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The Influence of γ -Ray Irradiation at Low Doses on Antioxidant Activity, Phenolic Compounds Profile, Chlorophylls and Structure of Cladode Powder (*Opuntia ficus indica*)

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Abstract

Cladode powder was treated at low doses (0.5 kGy, 1 kGy and 2 kGy) to investigate the effect of γ-irradiation on physicochemical properties, antioxidant activity and the bioactive profile. The main bioactive compounds were identified and quantified by High Performance Liquid Chromatography (HPLC). The degradation effect on the structure was studied by UV-visible spectrum, FT-IR and SEM analysis. The results indicated that γ-irradiation has no effect on the chemical composition (°Brix, moisture and ash) whereas significant ($p \leq 0.05$) increase in chlorophylls B and pheophytins content was revealed. Irradiation treatment increased the Hunter colour'L'and 'b'values while'a'value increased. Samples irradiated at 2 kGy contained high phenolics and flavonoids content (887.64 mg GAE/100 g DW; 302.5 mg CE/100 g DW) and antioxidant capacity (88.40% and 85.21% for DPPH and ABTS assays). The major bioactive compounds of cladode powder including phenolic acids, flavonoids identified were syringic acid, caffeic acid and rutin. Further, FT-IR, UV spectrum and SEM analysis indicated that γ-irradiation introduced no significant changes into the functional group and particle morphology. Therefore, the effects of γ-irradiation at low doses were considerable and would be effective to increase the shelf life and bioactive quality of cladode powder.

Keywords
Cladode powder; Gamma irradiation; Phenolic profile; Antioxidant activity; FT-IR; UV-Vis spectroscopy.

Introduction

Opuntia ficus indica is a Mexican plant belonging to the Cactaceae family with approximately 130 genera and 1500 species; it is widely distributed in arid and semi-arid regions of many countries including Morocco. This sustainable plant deserved special attention due to their agronomic advantages and new alternatives for producing added-value food. Cladode of *Opuntia ficus indica* has been considered a potential source of bioactive compounds, dietary fibre, vitamins, mineral and trace elements with high health-promoting benefits. Recently, due to their antioxidant activity, an increasing valorisation of cactus cladodes juice as concentrates or powders became a great priority for the food market. Thus, it is strongly desirable to have current knowledge of foodstuff processing and storage conditions.

Therefore, the processing and storage food conditions require the use of effective methods to prevent from spoiling. Unfortunately, traditional methods widely used for sterilization (Salting, Pasteurization, Canning and Adding chemicals) are difficult to apply to cladode powder. Thus, high energy irradiation (Gamma rays) is considered as a new opportunity with a fast and reliable technological process, established to improve the food quality and prolong its shelf life. Food and Agricultural Organization (FAO), International Atomic Energy Agency (IAEA) and World Health Organization (WHO) have been proved it as a safe method to sterilize plant product since did not induce a significant increase in temperature, do not affect the chemical composition and have no dependence on any type of catalysts [1,2]. Radiation treatment at doses of 2-7 kGy can potentially eliminate pathogenic bacteria and prevent spoilage microorganisms without compromising the safety, nutritional properties and sensory quality of the food. Although the levels of modification might vary depending on the material composition used, irradiation doses and radiation source. Moreover, many studies had showed that γ-irradiation strongly affected the total phenolic compounds and antioxidant properties of different plant materials [3,4]. Furthermore, the effect of γ-irradiation was also evaluated for polymers structure and morphology of particles. It was reported that gamma radiation induced the formation of free radicals responsible for the breakdown of hydrogen and glycosides bonds of starch macromolecules and consequently affected structural,

physicochemical, antioxidant, morphological and rheological properties of the wheat starch treated [5].

Despite the number of studies showing the changes in structure or composition of several fruits, vegetables and polymers, the effect of γ-irradiation on cladode powder has not been investigated. Moreover, data on the correlation between irradiation doses and changes in phenolics profile, antioxidant activity and microstructure of cladode powder are lacking. Thus, the present work was undertaken to investigate the effect of low doses irradiation (0.5, 1 and 2 kGy) on physicochemical properties, phenolics content, and the antioxidant capacity of cladode powder. The structure modifications were also evaluated.

Materials and Methods

Plant material

The spineless cladodes (*Opuntia ficus indica*) were collected from Oulad dlim region of Marrakesh (Morocco). Fresh cladodes were washed with distilled water and oven dried (Ecocell standard) at 60°C for 48 hr. The dried cladodes were ground using a hammer mill (Monbroy/2000 W) at 25°C for 15 min then screened through a mesh sieve of 500 µm. the samples were weighed (10 g) and packed in hermetic bags prior irradiation treatment.

