Egypt. J. Chem. **Vol. 65,** No. SI:13B pp. 347-351 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Effect Of Ultraviolet C In Chemical Composition, Molecular Structure And Antioxidants Of Yellow Mustard Medical Seeds

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Abstract

Ultraviolet rays affect the raw materials produced by the seeds of the plant. Some seeds contain a significant concentrations of primary and natural antioxidants and it is a major source of vitamins and minerals, for this reasons, the aim of this work is to study the effect of ultraviolet C in chemical and molecular structure, enzymatic and non-enzymatic antioxidants for *yellow mustard* seeds. The results show that, molecular structure such as arrangement, orientation, size and interconnections of molecules and bonds characteristic, chemical composition such as protein, carbohydrate, fiber and fat contents and chemical component such as oxygen and carbon atoms for *yellow mustard* seeds were effected after exposure to ultraviolet C for different periods times and dissimilar distances. Glutathione content as a non-enzymatic antioxidant in *yellow mustard* varied after exposed to ultraviolet C for 1, 2, 3 and 4 hours at 5 cm and 20 cm distances from UV source. Phenolic content increased in yellow mustard, while tocopherol content decreased after exposed to ultraviolet C for 1, 2, 3 and 4 hours at 5 cm and 20 cm distances from UV source. Flavonoids and proline contents as enzymatic antioxidants in *yellow mustard* varied after exposed to ultraviolet C for 1, 2, 3 and 4 hours at 5 cm and 20 cm distances from UV source. From this research results, it's obvious that, it can be control in medical bio-contents in medical seeds by change radiation doses and types.

Key words: enzymatic oxidant, ultraviolet C, carbohydrate, protein, fat, *yellow mustard* seeds

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1. Introduction

Radiation is an important environmental abiotic factor for plants. The nonionizing ultraviolet (UV) spectrum cannot ionize atoms (UV ranges between 1000 Å and 4000 Å; 1000 Å has been chosen as the boundary between non-ionizing and ionizing radiation), but can disrupt the interatomic bonds which form molecules, thereby breaking down molecules rather than atoms. Mustard is a plant, known since prehistoric times, that possesses a high level of bioactive ingredients, where mustard seeds used as condiments. Also the mustard plant is a member of the Brassicaceae family, where different varieties of mustard include white or yellow mustard, Oriental, brown or Indian mustard, and black mustard. Mustard is used as spice, food and medicine, as the medicinal plant used for the treatment of various diseases. The physiological, biochemical processes, leaf chlorophyll, protein content, and peroxidase enzyme activity in plants can be affected by UVC radiation [1, 2]. Values of the vitamins, enzyme activity and antioxidant activity of ammi majus and foeniculum vulgare seeds increased after exposure to UVC for different times at dissimilar distances [3, 4]. Growth behavior, molecular structure, enzymes and free radical of *Nigella Sativa* and *garden cress* changed after exposure to UVC for different times and dissimilar distances [5, 6]. UVs possess sufficient energy to break the chemical bonds causing photochemical reactions and inducing changes in plant metabolic enzyme, subsequently trigger the production of secondary metabolites [7- 9]. The objective of this research is to study the effect of UVC on molecular structure, chemical composition, non-enzymatic and enzymatic antioxidants for *yellow mustard* seeds.

2. Experimental methods

2.1. Determination of moisture content

Moisture content was determine according to the method described by AOAC (2019) where a known weight of air dried seeds (2g) was dried at 105 °C in an air drying oven to a constant weight. Then percentage of moisture content was calculated.

2.2. Ash content determination

Ash content was determine according to AOAC (2019), as follow: Exactly 2g of air dried seeds were place in a silica crucible and ignited at 600 ºC in a

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Receive Date: 11 March 2022, Revise Date: 03 June 2022, Accept Date: 26 June 2022
DOI: 10.21608/EJCHEM.2022.126763.5630

muffle furnace till a constant weight; the percentage of ash content is calculated.

