Original Research Article

EVALUATIONS OF RED CABBAGE (*BRASSICA OLERACEA* L. VAR. *CAPITATA* F. *RUBRA* DC.) EXTRACT BEHAVIOR UNDER HIGH DOSE GAMMA IRRADIATION

ABSTRACT

Aqueous solutions of different pH from red cabbage *(Brassica oleracea* L. var. *capitata* f. *rubra* DC.) extracts were prepared. Effects of different doses/dose rates of gamma irradiation on the red cabbage aqueous solutions were investigated. Solutions were exposed to 1- 10 kGy of gamma irradiation doses at different rates ranged between 0.42513 to 38.883 kGy/h. The absorbance changes, which are due to gamma irradiation exposures, were determined by a spectrophotometry analysis. Results demonstrate that under gamma irradiation exposure variations (doses and dose rates), the best linearity (R2>0.97) of red cabbage solution extract was at a pH=3.3. Results could be of great importance and obviously indicated the efficacy of a natural red cabbage dye as dosimetric indicators.

Keywords: *Red cabbage, Absorbance, Gamma Irradiation, Fitting, Dosimetry Indicator, pH.*

1. Introduction

Ionizing radiation is an important technology that has a wide arrays of beneficial applications in different trailed such as agriculture (preservation of food), medicine (sterilization of medical and pharmaceuticals products), environment, and industrial improvement. Application of ionizing radiation is an essential method for food irradiation especially in developing countries and recently for food decontamination from environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs) without changing in food proprieties, and moreover the increase of storage period of food make food more inoffensive [1-3]. It is well known, that ionizing radiation can alter physical, chemical, and biological properties of materials depending on absorbed doses; these processes could be an important indicator related to many radiation dosimetry applications. Consequently, the dosimetry is considered a confidence key parameter of a quality assurance for radiation processes and irradiated product controls. Dosimetry is devoted for many applications in numerous areas including environmental protection, nuclear safety, food sterilization and aliments security, medicine and health care [4].

Nowadays, world current development gives important ethics to natural sources particularly in food and medical applications [5], including natural coloured compounds namely ‘dyes’ such as anthocyanins, carotenoids, and betalains [6-8], these dyes are not only used in food applications, but also for reducing some important diseases such as diabetes, obesity and cancer [9, 10].

Several natural dyes are also used in dosimetry measurement since natural dyes are biodegradables, health preserving and have no clearance challenges to the environment. Contrary to synthetic dyes, which may be carcinogenic, and non-biodegradables, furthermore, the potential side effect of the chemicals used in food synthesis colorants and the existence of environmental pollutants [11]. Therefore, world attention to natural dyes as visual or dosimetric indicators have been increased [12]. Red cabbage contains the most sensitive pigment, which we can existing in food, named anthocyanin (Fig. 1).This pigment changes colour dependent on aqueous solution pH of the red cabbage extract. Xavier et al [13] found that, acetic acid 10%v/v is the best solvent for the extraction of anthocyanin and they studied the best conditions for the extraction and purification. Moreover, red cabbage can be used to monitor the level of hydrogen ions in the solution [14].

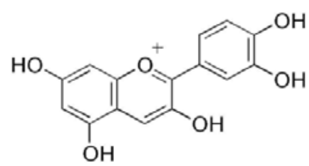

Fig. 1. Structure of cyanidin, type of anthocyanin in red cabbage [15]

The present study investigates the impact of dose and dose rate of ionizing radiation on the aqueous solution extract of a natural red cabbage. Also radiation effects on different range of pH on the dosimetric responses were also reported.

2. Materials and methods

2.1. Sample preparation

The red cabbage extract was prepared in aqueous solution at different pH. The fresh harvested red cabbage samples were manually ground into small pieces of ~ 0.6 cm^2. 15g of ground red cabbages was added to the aqueous solution and left for 24h at room temperature before filtration (B_1, B_2, N, A_1, A_2), while the other two samples A_{1-H}, B_{2-H} were obtained by heating the red cabbage pieces in the solution for one hour at 60 ^oC. The different pH solutions were prepared using glacial acetic acid and sodium hydroxide (purchased from CARLO ERBA, France). The different pH values used in this study are listed in Table (1).