Irradiation treatment

Samples of cladode powder were subjected to gamma radiation at low doses 0.5, 1 and 2 kGy. The irradiation rays were cobalt radio-isotopic source (60 Co) of BOUKHALEF ionization station located in the National Institute for Agricultural Research (INRA) (Tangier, Morocco). The irradiation process was accomplished at the chamber by routine Fricke dosimeters with a dose rate of 1.05 Gy/7 min. The control (0 kGy) and irradiated samples were stored at 4 °C until analysis.

Physicochemical properties

Unirradiated and irradiated samples were analysed for moisture, ash, total soluble solids, pH and titratable acidity. The moisture content of samples was determined by drying in a hot air oven at 105°C to constant weight. Ash determined by incineration at 550°C for 3 hours and calculated as the difference in masses [6]. For pH and soluble solids (°Brix) measurement, 1 g of each sample were added separately to 10 mL of distilled water, homogenized and filtered then measured with a pH meter (Hanna Instruments) and refractometer (DR 6000, A. Krus Optronic GmbH, Hamburg, Germany). Titratable acidity was assessed by titrating 10 mL of aliquot sample to a pH 8.2 with 0.1 N NaOH solutions. Results were expressed as % citric acid using the formula follows % = [(N*V*mEq citric acid)/W]. N: the concentration of NaOH; V: the volume of NaOH; W: the weight of sample; and mEq: the citric acid mEq (0.064). All samples were analysed in triplicates.

Chlorophylls determination

A powder sample (0.1 g) was added to 10 mL of acetone and the homogenates were centrifuged. The absorbance of the supernatant was determined at 662, 645, 470, 653 and 654 nm by a UV-2100 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The chlorophyll (A and B) content, total carotenoids and pheophytins were expressed as milligrams of pigment per gram of dry weight [7]. Experiment was carried out in triplicate.

Colour measurement

Colour of native and irradiated cladode powder was determined using High quality colorimeter NH 300 (KEJIAN, Chine) and expressed on the 'L' (lightness), 'a' (redness) and 'b' (yellowness) values. In order to analyse the results, the values ΔE* and ΔL* were calculated for each sample, where ΔE* represents the total colour difference, and ΔL* is the difference in lightness and darkness (+ = lighter, - = darker). These values are calculated using the following equations reported by Vujcic et al. [8]

$$\Delta E^* = [(\Delta L^*)\,2 + (\Delta a^*)\,2 + (\Delta b^*)2]1/2, \quad (1)$$

$$\Delta L^* = L^*\text{irradiated sample} - L^*\text{non-irradiated sample}, \quad (2)$$

$$\Delta a^* = a^*\text{irradiated sample} - a^*\text{non-irradiated sample}, \quad (3)$$

$$\Delta b^* = b^*\text{irradiated sample} - b^*\text{non-irradiated sample}. \quad (4)$$

Determination of total phenolic content and total flavonoids content

Samples (2 gm) subjected to an extraction with 20 mL of 80% methanol. The mixture was agitated twice during 2 hr at room temperature in darkness and then filtered. The two filtrates were combined, concentrated under vacuum using a rotary evaporator and finally reconstituted in 10 mL of pure methanol and stored at 4°C until analysis. Total Phenolic Content (TPC) was determined as follow: 0.5 ml of extract was mixed with 1.5 mL Folin-Ciocalteu reagent (diluted ten times). After 5 min, 1.5 mL of sodium carbonate (6%) were added. The mixture was incubated in darkness for 1 h and then the absorbance was measured at 760 nm against a blank. The results are expressed as mg equivalent of Gallic acid (GAE) per 100 g of dry weight [9]. For the Total Flavonoids Content (TFC) determination, samples aliquot of 1.5 mL was added to 1.5 mL of AlCl3 reagent (2%). After 30 min of incubation the absorbance was recorded at 430 nm against a blank. The flavonoid contents were calculated using a standard calibration curve, prepared from catechin. The content of flavonoids was expressed as mg of catechin (CE)/100 g of dry weight [9].

The identification and quantification of phenolic compounds was carried out using a high-performance liquid chromatography (Knauer) equipped with a (K-1001) pump and a 215 PDA detector (UV-VIS) operating at 280 nm. The column was C 18 Eurospher II 100-5, and the temperature was maintained at 25°C. The flow rate was 1 mL/min and the injected sample volume was 10 µL. A mixture of acidified water (A) and Acetonitrile (B) was used as a mobile phase for a total running time of 60 min. The identification of phenolic compounds was

fulfilled by comparison of retention times and UV-VIS spectra with the standards.

