Journal of Home Economics
Volume 27, Number (2), 2017
Journal of Home Economics
http://homeEcon.menofia.edu.eg
ISSN 1110-2578

Influence of ultraviolet radiation treatment on the stability of bioactive compounds and antioxidant activity in leafy vegetables throughout refrigerated storage

Nahed Shehata [1] ,Eman Negm[2]

[1]Department of Home Economics, Faculty of Specific Education, Zagazig University, Zagazig, Egypt, [2]Department of Food Technology, Faculty of Agriculture, Cairo University, Cairo, Egypt

Abstract: Different vegetables are considered as sources of human health promoting components. Leaf vegetables are widely used in human diet for many reasons including their very high in bioactive compounds with physiological significant roles. Irradiation is a non-thermal preservation method which involves the exposure of food materials or products to radiation, for example ultraviolet (UV-C) rays. This research was carried out to study the influence of ultraviolet radiation treatment on the stability of bioactive compounds and antioxidant activity in leafy vegetables throughout refrigerated storage. The analysis of variance for the total phenolics (TP), carotenoids (TC) and chlorophyll (TCP) levels indicates that their values were significantly ($P < 0.05$) affected by both the UV-C radiation and the refrigeration storage period. Initial TP, TC and TCP values for all UV-treated samples were significantly lower ($P < 0.05$) than those for the control samples. When all leafy vegetables were included in the statistical analysis, there was a positive significant ($p \leq 0.05$) relationship between total phenolics ($r^2 = 0.584$), total carotenoids ($r^2 = 0.385$), total chlorophyll ($r^2 = 0.312$) and antioxidant activity in control and UV-treated samples throughout refrigerated storage. Also, bacteriological analysis indicated that total aerobic bacterial growth (TABG) of spinach, lettuce and chicory was recorded 5.51, 4.88 and 4.56 $\log_{10}$ cfu/g which increased by rates of 58.11, 61.47 and 69.30% for the untreated samples and 31.80, 34.15 and 34.93% for the UV treated samples after 8 days of refrigeration at 4 ^{0}C, respectively. In conclusion, UV-C treatment brings some benefits to the fresh cut industry of leafy vegetables (spinach, lettuce and chicory) including prolonged their shelf life based on total microbial counts but some reducing in an important bioactive compounds i.e. phenolics, carotenoids and chlorophyll have been reduced. Such notice should be taken in our consideration when the UV-treated leafy vegetables will be used as functional foods and/or in diets planning.

Keywords: UV-C, phenolics, carotenoids, chlorophyll**,** spinach, lettuce, chicory, total aerobic bacterial growth

Introduction

Vegetables play an important role in human diets, as they support the normal functioning of the different body systems. They provide our cells with vitamins, minerals, fiber, essential oils and phytonutrients. Vegetables contain low amounts of fat and calories (Banerjee *et al.*, 2012). Leaf vegetables came from very wide variety of plants and they are plants with edible leaves. Each of us knows lettuce and spinach, as well as mustard, but also early springtime nettles are valuable source of vitamin C. Green leafy vegetables are popularly used for food, being a rich source of β-carotene, ascorbic acid, minerals and dietary fiber. One of the most popular vegetable is spinach, lettuce and chicory. They are cultivated worldwide, and are some of the most consumed green leafy vegetables in the raw form for its taste and high nutritive value, being regarded as an important source of phytochemicals, including carotenoids, phenolics and chlorophyll in the diet (Dragan et al., 2011; Goh et al., 2012 and Chang *et al.,* 2013).

The increased demand of fresh minimally processed vegetables has led to an increase in the quantity and variety of produce available to the consumer in the markets. Since the last decade, spinach, lettuce and chicory and other analogous vegetables are still the most consumed fresh processed vegetables (Nguyen-the and Carlin, 1994). The limited shelf-life of fresh processed leafy vegetables is one of the greatest problems faced by commercial marketers mainly due to the natural variability in the material. Worldwide, all fresh-cut products are by necessity handled in modified atmosphere packaging to achieve the necessary post-harvest lifespan. However, it is very difficult to identify an optimal storage atmosphere with the current techniques and methodologies. Thus, efforts are made to optimize processing to reduce losses from microbial spoilage as well as the design of moderate but workable alternative preservation treatments.