Table 1. Extracted colours description

	B_1	B_{2-H}	B_2	N	A_1	A_{1-H}	A_2
pH	12.1	12.1	13	6.9	3.3	3.3	3.6
Extraction solution	0.15 N NaOH	0.15 N NaOH	0.5 N NaOH	distilled water	1%v/v CH_3COOH	1%v/v CH_3COOH	4%v/v CH_3COOH
Colour	yellow	yellow	yellow	red	red	red	red
λ_{max}	325	325	318	528	531	531	530

2.2. pH measurement

pH values of the solutions were determined using an HI 8521pH meter (Hanna Instruments, Woonsocket, RI, USA). The pH meter was calibrated using a solution of known pH (4, 7 and 9).

2.3. Irradiation treatment

The gamma irradiation processes were performed under general conditions, (air atmosphere and ambient temperature) and by using two different source of gamma irradiation. The first, was carried out by means of a gamma cell (dose rate 0.425130kGy/h, trans. dose 2.05 Gy) while, the second, was carried out using a ^{60}Co Gamma irradiator (Russian Type: ROBO) (200 kCi). Samples were exposed to 1, 2, 5, 10 kGy doses at different dose rates (2.2470, 9.2585, 20.9714, 31.5389, 38.8829kGy/h) of gamma irradiation. The two gamma facilities were calibrated by Fricke using the standard (ISO/ASTM 51026, 2005. Standard Practice, Fricke reference Standard Dosimetry System) [16].

2.4. Absorption spectral:

Absorption spectra of unirradiated and irradiated solutions of red cabbage extracts were measured using a UV-VIS spectrophotometer at wavelength range from 400-700 nm (resolution: 1 nm, reference: solution of same pH without red cabbage). The maximum of absorbance was found at 531 nm for all samples. Consequently, the relation between the absorbance at 531 nm and the absorbed irradiation dose/ irradiation dose rate has been considered.

2.5. Mathematical model:

Two mathematical models were developed in this study to evaluate the effect of irradiation on the extract solution.

First model was the linear trend (Eq. (1)) to describe the relation between the absorbance and the irradiation dose rate at specified dose:

$$A_0 - A_{d.r.} = \theta . D_r + \vartheta \qquad (1)$$

Where: A_0: Absorbance of un-irradiated sample

$A_{d.r.}$: Absorbance of irradiated sample at specific dose rate.

D_r: Gamma irradiation dose rate

θ, ϑ: constants

The second model was exponential model that describe the relation between the absorbed irradiation dose and the absorbance at each dose rate:

$$A = A_{m.D.r.} + \gamma_0 \, e^{-R_0 \cdot D} \qquad (2)$$

Where: D: absorbed dose, $A_{m.D.r.}$, γ_0, R_0 : apparent constants

3. Results and discussion

3.1. Absorption spectra

It is known that, red cabbage contains several dyes as anthocyanin (which have a maximum of absorbance at 531 nm) that can react with the gamma irradiation and produce some change in the intensity of the colour similar to artificial dyes as tartrazine, ethyl violet- bromophenol blue and methyl orange [17-19].

The result of red cabbage in alkaline solution did not give a good correlation between the intensity of absorption peak and gamma irradiation doses, and only red cabbage in acidic solution that has a maximum of absorbance at 531 nm corresponding to the red colour of the anthocyanin gave a good correlation. This result was in agreement with result reported by Shahid et al. [14].

The irradiation in alkaline solution (basic conditions) shows that no changes were found and turbidity in the formed solution has been seen very clearly. As shown in Fig. 1, the anthocyanin type structure presented in red cabbage has five acidity hydrogens (pKa= 3.8). The interpretation for that behaviour under basic conditions is due the formation of anions, which are stabilized by resonance and having negative charge on highly electronegative atoms. Therefore the formed salt after the addition of sodium hydroxide is very stable, whereas. the formed five RO- groups are stabilizing the formed complex as the result of irradiation under basic conditions which is sort of acid-base equilibrium.