Evaluation of antioxidant activity

The antioxidant potential of all extracts was determined using DPPH and ABTS assays. 1 mL of 100 mM DPPH solution in methanol was mixed with 1 mL samples extract. The reaction mixture was incubated in the dark for 30 min and its optical density was recorded at 517 nm against the blank. The results obtained were expressed as % inhibition of DPPH based on the following formula: % Inhibition of DPPH = (A control-A sample)/A control*100. A control is the absorbance of the DPPH solution without sample extract and A sample is the absorbance of the sample with DPPH solution [10]. For ABTS assay, the ABTS+ radical was generated by reacting 7 mM ABTS+ and 2.45 mM potassium persulphate. After incubation at room temperature in the dark for 16 h, the solution was diluted to get an absorbance of 0.70 ± 0.02 at 734 nm. The ABTS+solution (1 mL) was added of the test sample (10 µL), mixed thoroughly and incubated for 30 min. The absorbance of the reactive mixture was measured at 734 nm. The % ABTS inhibition was calculated based on control and sample Optic Densities (ODs) [11].

Structural analysis

UV-Visible Spectroscopy: Spectra of samples were obtained with an UV-Visible spectrophotometer (UV-2550, Shimadzu), using a 1 cm thick quartz cell. The spectrophotometer was equipped with a deuterium lamp and halogen lamp for UV measurements. The measurements were made between 450 and 700 nm, in steps of 1 nm. Repeatability (for absorption value and wavelength shift) was corroborated acquiring the spectrum three times for each sample. All spectra were recorded using methanol as blank. Methanolic extracts were diluted such that the absorption fitted the linear range of the instrument.

Fourier transform infrared (FT-IR) spectral analysis: The Fourier transform infrared (FT-IR) spectra of unirradiated and irradiated samples were determined using a FT-IR spectrometer (VERTEX 70, Bruker) equipped with standard internal source and room temperature DTGS detector. The powder of samples was thoroughly mixed with anhydrous potassium bromide (KBr) at a ratio of 10 mg sample to 90 mg KBr and then pressed into a KBr pellet. The pellet was scanned at room temperature in the spectra range of 400-4000 cm-1 with a resolution of 4 cm-1.

Scanning electron microscopy: SEM and area-restricted x-ray analysis by energy dispersive spectrometry were carried out with a TESCAN VEGA 3 instrument. Samples were prepared for SEM viewing either unirradiated or irradiated samples by gamma irradiation at 0.5, 1 and 2 kGy. Each sample was carbon sputtered before being digitized and photographed at various magnifications. An acceleration potential of 20 kV was used in the image acquisition micrograph.

Statistical analysis

Data collected are an average of three independent determinations, each carried out in triplicate. Analysis of Variance (ANOVA) was conducted in order to determine the effect of gamma irradiation on powders properties. The comparison of sample means was performed by Turkey test at 5% significance level. PCA analysis was performed to visualize the relationship between treatment doses and all properties of cladode powders. Data was analysed using XLSTAT software (2016, France).

Results and Discussion

Physicochemical properties

The physicochemical properties of irradiated and unirradiated cladode powder are summarized in Table 1. The results showed no significant effect ($p<0.05$) of gamma irradiation was observed on the moisture, ash contents and solids soluble for the treated powders. Whereas, it was observed a decrease in pH values with an increase in titratable acidity by irradiation doses. The low moisture content in the nopal powder is due to the drying process; this avoids the proliferation of microorganisms and indicates better shelf stability. These results were in agreement with those of Rodríguez-Garcia et al. [12] for cladode at maturation stages of 52 days. According to Liu et al. [13] the moisture and ash contents of two different peanut cultivars were not affected by high irradiation (10 kGy). The pH results were in agreement with those of Sofi et al. [14] and Suriya et al. [15], who reported a decrease in pH upon the increase in irradiation dose for broad bean starch and *Amorphophallus paeoniifolius* flour's, respectively. While Mahrouz et al. [16] showed that clementines treated at 0.3 kGy have no significant impact on the titratable acidity. In addition, increased acidity and decreased pH of cladode powders may be possible because of the formation of free radicals following irradiation, which led to the breakdown of glycosides linkages in the plant molecule.

Table 1: Physicochemical properties of unirradiated and irradiated powder cladode.

Parameters	Control	0.5 kGy	1 kGy	2 kGy
Moisture (%)	7.95 ± 1.90 a	7.92 ± 1.19 a	7.62 ± 1.86 a	7.90 ± 0.92 a
Ash (%)	23.24 ± 0.47a	22.30 ± 0.6a	22.35 ± 0.54a	22.34 ± 0.33 a
Ph	5.37 ± 0.06 a	5.20 ± 0.18 a	5.22 ± 0.02 a	5.18 ± 0.01 a
Solids soluble (°Brix)	4.53 ± 0.12 a	4.20 ± 0.20 a	4.47 ± 0.12 a	4.33 ± 0.12 a
Titratable acidity (% citric acid)	0.68 ± 0.07 b	0.69 ± 0.06 a	0.70 ± 0.06 b	0.78 ± 0.06 b