2.3. Determination of crude fiber content

Crude fiber is estimated according to the method described by AOAC (2019). A known weight of the air dried seeds (2g) is mixed with 0.5g asbestos, then 200 ml of sulphuric acid (1.25%v/v), are added. The mixture is boiled under reflex for 30 minutes, followed by filtration through Gooch crucible. The residue is boiled again with aqueous sodium hydroxide solution (200 ml, 1.25%w/v) for 30 minutes, and then filtration was repeated in the same manner. Finally the residue is washed with hot water followed by diethyl ether and dried at 110 ºC to constant weight. The content of Gooch crucible was then ignited in the muffle furnace at 600 ºC to a constant weight. Fiber content is calculated by subtraction of ash content from the weight of digested sample. Percentage of crude fiber content was then calculated.

2.4. Crude protein content determination

Crude protein content was determined by the official Kjeldahl method described in AOAC (2019), as follow: A known weight of air dried seeds (0.5g) was digested with 8ml. of concentrated sulphuric acid in Kjeldahl flask in the presence of (2.14g) digestion mixture [1kg potassium sulphate and 60g of mercuric oxide (red)]. After digestion the solution was treated with 10ml. of 40% sodium hydroxide solution. The liberated NH3 was received into 10ml. of 1% boric acid in the presence of 2 drops of Tachero indicator (1.25g methyl red+0.32g methylene blue in one liter of 90% ethanol). The received ammonia was titrated with 0.01N sulphuric acid. The percentage of total nitrogen was estimated and the crude protein content was calculated by using 6.25 as a factor of protein.

2.5. Determination of carbohydrates:

Carbohydrates content was calculated by difference from the following equation: Carbohydrates content % = 100 - [% protein + % ash + % lipids + % fiber].

2.6. GSH determination

At environmental conditions, Temp 25˚C, Humidity 38% rH, UV/Vis spectrophotometer, Jenway, England was used to determine GSH in *yellow mustard* seeds.

2.7. Determination of total phenols

Total phenols were determined colorimetric by Folin-Ciocalteu reagent where, total phenolic content is calculated from the regression equation of the standard plot(y=3.005×-993.56, r2=0.9974) and was expressed as mg Gallic acid equivalent/100g sample.

2.8. Total flavonoids determination

Aluminum chloride colorimetric method was used to determine flavonoid content in *yellow mustard* seeds. 1 ml of sample extract was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride. 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 minutes. The absorbance was measured at 420 nm. Rutin was used as standard (1mg/ml). Flavonoid content was calculated from the regression equation of the standard plot (y=16.122×-340.23, r2=0.9777) and are expressed as (mg Rutin equivalent/100g sample).

2.9. Determination of Proline

The Proline concentration in *yellow mustard* seeds is determined after extraction with 3% (W/V) aqueous sulfosalicylic acid from a standard curve using D-Proline (y=36.738x+1.2739, r2=0.9777). UV/Vis spectrophotometer, Jenway, England was used to determine proline at environmental conditions, Temp 25˚C, Humidity 38% rH.

2.10. Structure

Structure of *yellow mustard* seeds was studied by scanning electron microscope (JEOL JSM-6510LV, Japan) and molecular structure was studied by Nicolet™ iS™ 10 FT-IR Spectrometer from USA.

2.11. Statistical analysis

Data were analyzed by SPSS software using analysis of variance (ANOVA) and differences among means were determined for significance at $P < 0.05$ using Tukey's test.

3. Results and discussions

3.1. Glutathione content

Table 1 showed that, glutathione content in *yellow mustard* seeds varied after exposure to UVC for different times and dissimilar distances. A significant change, P<0.001, in glutathione content where decreased by 42.7% and 23.9%, and increased by 22.47% and 32.6% after exposed to UVC for one and four hours, and two and three hours respectively at 5 cm distance. It decreased by 21.1% after one hour exposure then increased by 21.02%, 14.22%, and 15.14% after exposed for two, three and four hours at distance 20 cm from UV source. Variation on glutathione content in *yellow mustard* after exposure to UVC, was caused by hermetic type of response of glutathione under applied doses from radiation [10, 11].