Irradiation is a non-thermal preservation method which involves the exposure of food materials or products to radiation, for example ultraviolet (UV-C) rays. This treatment brings some benefits to the fresh cut industry as its use is approved by the code of Food and Drug Administration (FDA) in the USA on food products to control surface micro-organisms, does not leave a residue, and does not require extensive safety equipment (Yaun *et al., 2004).* UV-C light at 254 nm is lethal to most microorganisms (Bintsis *et al.*, 2000) such as *Pseudomonas spp*. on fresh meat (Stermer *et al.*, 1987) and Salmonella typhimurium on fish (Huang and Toledo, 1982), and it also can be applied to prolong shelf life of food such as orange juice (Tran and Farid, 2004). UV-C was reported to result in 90.99% inactivation of total viable bacteria and total inactivation of yeast and mold in pineapple juice (Noranizan *et al*., 2011).

Given the great benefits provided by antioxidants, refrigerated leafy vegetables that retained high amount of antioxidants from the fresh vegetables are in demand. However, some of the bioactive compounds i.e. antioxidants are destroyed by UV treatment (Pan *et al*., 2004; Koutchma, 2009 and Goh et al., 2012). The losses of such bioactive compounds may lead to colourless products, such as reported by Chen e*t al*. (1995) in carrot juice, and thus causes lower quality products. Therefore, the objective of this study was to study the influence of UV-C treatments on the bioactive compounds stability and antioxidant activity of leafy vegetables throughout 8 days of refrigerated storage.

Materials and Methods

Materials

Selected leafy vegetables, spinach (*Spinacia oleracea*), lettuce (*Lactuca sativa*) and chicory (*Cichorium intybus*) were obtained from the Egyptian local markets, Cairo, Egypt. All chemicals, reagents and solvents were of analytical grade and purchased from El-Ghomhorya for Drugs, Chemicals and Medical Instruments Trading Co. (Cairo, Egypt).

UV-C radiation treatments

The UV-C radiation device consisted of one bank of three stainless-steel reflectors with unfiltered germicidal emitting lamps (Atlanta Light Bulbs Inc., Tucker, Georgia) located 15 cm above the radiation vessel. The emitted light was in the UV-C (220–290 nm, with peak radiation at approximately 254nm) region. All of the Occupational Safety Procedures for users was taken in the consideration through enclosed the UV-C lamps, reflectors, and treatment area in a wooden box supported by metal frame and covered with stainless steel cover. The UV lamps were allowed to stabilize by turning them to 30 min. Leafy vegetables i.e. spinach, lettuce and chicory were then placed over a tray (50 x 40 cm, LxW) for the UV-C treatments. The tray consisted in a polystyrene net. The UV-C radiation dose selected for these experiments was: 8.0 kJm^2. Non-radiated spanish, lettuce and chicory were considered as the control. Radiation of the product was carried out in the air conditioning room at 18 ^{0}C to avoid a temperature increase during the UV-C treatment. After radiation, 100 g of spinach, lettuce and chicory were packaged and sealed in polypropylene bags and stored at 4 ^{0}C for 8 days.

Determination of total phenolics content

Total phenolics in plant parts extracts were determined using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Two hundred milligrams of sample was extracted for 2 h with 2 ml of 80% MeOH containing 1% hydrochloric acid at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000g for 15 min and the supernatant decanted into 4 ml vials. The pellets were combined and used for total phenolics assay. One hundred microliters of extract was mixed with 0.75 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 ^{0}C for 5 min; 0.75 ml of sodium bicarbonate (60 g/l) solution was added to the mixture after 90 min at 22 ^{0}C, absorbance was measured at 725 nm. Results are expressed as ferulic and equivalents.