Chlorophylls and colour determination

The results presented in Table 2 showed that the content of the pigments was affected by gamma irradiation and depends on the radiation dose applied. The contents of chlorophyll A were significantly higher in powder irradiated by 0.5 kGy compared to the control. In contrast, samples irradiated by 1 and 2 kGy showed a decline in chlorophyll a as compared to the

non-irradiated powder. Further, chlorophyll B was lower than chlorophyll A in all doses including control. Besides, low doses of gamma radiation elevated chlorophyll B and pheophytins in comparison to the control level. However, the total carotenoids content has no significant difference between irradiated and unirradiated samples. This is in line with the results of Amirikhah et al.[17] who also found positive effects of low doses (up to 15 k rad) on the contents of chlorophyll B in the host plant (*Epichloe endophyte*). Whereas, the study of irradiated *Vigna radiata* (L.) from 10 to 100 Gy showed that chlorophyll A and chlorophyll B content decreased with increasing gamma exposure [18]. The increase in pheophytin content could be due to degrading chlorophyll molecules (especially chlorophyll A) involving free radical reactions and the cleavage of carbon-carbon (double bond) and carbon-hydrogen bonds under conditions of high-energy radiation.

The colour values for control and irradiated powders are shown in Table 2. Cladode powder had an intense yellowish tonality under 2 kGy irradiation dose, with L* values increased upon irradiation from 81.46 to 85.49. In addition, the yellowness (b*) values increased from 12.34 to 13.33 whereas gamma irradiation had no effect on the redness (a) value (2.15-2.10). On the contrary, L*, a* and b* values reported by Ayadi et al. (2009) [19] and Ramírez-Moreno et al. (2013) [20] showed lower values than our data (L* = 67.49 and 73.53; a* = -8.17 and 8.45; b* = 25.15 and 25.35) for spiny and spineless cladodes powders. Jo et al. (2003) [21] reported that tea 89.98 at 25 °C), while a* and b* value decreased by irradiation from 10.63 to 0.47 and from 102.89 to 40.89 respectively. In contrast, Lee and Kim,[22] reported that irradiation of brown rice decreased the a* and b* values, while the L* values increased up to dose of 10 kGy. The colour change could be due to double bonds of cladode polysaccharides formed after main chain scission or hydrogen abstraction reaction during the irradiation process. The mean colour difference ΔE was recorded in the range of 2.30-4.10 with a strong correlation at 1 kGy and 2 kGy (r=0.59 and r=0.33 respectively). It was confirmed that visually perceptible colour differences were observed among studied samples up to a dose of 2 kGy. These finding could be explained by the breakup of some chemical bonds in organic molecules under the influence of gamma irradiation. Moreover, noticeable and quantitative colour differences were also seen for ΔL* and Δb* with a strong correlation for 1 kGy and 2 kGy (r=0.62 and r=0.49). Although a weak correlation between gamma irradiation and Δa* values were observed (r=0.11). This parameter did not contribute to the establishment of significant differences of colour while the lightness was mainly responsible for the discrepancy between unirradiated and irradiated powders. Therefore, irradiated cladode powders could be useful as an additive to confer vivid colours and the irradiation technology may be used to improve powders brightness.

Table 2: Chlorophylls (a, b), carotenoids, pheophytins content and color differences for irradiated cladode powder at low doses of gamma rays.

Parameters	Control	Irradiation doses (kGy)		
		0.5	1	2
Chlorophylls A (mg/100 g dry weight)	12.07 ± 0.01b	14.12 ± 0.01a	12.37 ± 0.00b	12.15 ± 0.01b
Chlorophylls B (mg/100 g dry weight)	5.18 ± 0.02b	8.97 ± 0.00a	9.24 ± 0.02a	10.87 ± 0.06a
Carotenoids (mg/g dryweight)	7.48 ± 0.85a	9.07 ± 0.28a	8.57 ± 0.12a	9.47 ± 0.24a
Pheophytins (mg/g dry weight)	0.69 ± 0.19b	1.23 ± 0.25a	1.27 ± 0.48a	1.40 ± 0.06a
L*	81.46 ± 0.45c	83.60 ± 0.83b	84.66 ± 0.17a	85.49 ± 0.15ab
a*	2.15 ± 0.29a	2.20 ± 0.04a	2.17 ± 0.03a	2.10 ± 0.02a
b*	12.34 ± 0.51b	12.93 ± 0.24ab	12.99 ± 0.28ab	13.33 ± 0.19a
ΔE	-	2.30 ± 0.62b	3.38 ± 0.53a	4.10 ± 0.56ab
ΔL*	-	2.14 ± 0.54b	3.20 ± 0.62a	4.03 ± 0.56ab
Δa*	-	0.05 ± 0.29a	0.03 ± 0.28a	-0.04 ± 0.30a
Δb*	-	0.59 ± 0.75a	0.65 ± 0.31a	0.99 ± 0.32a