Table 1**:** glutathione content in *yellow mustard* seeds after exposure to UVC

Exposure time (h)	GSH (mg/ 100g)	
Zero (Control)	72.66	
	5 cm	20 cm
1	41.66	57.33
2	88.99	87.93
3	96.32	82.99
4	55.33	83.66

4.2. Phenolic content

Phenolic content in *yellow mustard* seeds increased after exposure to UVC for different times at dissimilar distances as listed Table 2. A variable increase in

Phenolic content happened and this result agree with other study [12]. A significant change, P<0.001, in phenolic content in untreated yellow mustard seeds, where increased from (409.5 mg/100g) to (682.6 mg/100gm) and (489.3 mg/100gm) after exposure to UVC for 1 and 3 hours at 5cm and 20 cm distances from UV source respectively. UVC caused a change in phenolic content because it's effected on accumulation of phenolic compounds, and capable for breaking hydroxyl bonds of poly phenols thereby releasing soluble phenols of low molecular weight. Also UVC changed protein content which used as a base for phenolic transfer, resulting in variance in phenolic content quantity. The change in hydroxyl group, O-H, which play an important role for carrying protein or for phenolic compounds transfer, resulting in variance in phenolic content.

Table 2: total phenolic content of *yellow mustard* seeds after exposure to UVC

Exposure time (h)	Total phenolic (mg/ 100g)	
Zero (Control)	409.48	
	5 cm	20 cm
1	682.60	434.29
2	423.63	431.85
3	541.17	489.33
4	411.57	428.60

3.3. Flavonoids content

Flavonoids content in *yellow mustard* seeds decreased after exposure to UVC for dissimilar distances and different period times as shown in Table 3. Where these results agree with other studies [13-15]. Ultraviolet C own enough energy for broking the chemical bonds that cause photochemical reactions, which leads to a variation in flavonoid content for yellow mustard seeds. UV effected on bio compounds of seeds then effect on their structure phenol compounds which leads to a change in flavonoid content.

Table 3: flavonoids content of *yellow mustard* seeds after exposure to UVC

Exposure time (h)	Total flavonoids (mg/ 100g)	
Zero (Control)	297.6	
	5 cm	20 cm
1	279.66	207.70
2	295.62	290.28
3	220.41	246.47
4	151.79	252.82

3.4. Proline content

Total proline content in *yellow mustard* seeds deceased after exposure to UVC at dissimilar distances for different times as listed in Table 4. There is a little variation occurred for proline content in *yellow mustard* after exposure to UVC due a change in molecular and chemical composition.

Table 4: proline content in *yellow mustard* seeds after exposed to UVC

Exposure time (h)	Total proline (mg/ 100g)	
Zero (Control)	925.84	
	5 cm	20 cm
1	890.96	920.64
2	920.23	866.01
3	923.21	880.01
4	912.55	916.49

3.5. Tocopherol content

Tocopherol content for *yellow mustard* seeds decreased after exposed to UVC as presented in Table 5, where decreased by 68.6%, 26.7%, 16.95% and 43.86% after exposed by UVC for 1, 2, 3 and 4 hours, then by 33.86%, 12.22%, 31.35% and 20.7% after exposed by UVC for one, two, three and four hours at 5 cm and 20 cm distances respectively.

Table 5: tocopherol content in *yellow mustard* seeds after exposed to UVC

Exposure time (h)	Tocopherol (mg/ kg)	
Zero (Control)	2029.19	
	5 cm	20 cm
1	1391.52	1342.09
2	1488.46	1780.75
3	1684.96	1393.30
4	1138.92	1608.91

3.6. Chemical composition

Chemical composition for plant seeds changed due to environmental conditions. The results presented in Table 6, show
that carbohydrates in *yellow mustard* seeds increased by 92.6% with decreasing protein, fats, moisture, total fibers and ASH by 15.2%, 32.7%, 13.7%, 6.1 and 0.2% respectively after exposure to UVC for one hour at 5 cm distance. Chemical composition of *yellow mustard* seeds changed after exposed to UVC, because UVC react rapidly with almost all structural and functional organic molecules [16] then cause a change in its bio-component such as protein, carbohydrate etc....

Table 6: chemical composition of *yellow mustard* after exposed to UVC at 5 and 20 cm for different period times

Time	Chemical composition of *yellow mustard* (5 cm) Carbohydrates	Protein	Fats	Moisture	Fibers
control	16.54%	14.5 %	32.7 %	5.76%	26.52%
1	31.86%	12.3 %	22.0 %	4.97%	24.90%
4	15.50%	25.7 %	27.1 %	5.11%	23.30%

Time	Chemical composition of *yellow mustard* (20 cm) Carbohydrates	Protein	Fats	Moisture	Fibers
control	16.54%	14.50 %	32.7 %	5.76%	26.52 %
1	17.40%	11.28 %	35.0 %	5.73%	26.80 %
4	15.03%	19.70 %	37.0 %	4.81%	19.60 %

4.7. Structure

Figure 1 shows scanning electron micrographs, SEM, of *yellow mustard* seeds after exposed to UVC for 1 and 4 hours at 5 cm and 20 cm distances. The micrographs showed a change in internal structure, such as shape or size or orientation or interconnection of molecules for *yellow mustard* seeds after exposed to UVC, due to the action of UVC ray on it, as break or modify bonds or form free radical and this agree with other studies [17, 18].