Moreover, the extraction using temperature gave unclear solution and caused an offset in the background of the UV spectra, which make it difficult to distinguish the changes in the value of the absorbance in contrast to irradiation.

Fig. 2 shows the changes in the absorbance at 531 nm due to the irradiation process. The absorbance at 531 nm decreased gradually with the increase of the dose of Gamma radiation. Moreover, there is no shifting in the position of the peak of absorption. The decrease in the absorbance at 531 nm is probably due to the red cabbage degradation by absorbed irradiation dose. Moreover, this decrease was also affected by the irradiation dose rate. The decrease of the absorbance was smaller at high dose rate for same absorbed dose, and vice versa. whereas, at a high dose rate, the time of irradiation will be short causing less degradation in the red cabbage solution and less decrease in the absorbance.

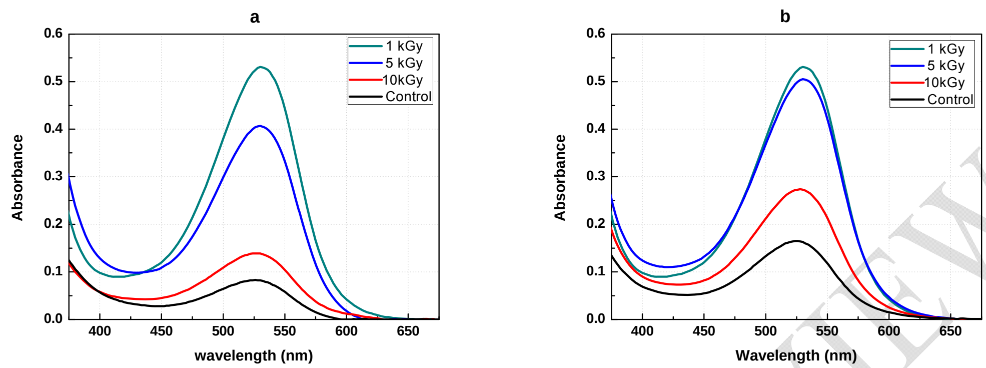

Fig. 2. Variation of absorption spectrums of red cabbage solution (pH=3.6) with respect to gamma radiation dose (from 1 to 10 kGy) at different dose rate: (a) 9.2585 kGy/h (a), 38.8829 kGy/h (b)

3.2. Effectof irradiation dose rate:

The behaviour of this solution under different irradiation dose rate, which varies between 0.43 and 38.88 kGy/h, was found to go through a linear tendency of photo- degradation (absorbance decrease) [16], as in the Eq. (1)

$$A_o - A_{d.r.} = \theta_1 D_r + \theta \qquad (1)$$

Fig. 3 demonstrates that, similar trend was also observed at different absorbed doses (1, 5, 10 kGy). Table (2) displays the value of slope of the linear fitting and the crossing point. The correlation coefficient (R^2) of the linear regression model has a value > 0.99. The negative values of slope indicate that the difference of absorbance between irradiated and unirradiated sample (A_o - $A_{d.r.}$) decreased with the increase of applied dose rates.

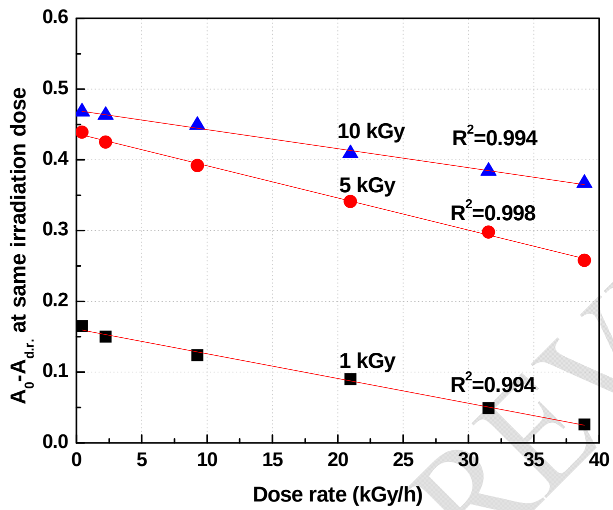

Fig. 3. Variation of the absorbance of red cabbage extract in acidic solution A_2 with irradiation dose rate, Dose rate 0.42513, 2.2470, 9.2585, 20.9714, 31.5389, 38.8829 kGy/h at irradiation dose 1, 5, 10 kGy