Total bioactive compounds

Phenolics analysis was commonly used as quality indicator of nutraceutical plants. The determination of phenolics content and antioxidant activity by DPPH and ABTS assays are shown in Figure 1. As compared with control, the Total Phenolic Content (TPC) of the irradiated samples at 0.5 kGy remains similar (510.02 and 577.15 GAE/100 g DW). However, the irradiation at 1 and 2 kGy resulted in high concentrations (806.53 and 887.65 mg GAE/100 g DW respectively). The degradation of larger phenolic compounds into smaller ones could explain the increased phenolics content in gamma-irradiated powders. In fact, the irradiation rays break down the chemical bonds inducing the release of low molecular weight and soluble phenols. It was found that Total Flavonoid Content (TFC) was significantly different among the samples and the powder irradiated at 1 kGy had the highest level 305.33 CE/100 g DW. While TFC remains constant at 2 kGy (249.17 to 281.33). Pearson correlation showed a high correlation between TPC and 2 kGy doses with r=0.71 and between TFC and 1 kGy doses with r=0.91. This was consistent with the report of Oufedjikh et al. [23] who were found that gamma irradiation induced the biosynthesis of flavonoids extracted from clementine peel during their storage 14 days of storage. Furthermore, Harrison and were [24] confirmed that gamma irradiation of almond skins increased the total phenolics content as well as antioxidant activity of extracts at irradiation levels of 4 kGy. Fan [4] was found that gamma-irradiation increased the phenolics content and antioxidant capacity of three vegetables tissues (romaine, lettuce and endive) at 4 and 8 days of storage. Our finding was also confirmed by previous studies for different plant material treated with various gamma irradiation doses [25-27]. The differences in effects were attributed to the different phenolics

compounds present in the various spices. In addition, the concentration of these phenolics compounds may be dependent on the time of evaluation, the dose administered, technological criteria, as well as the specific nature of the product. Hence, gamma irradiation technology could enhance the phenolic qualitative composition and improve the bioactivity of cladode powder.

As shown in Figure 1, the radical scavenging (DPPH) and (ABTS) of powders extracts increased as a result of gamma irradiation at 1 and 2 kGy, which could justify the observed increase in phenolics compounds content. In fact, it was observed a strong correlation between antioxidant activity and phenolics content with r=0.94 and r=0.96 for DPPH and ABTS respectively. The irradiated samples were able to inhibit the activity of DPPH radicals in a dose-dependent manner and the inhibition ratio has reached 85.21%. Besides, powder exposed to the irradiation level of 2 kGy was most effective at reducing the absorbance of ABTS+ by 85.21% in comparison to DPPH. The current finding was basically in agreement with previous studies [28,29]. While, Mohamed Ibrahim et al. [30] was found that gamma irradiation had no effect on the antioxidant properties of *Thapsia Garganica L*. roots extracts.

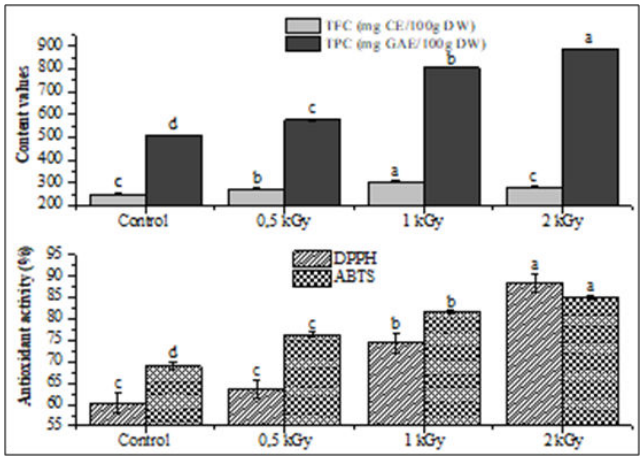

Figure 1: Total phenolics content, total flavonoids content and antioxidant activity of irradiated samples compared to the control (unirradiated sample).

The effect of gamma irradiation on individual phenolics compounds was analyzed by HPLC. The identified phenolics and flavonoid compounds are listed in Table 3. HPLC analyses were performed on methanol extracts of control and γ-irradiated samples. Five phenolics acids and one flavonoid compound were identified before and after irradiation. The individual phenolics compound predominant in the control sample with the greatest concentration was gallic acid followed by syringic acid and rutin. Gallic acid and L-Tyrosol decreased by irradiation from 661.99 to 504.52 mg/100 g and from 18.81 to 11.88 mg/100 g respectively, with a negative correlation at 2 kGy (r=-0.95 and r=-0.97). Whereas, rutin and caffeic acid increased by irradiation from 34.98 to 43.42, and from 4.28 to 7.39 mg/100 g respectively. It was observed that syringic acid was the most effected by gamma irradiation at 2 kGy by reaching 259.92 mg/100 g compared to the non-irradiated sample. However, vanillic acid obtained after irradiation is approximately similar for different doses compared with the control. Our results were higher than those found by Guevara-Figueroa et al. [31] for gallic acid and lower for rutin extracted from two commercial and eight wild varieties of nopal powders.