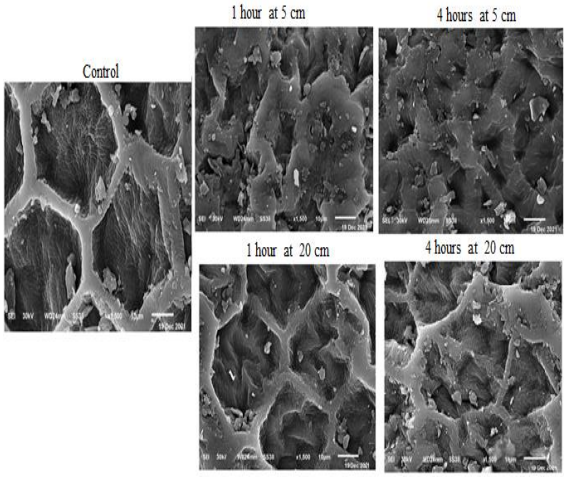

Figure 1: SEM of *yellow mustard* seeds after exposed to UVC

Molecular structure (IR analysis)

IR spectrum of *yellow mustard* it is a plot of wave number (X- axis) vs. present transmittance (Y- axis) as shown in Figure 2. IR analysis of *yellow mustard* show the transmittance intensity is increased after exposure to UVC for 1, 4 and 1 hours at 5 cm and 20 cm from source. Also there is a significant change in the main peak position, O-H, after exposure to UVC for 4 hours at 20 cm and little variation for other exposure times at 5 and 20 cm distance. Where break or modified position of molecular bonds after exposed to UVC. However, the change of some bands after exposure to UVC caused either degradation or switching off of the transcription- translation machinery during radiation exposure [19].

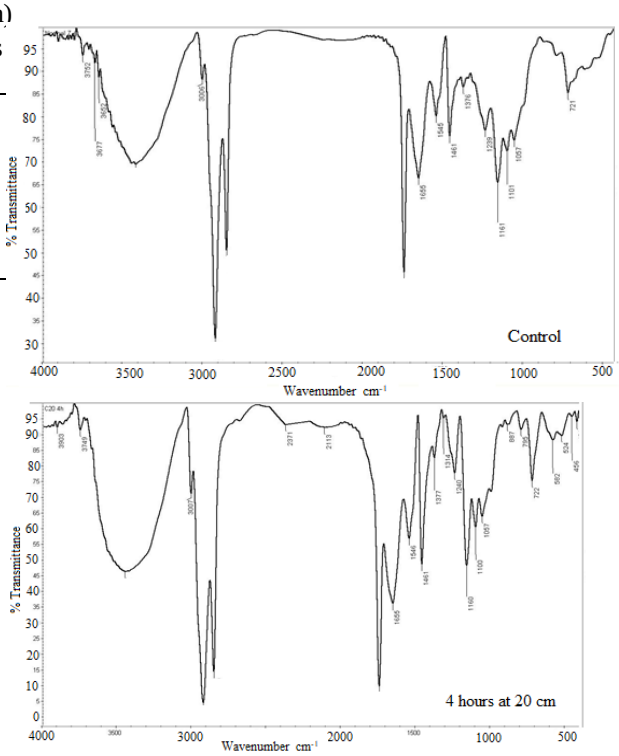

Figure 2: IR spectrum of *yellow mustard* after exposed to UVC

Conclusion

Molecular structure such as accumulation or arrangements or orientations or interconnection of molecules and chemical composition such as carbohydrate, protein, fat and total fiber of *yellow mustard* seeds changed after exposure to UVC for different times at 5 cm and 20 cm distances. Also enzymatic and non-enzymatic antioxidants such as GSH, total phenolic and flavonoids of in *yellow mustard* seeds changed after exposure to UVC for different times at 5 cm and 20 cm distances.

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