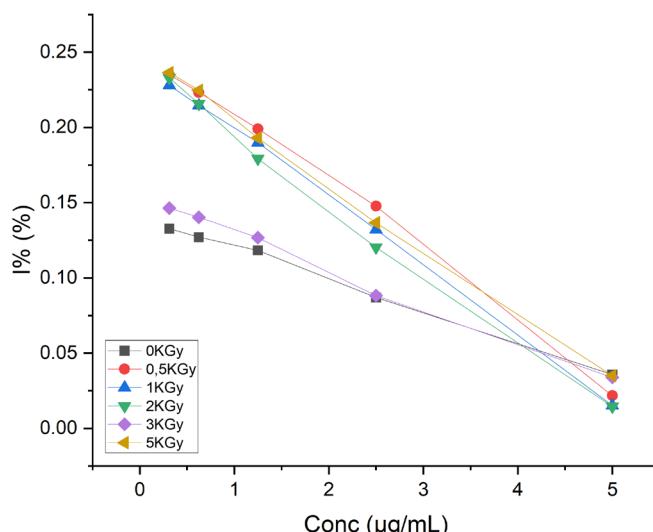
## RESULTS AND DISCUSSION

### Antioxidant activity

The DPPH method uses a stable organic radical 1,1-diphenyl-2-picrylhydrazyl to evaluate free radical scavenging activity, typically expressed as  $IC_{50}$  as the concentration of antioxidants required to reduce the concentration of  $[DPPH]_0$  by 50 %. A low  $IC_{50}$  value indicates stronger antioxidant activity, reflecting a higher ability to scavenge DPPH free radicals and reduce oxidation processes. The linear regression lines in figure 1 show superiority with a correlation coefficient of  $R^2 > 0,99$ . The results from table 1 show that when the concentration increases and the irradiation doses increase, the DPPH free radical scanning ability of the survey samples has a certain variation. In the test samples, at 2KGy showed the best antioxidant effect with  $IC_{50}$  values of  $2,545 \pm 1,000 \mu\text{g/mL}$ , followed by 0,5KGy and 1KGy with  $IC_{50}$  values of  $2,889 \pm 6,789 \mu\text{g/mL}$  and  $2,672 \pm 1,748 \mu\text{g/mL}$ , respectively. At 3KGy radiation dose, the asymptotic value is close to the control (non-irradiated) but there is still a fluctuation with  $IC_{50}$  values of  $3,282 \pm 4,403 \mu\text{g/mL}$  compared to  $3,395 \pm 5,733 \mu\text{g/mL}$ .

**Table 1.** DPPH free radical scavenging capacity of Vitamin C by irradiated doses

Irradiation doses	IC50 ( $\mu\text{g/mL}$ )
0KGy	$3,395 \pm 5,733$
0,5KGy	$2,889 \pm 6,789$
1KGy	$2,672 \pm 1,748$
2KGy	$2,545 \pm 1,000$
3KGy	$3,282 \pm 4,403$
5KGy	$2,907 \pm 3,671$