The increase of some phenolics acids by irradiation could be explained by the degradation of gallic acid as a radiolytic product. Indeed, Madureira et al. [32] was verified that gallic acid is a radiolytic product of syringic, vanillic and protocatechuic acids, in both isolated and mixture solutions, treated by gamma radiation and under different pH and atmospheres. Further, the phenolics release may be responsible for the improved antioxidant activity in the cladode extracts, and could also be linked with the types, the position and number of hydroxyl groups present in phenolics acids. Although it is considered that some bonds can be broken resulting in smaller molecules, the degradation of molecules as L-tyrosol during the irradiation could be occurred by unknown mechanisms. The irradiation effect was related to the intensity and characteristics of each individual compound. Besides, it could be linked to the radiation dose administered, the variety or the plant environment and the extraction procedure used. Thus, the irradiated cladode may be useful for functional food applications with high pharmacological efficacy.

Table 3: Quantification of individual phenolics compounds of unirradiated and irradiated powders determined by HPLC-DAD (mg/100 g of dry matter).

Phenolics profile	Unirradiated	Irradiation doses (kGy)		
		0.5	1	2
Gallic acid	661,99 ± 1.95a	464,12 ± 4.02d	525.23 ± 3.74b	504.52 ± 6.97c
L-tyrosol	18,81 ± 0.20a	20.44 ± 1.19a	19.22 ± 0.57a	11.88 ± 0.22b
Caffeic acid	21,64 ± 0.48c	20.29 ± 1.16c	27.70 ± 0.78a	23.85 ± 0.27b
Syringic acid	46,98 ± 0.42d	238.40 ± 6.83b	213.04 ± 4.14c	259.92 ± 1.47a
Vanillic acid	4,12 ± 0.38a	4.34 ± 0.42a	4.48 ± 0.12a	4.31 ± 0.25a
Rutin	34,98 ± 0.31d	41.02 ± 0.15c	44.97 ± 0.20a	43.42 ± 0.08
Phenolics sum	792 ± 3.75	794.25 ± 14.08	842.29 ± 9.77	855.29 ± 9.66

Principal component analysis (PCA)

For the better interpretation of the results, mean values of cladode properties data were subjected to principal component analysis. The component patterns and component score plots are shown in Figure 2. Two principal components explained 62.77% of the total variance for irradiated and unirradiated powder. A component score shows clear segregation of irradiated powders on the right part of the plot, while the non-irradiated sample is located on the negative side of the first principal component, thus the first component has a stronger discrimination power than the second component. Thus, irradiation at 2 kGy might be beneficial for enhancement of the

phenolics profile and antioxidant activity since all of the bioactive compounds have been identified as an important antioxidant compound in cladode powder. In this study, a positive correlation was found between the γ-irradiation doses and antioxidant activities, phenolics profile and chlorophylls content. PCA was an appropriate approach to verify the differences between the gamma radiation doses in samples of cladode powder.