Table 2. Values of the constant of the linear fitting

Dose (kGy)	θ	ϑ	R^2
1	-0.0035	0.161	0.994
5	-0.0046	0.437	0.998
10	-0.0027	0.470	0.994

3.3. Effect of absorbed irradiation dose:

Sample solution exposed to different dose rates was found to be discoloured with the increase of the absorbed dose. Fig. 4 shows the relation between the absorbed dose and the variation of the absorbance at 531 nm. The relation between the absorbed dose and the absorbance follows an exponential model that can be expressed in the following Eq. (2):

$$A = A_{sat.} + \gamma_0 \, e^{-k_s \cdot D} \quad (2)$$

This model was found to be appropriate for all studied dose rates (Fig. 4). The correlation coefficient (R^2) shows a strong relation within the range between (0.944- 0.997).

Constant value of the model was listed in the Table (3). The value of the constant of γ_0 had a constant value for all dose rates of 0,456. While the constants of R_0 were correlated and had a linear relation by the dose rate effects. The coefficient of correlation had a value of 0.954, which shows a good relation between these constants. Moreover, Fig. 5 clearly shows that R_0 was not a real constant, but it is correlated to the irradiation dose rate.

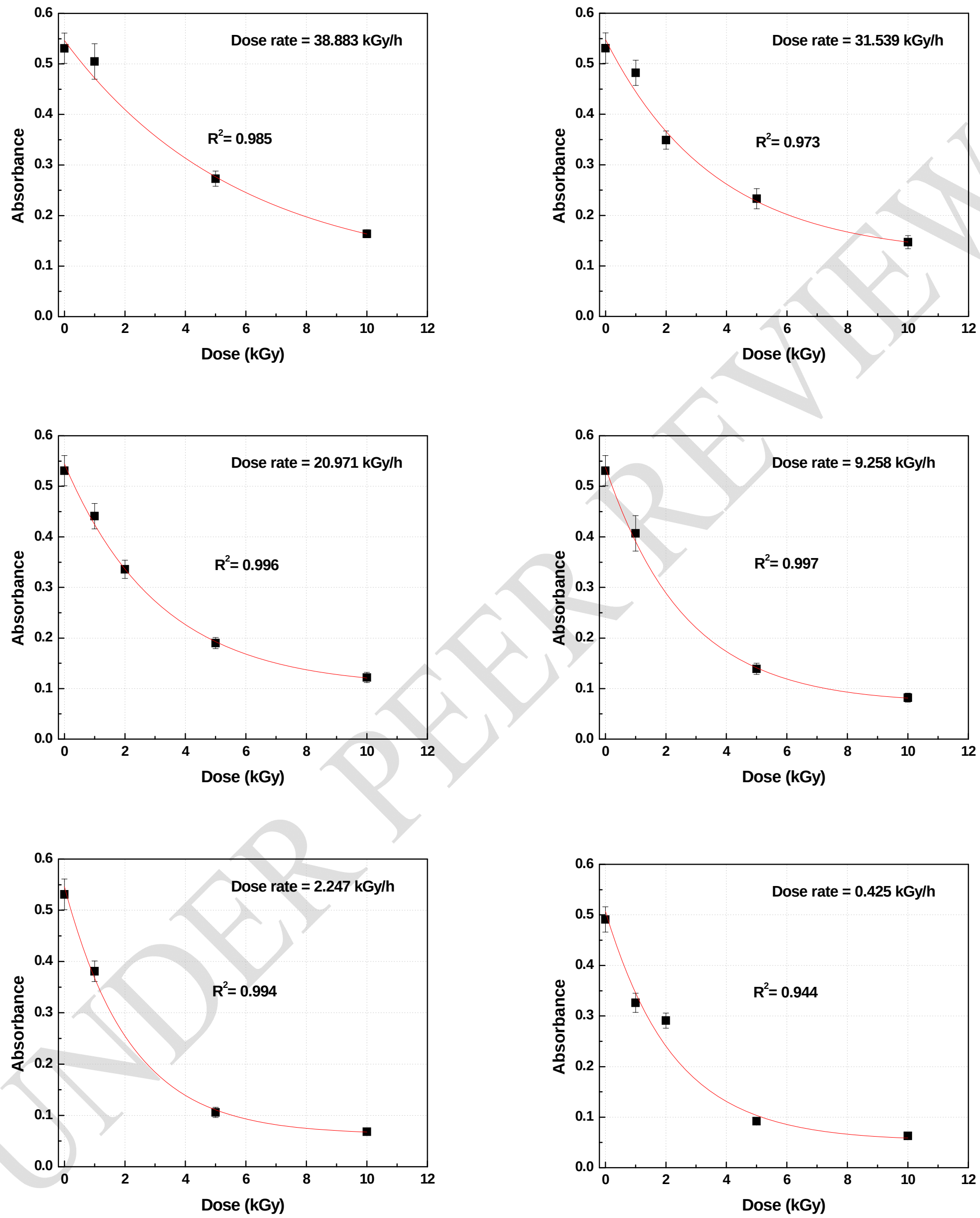

Fig. 4. Variation of absorbance of red cabbage in acidic solution A2 with irradiation dose at different dose rate 0.42513, 2.2470, 9.2585, 20.9714, 31.5389, 38.8829 kGy/h