**Figure 1.** Linear regression of concentrations of irradiation doses in I%

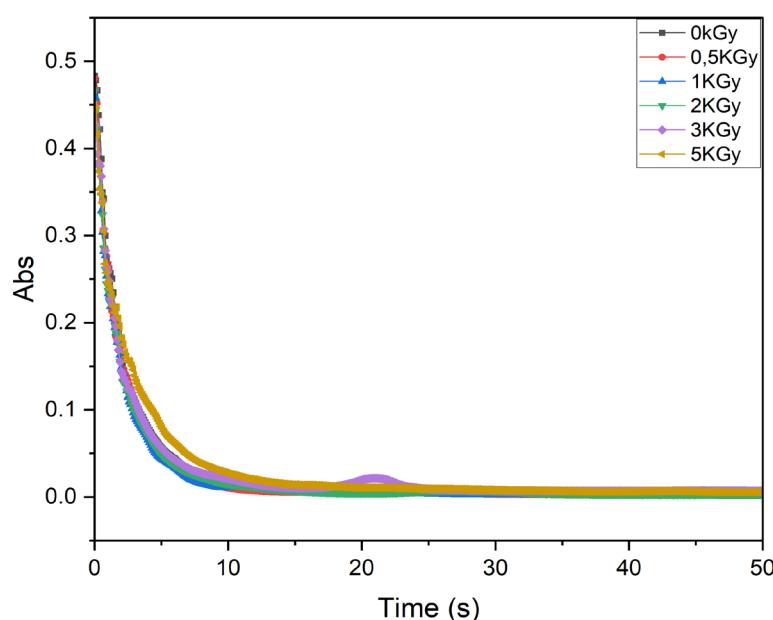
### $K_1$ reactive kinetic constant

Based on the results of previous research, it is indicated that vitamin C reacts very quickly with DPPH free radicals without adverse reactions occurring mainly due to the rapidly oxidized Ascorbyl radicals forming Dehydroascorbic Acid.<sup>(12)</sup> Figures 2 and 3 show a very rapid decrease in photoabsorption and DPPH concentrations consistent with the rapid free radical binding properties of Vitamin C, as the reaction occurs so quickly that the UV-VIS spectrophotometer must be sufficiently sensitive and state-of-the-art. The reaction is then monitored by recording the maximum optical absorption signal at 517nm every 0,1s, the system will automatically record a value that ensures the number of metrics to perform regression. Experiments are repeated 3 times in 1 day to avoid experimental errors.

**Table 2.** Kinetic values of 0,1mM DPPH reaction with 0,1mM Vitamin C

Irradiation doses	$K_1$ ( $M^{-1}s^{-1}$ )
0KGy	$12018 \pm 9,685$
0,5KGy	$10546 \pm 3,810$
1KGy	$13783,333 \pm 2,080$
2KGy	$12169 \pm 8,389$
3KGy	$10800 \pm 1,417$
5KGy	$7873,3 \pm 6,777$

The linear regression approach with the graph is a straight line  $y=K_1 t+b$  (1) for the reaction rate constants of the irradiation doses respectively summed up in table 2. Such high values are typical for fast interactions and reactions with DPPH radicals. At 0KGy with  $K_1=12018 \pm 9,685 M^{-1}s^{-1}$ , the reaction occurred rapidly, but from the irradiation doses, there was an effect on the chemical structure, reducing the ability to reduce free radicals as in 0,5KGy with  $K_1=10546 \pm 3,810 M^{-1}s^{-1}$ , gamma radiation had a great impact on antioxidant activities as well as reducing the ability to react with DPPH free radicals. However, at a sharply increased dose of 1KGy, there was a stimulation of free radical reduction functional groups to form derivatives with higher activity, which increased free radical scavenging activity, thereby increasing the reaction speed. When the irradiation dose was increased to 2 KGy, the  $K_1$  value dropped back to approximately the same level as the control sample, indicating that the stimulation effect had diminished and the destructive effect of radiation began to prevail. At doses higher than 3 KGy and 5 KGy, the rate constants decreased to  $10800 \pm 1,417$  and  $7873 \pm 6,777$ , respectively  $M^{-1}s^{-1}$ , which clearly reflects the structural destruction of antioxidant compounds, showed that high-dose irradiation caused a drastic impairment of the antioxidant activity of the sample.