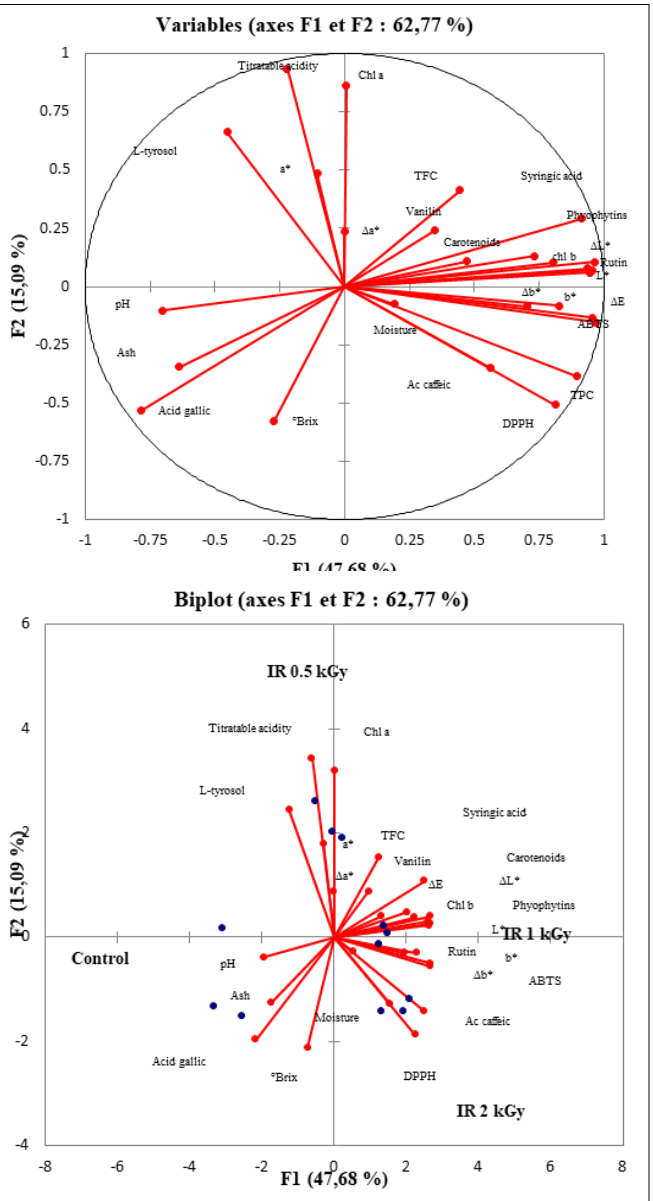

Figure 2: Total phenolics content, total flavonoids content and activity of irradiated samples compared control (unirradiated sample).

Structure analysis

UV-visible spectrum: The absorption spectroscopic analysis was used to investigate the optical properties of the powders extract and results were illustrated in Figure 3. The spectra of the irradiated samples showed absorption bands at 503, 536 and 607 nm, with a distinct absorption peak at 665 nm of all irradiated samples and their intensities increase with irradiation dose. This is due to phenolics substances that could strongly absorb UV light in the range of 665-767 nm and certain compounds with coloured nature leading to absorption features in the visible range. The gamma irradiation could induce the generation of free radicals in organic compounds, mostly in unstable chemical bonds. Indeed, carbonyl groups or double bond could be formed after the main chain scission of the polymer followed by the ring-opening of phenolics compounds. These results were in agreement with the increase of phenolics and flavonoid content discussed below. This optical behaviour observed for irradiated powders makes UV-visible spectroscopy a suitable technique to investigate and quantify plant phenolics compounds. Further, the possibility of measurement as a gamma dosimeter for phenolics compounds in the range of 650 to 700 nm could be a new procedure for phenolics analysis.

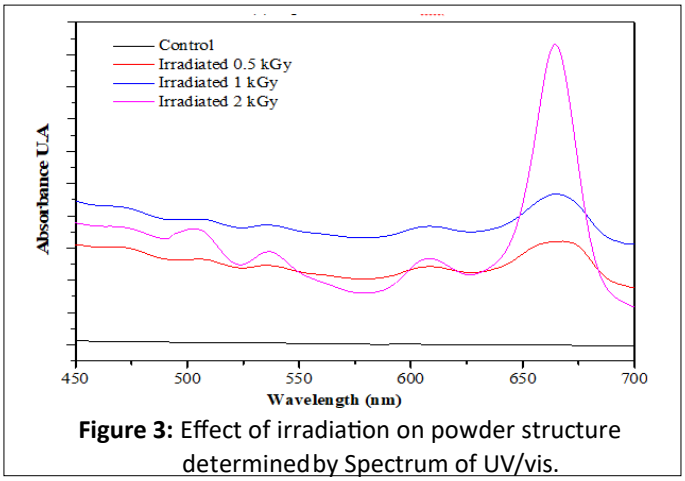

Figure 3: Effect of irradiation on powder structure determined by Spectrum of UV/vis.

FT-IR spectrum results: FT-IR spectroscopy was performed on irradiated and non-irradiated powders between 450 and 4000 cm^{-1} in order to characterize their major functional groups and structure modification. The FT-IR spectra of all samples are shown in Figure 4. Spectroscopy revealed that the spectral patterns of control and irradiated powders did not change, and no new functional groups were introduced by irradiation treatment. However, a variation in the absorption intensity was observed at 2 kGy doses in 3387, 2917, 1617, 1429, 1317, 1248, 1061, 774 and 661 cm^{-1} bands. These absorption peaks could be the main characteristic of pectin, cellulose, lignin and hemicellulose extracted from cladode fibres [33, 34]. The intensity changes may be due to the breaking of chemical bonds by irradiation, resulting in the formation of unstable and converted species. The broad absorption peak at 3387 cm^{-1} was related to the stretching frequency of O-H group. These groups may be derived from several components that have one or more hydroxyl groups in their structure. The small band at 2917 cm^{-1} was attributed to the C-H stretching and bending vibrations. Furthermore, the strong peak at 1617 cm^{-1} is due to the stretching vibrations of C-O bonds and asymmetrical COO– stretching vibration while 1429 cm-1 was the symmetrical deformation vibration of C-H and the band at 1317 cm-1 was ascribed to O-C-H, C-C-H and C-O-H. Further, the band at around 1248 cm^{-1} has been designated as the stretching vibrations of C-O-C and the band at 1061 cm^{-1} was attributed to the coupling of C-O, O-H and C-C bonds. Whereas bands at 774 and 661 cm^{-1} are due to the bending vibrations of C-H. The obtained spectrum of

both control and irradiated powder corresponds to a typical pattern when compared with others FI-IR spectra of nature polysaccharides reported by Lefsih et al. [34] for three pectic fractions of cladode with a strong absorption peak at 1618 cm^{-1} assigned to the vibration of COO-group of galacturonic acid. Hence, control and irradiated samples have a similar pattern of FT-IR spectra which indicated that gamma irradiation had no significant effect on functional groups of cladode powder.