Table 3. Values of the constant of the fitting

Dose rate (kGy/h)	$A_{D.r.}$	γ_0	R_0
38.88289	0.082±0.051	0.464±0.048	0.174±0.050
31.53895	0.120±0.032	0.427±0.036	0.276±0.074
20.9714	0.102±0.010	0.442±0.015	0.318±0.027
9.25847	0.071±0.007	0.467±0.016	0.379±0.030
2.24695	0.063±0.005	0.483±0.024	0.461±0.051
0.42513	0.053±0.018	0.454±0.059	0.442±0.103
average		0.456	

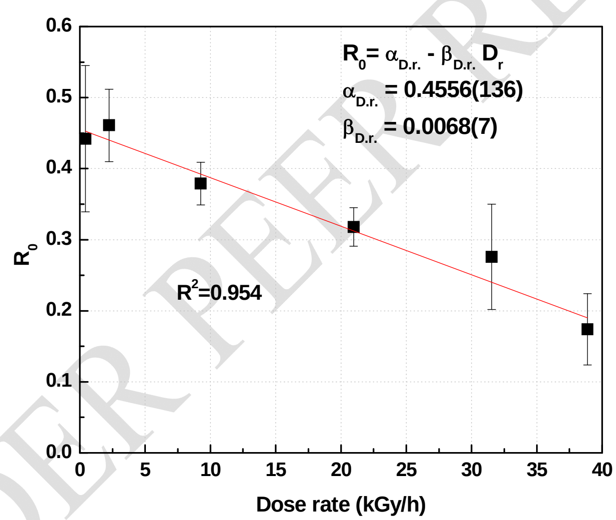

Fig. 5. Linear relation between the constant R_0 for the exponential model and irradiation dose at different dose rate

In addition, the constants $A_{D.r.}$ of the model at different dose rate were also correlated via a linear model as demonstrated in the Fig. 6.

The abovementioned correlations allow modifying the proposed exponential model, correlated the red cabbage absorbance with the absorbed dose, and rewrite the equation as follow in the Eq. (3) and Eq. (4):

$$A = (\alpha_{\infty,D_r} + \beta_{\infty,D_r}.D_r) + 0.456 \times e^{-(0.436+\beta_{D_r}.D_r).D} \qquad (3)$$

Where:

α_{∞,D_r} = 0.0567(24)

β_{∞,D_r} = 0.0020(2)

β_{D_r} = 0.0068(7)

Then

$$A = (0.0567 + 0.0020.D_r) + 0.456 \times e^{-(0.436+0.0068.D_r).D} \qquad (4)$$

The relative equation above shows that the absorbance of the red cabbage solution is simultaneously subjected to the variation in absorbed dose and dose rate of gamma irradiation.