**Figure 2.** Decrease in optical absorption over time

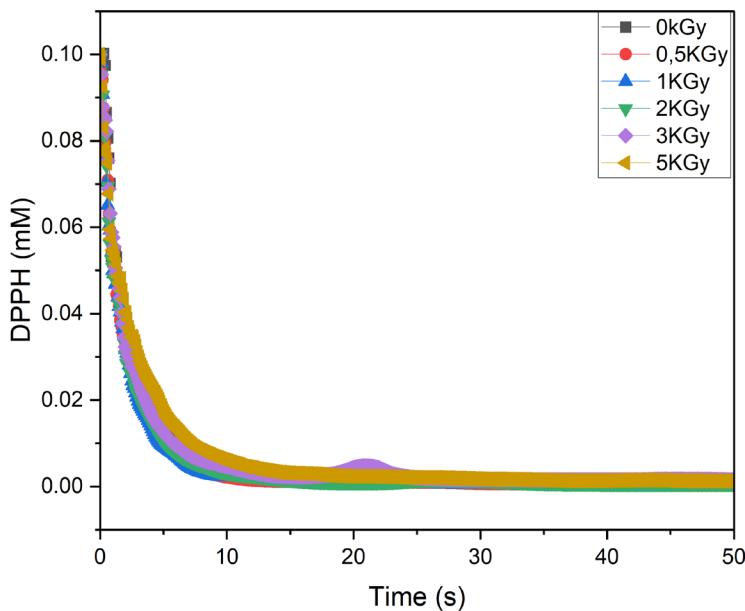


Figure 3. Decrease in DPPH concentration over time

Figures 2 and 3 show the decline in photoabsorption and DPPH concentration over time for the solid samples irradiated at doses ranging from 0-5 KGy. In all samples, the initial absorption decreases rapidly in the first 10 seconds and then gradually approach to equilibrium. This behavior reflects the rapid initial scavenging of DPPH radicals that takes place immediately after mixing two equal volumetric portions, consistent with the secondary reaction mechanism between vitamin C and DPPH. Differences among radiation doses are most evident in the early stages. The 1 KGy sample exhibits a faster absorption degradation than the control, demonstrating enhanced free radical scavenging. This suggested that low dose irradiation may activate or generate intermediates with increased antioxidant activity. The 0,5 KGy and 2 KGy samples showed the same or only slightly higher reaction rates compared to the control, indicating a moderate stimulation effect. In contrast, the 3 KGy and 5 KGy samples exhibit lower reaction rates, slower decay curves, and higher residual DPPH concentrations than the other samples. This suggests that high-dose irradiation causes adverse destruction or structural alteration of antioxidant compounds, resulting in a decrease in scavenging efficiency. In summary, the data highlight the importance of radiation dose optimization in food and herbal medicine preservation. Appropriately selected doses can maintain or even enhance biological activity, whereas excessive doses may reduce the product quality and antioxidant effectiveness.

#### FT-IR Spectrum

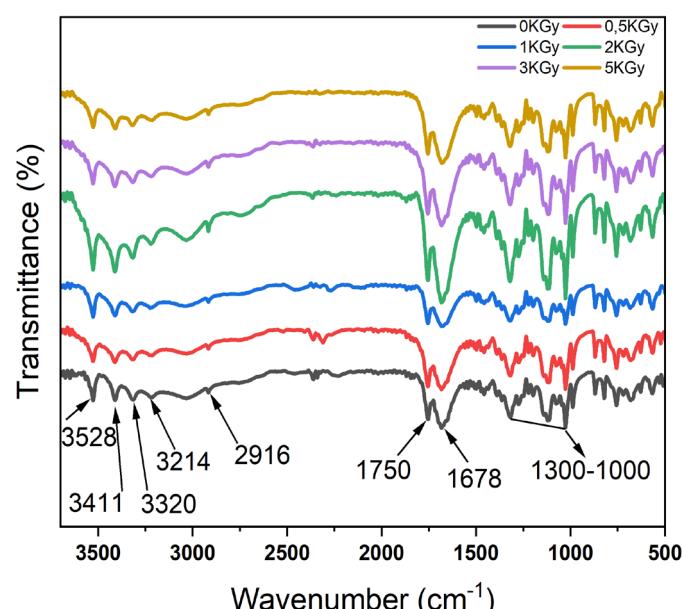


Figure 4. FT-IR spectra of irradiation doses

The FT-IR spectrum of the initial unirradiated Vitamin C is denoted as 0KGy and the irradiated Vitamin C is shown in figure 4. The appearance of characteristic peaks in the spectrum of Vitamin C should be most focused on 3600-3200 cm<sup>-1</sup> representing hydroxyl (-OH) groups, groups with strong hydrogen bonds between molecules, an increase in absorption peak intensity, which may be due to an increase in the -OH bond on the chemical structure of Vitamin C due to the penetration of gamma rays, the wave number 1750cm<sup>-1</sup> is the C=O bond of the 5-sided lactone ring and 1678cm<sup>-1</sup> is the C=C group conjugated to the ester, 2916cm<sup>-1</sup> is characterized by the oscillation of the C-H group (Carbon outside the ring), in the region of 1300-1000cm<sup>-1</sup> is characterized by the oscillations of the C-O-C group. The irradiation doses of 0,5Kgy and 1Kgy fluctuated but did not have much effect, and when increasing the irradiation doses from 2-5Kgy, there was a variation in optical transmittance in some characteristic peak regions, which can be explained that under the effect of gamma rays, it can decompose the substance into small pieces or polarize the bonds, however, the absorbent tapes do not Shifting out high wave numbers suggests that gamma rays are destructive or alter the binding oscillations of the infrared active groups of Vitamin C.

## CONCLUSION

The DPPH method is a rapid technique for determining the antioxidant activity and kinetics of the reaction between the Vitamin C, a specific antioxidant agent with DPPH. Though there have been many studies on the structural changes caused by gamma radiation on antioxidants, but non have investigated the effects of gamma rays on Vitamin C at solid state. Therefore, solid vitamin C has been irradiated in this study. UV-VIS spectrophotometry showed that the reaction rate of Vitamin C with DPPH free radicals was significantly enhanced at an irradiation dose of 1Kgy, exhibiting a very fast reaction rate. However, at 2KGy, the antioxidant capacity was even higher than that at 1Kgy. These results confirmed that at irradiation at 1-2KGy stimulates the free radical scavenging ability of Vitamin C, thereby significantly increasing its antioxidant activity. At higher radiation doses, structural degradation and transformation occur, directly affected the chemical properties of vitamin C.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORSHIP CONTRIBUTION

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*Research:* Vuong Van Loc.

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