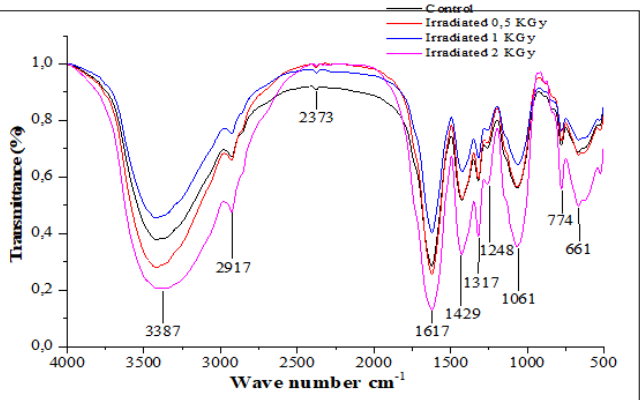

Figure 4: Effect of irradiation on powder structure determined by FT-IR Spectrum.

Image morphology (SEM): The microstructure of cladode powders following gamma radiation is shown in Figure 5. Micrographs showed an organic and heterogeneous material composition, with particle agglomerates that have irregular shapes and sizes characterizing the cladode powder. In fact, this morphology highly correlates with the fibril structure of cladode which seems to correspond to some bio-macromolecules as cellulose and lignin. The morphology of the granules did not change following gamma radiation while the fibrous structure was destroyed. In the irradiated samples at 1 kGy (C) and 2 kGy (D), an accumulation of smaller fragments was observed, may resulted from cracks or irregularities of the powder surface, compared to the control sample A. These changes could be assigned to disintegration and decomposition of polysaccharide caused by these highly energetic and penetrating radiations. Some researchers have also reported that the deformation of the granular structure appeared to be dose-dependent [35-37]. These findings concur with those reported by Abu et al. [38] for cowpea starch treated up to 50 kGy irradiation without any visible physical effect on granules.

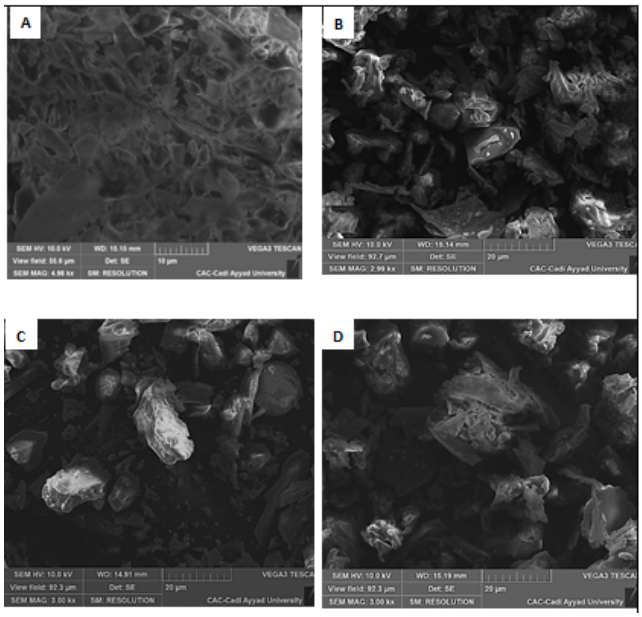

Figure 5: Effect of irradiation on powder structure determine by Scanning electron micrographs: A (control), B (0.5 kGy), C ()and D (2 kGy).

Conclusion

Our results suggest that irradiation increased phenolics content, flavonoids content, antioxidant capacity and lightness of cladode powder and the increase was more profound for 2 kGy doses. Hunter colour 'L' and 'b' values increased while 'a' value increased by irradiation. However non change was observed for chlorophylls A, B and carotenoids. Treating samples with low gamma irradiation has induced an improvement in the antioxidant activity and some of the bioactive compounds profiled in this work. FT-IR and SEM results revealed that no new functional groups were introduced by irradiation treatment. While UV visible spectra could be new way to identify the highest content of bioactive compounds.

In conclusion, irradiation technology can be used to enhance the bioactive quality, antioxidant activity, colour without any structure degradation or adverse change in physicochemical properties. This processing method at low doses may provide good information to the cosmetic and pharmaceutical industry, as well as the food industry, which is looking for value added materials.

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