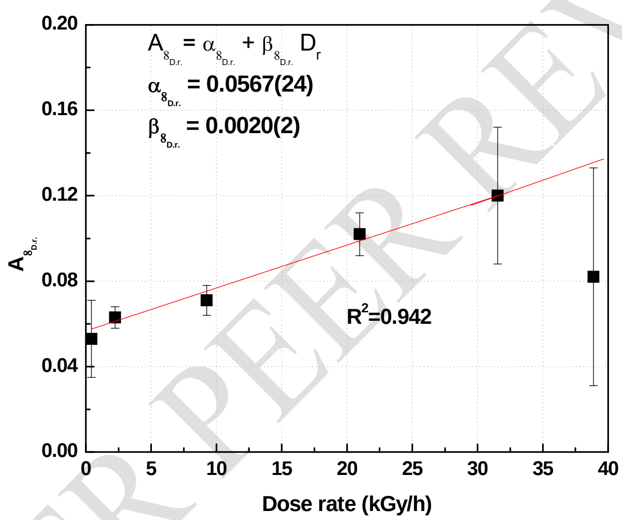

Fig. 6. Linear relation between the constants$A_0$of the exponential model between absorbance and irradiation dose at different dose rate

Moreover, the dose rate and the absorbed dose have a relation depending on the time of the radiation process as follow in Eq. (5):

$$D\,[kGy] = D_r\left[\frac{kGy}{hour}\right] * \frac{t\,[s]}{3600} \qquad (5)$$

By consequence, the absorbance of red cabbage extract measurements gives a direct indicator about the irradiation dose during a specified time as shown in the following equation Eq. (6):

$$A = (0.0567 + 0.0020.D_r) + 0.456 \times e^{-\frac{t}{3600}.D_r.(0.436+0.0068.D_r)} \qquad (6)$$

3.4. Effect of irradiation on pH of the acidic solution of red cabbage

The pH value of irradiated and un-irradiated acidic solution of red cabbage was determined at different doses and different dose rate. Table (4) shows a slight variation indicating that no effect of gamma irradiation on acidic propriety of the solution and thus the acidity remains stable during the whole process of irradiation.

Table 4. Variation of pH vs. irradiation dose and irradiation dose rate

Dose kGy	**0**	**1**					**5**					**10**				
Dose rate kGy/h	**-**	**2.2**	**9.3**	**21.0**	**31.5**	**38.9**	**2.2**	**9.3**	**21.0**	**31.5**	**38.9**	**2.2**	**9.3**	**21.0**	**31.5**	**38.9**
A_1	3.3	3.3	3.3	3.2	3.3	3.4	3.2	3.3	3.2	3.2	3.2	3.1	3.1	3.1	3.4	3.3
A_2	3.6	3.6	3.7	3.6	3.7	3.7	3.7	3.6	3.7	3.6	3.6	3.7	3.6	3.7	3.7	3.7
$A_{1\text{-}H}$	3.3	3.3	3.3	3.0	3.3	3.5	3.2	3.3	3.2	3.1	3.1	3.2	3.5	3.2	3.3	3.2

4. Conclusions

In this study, pH of different red cabbage (*Brassica oleracea* L. var. *capitate* f. *rubra* DC.) extract aqueous solutions were prepared. Acidic solution of pH= 3.3 showed good relations between absorbance and absorbed dose or dose rate during the irradiation process by gamma rays. An equation was concluded that correlates absorbance with irradiation dose from 1 to 10 kGy at dose rates ranged from 0.42513 to 38.883 kGy/h. The equation shows a possible application of red cabbage extract as a dosimetry indicator. Further studies are required on the response of constituents of red cabbage extract via irradiation and on the stability of solutions with the time and temperature variations, before reaching a firm conclusion in order to consider that, the red cabbage solution has a possible potential for applications as a natural dosimeter indicator.

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