Determination of total carotenoids content

Total carotenoids content (TC) analysis was performed according to Moore (2003). The wavelength used was 450 nm and the TC was calculated using extinction coefficient of 2500 according to the method of Lima *et al*., (2005).

Determination of total chlorophyll content

Total chlorophyll (TCP) content was determined directly in vegetables leaves by using portable chlorophyll meter hand-hold plant nutrient meter hand-held chlorophyll analyzer, Model, Chlorophyll meter SPAD-502Plus, Sasha, China.

Antioxidant activity

Antioxidant activity of plant extract and standards (α-tocopherol, BHA, and BHT; Sigma Chemical Co., St. Louis, Mo) was determined according to the β-carotene bleaching method following a modification of the procedure described by Marco (1968). For a typical assay, 1mL of β-carotene (Sigma) solution, 0.2 mg/mL in chloroform, was added to round-bottom flasks (50 mL) containing 0.02 mL of linoleic acid (J.T. Baker Chemical Co., Phillipsburg, NJ) and 0.2 mL of Tween 20 (BDH

Chemical Co., Toronto, On). Each mixture was then dosed with 0.2 mL of 80% MeOH (as control) or corresponding plant extract or standard. After evaporation to dryness under vacuum at room temperature, oxygenated distilled water (50 ml) was added and the mixture was shaken to form a liposome solution. The samples were then subjected to thermal autooxidation at 50 ^{0}C for 2 h. The absorbance of the solution at 470 nm was monitored on a spectrophotometer (Beckman DU-50) by taking measurements at 10 min intervals, and the rate of bleaching of β-carotene was calculated by fitting linear regression to data over time. All samples were assayed in triplicate. Various concentrations of BHT, BHA, and α-tocopherol in 80% methanol was used as the control. Antioxidant activity (AA, %) was calculated as percent inhibition relative to control using the equation of Al-Saikhan *et al.,* (1995) as follow:

$$AA= (R_{control} - R_{sample}) / R_{control} \times 100$$

Where: $R_{control}$ and R_{sample} were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively.

Determination of total aerobic bacteria (TAB)

TAB was determined by plating suitable dilution in duplicates using nutrient agar medium (Difco Manual 1966). This medium consists of beef extract, bacto peptone, agar and sodium chloride by 3, 5, 15, and 5 g/L, respectively and completed by distilled water to1000 ml then the pH adjusted to 7. Plates were incubated at 32ºC for 3 days before counting and recording the results.

Statistical analysis

Statistical analysis was performed with the Student *t*-test and MINITAB program (Minitab Inc., State College, PA).

Results and Discussions

The effect of UV treatments on the total phenolics levels (TP) of leafy vegetables throughout refrigerated storage

Data in Table (1) and Figure (1) show the effect of UV treatments on the total phenolic levels (TP) of leafy vegetables throughout refrigerated storage. The TPC of tested leafy vegetables i.e. spinach, lettuce and chicory decreased during storage. The analysis of variance for the TP data indicates that the TP values were significantly affected ($P < 0.05$) by both the UV-C radiation and the storage period. Initial TP values for all UV-treated samples were significantly lower than those for the control (P < 0.05). For the control samples, the TP of spinach, lettuce and chicory was recorded 462.56, 286.40 and 405.92 mg GAE/100 g FW, which decreased to 398.45, 231.38 and 377.54 mg GAE/100 g FW (-13.86, -19.21 and -6.99 % a percent of control samples) at the end of storage period (8 days at 4 ^{0}C), respectively. All the UV- treatment was effective in significant (P ≤ 0.05) decreasing the level of the TP in vegetable samples after storage periods. The decreasing rates for the all tested vegetables i.e. spinach, lettuce and chicory were -30.15, --25.92 and -24.02% (as a percent of control samples). The opposite direction was recorded for some phenolics member (e.g. flavonoids) in UV treated fruit juices such as starfruit, citrus and pineapple (Arcas *et al*., 2000; Bhat *et al.*, 2011 and Goh *et al.*, 2012).The increment of total flavonoidsis due to the increased biosynthesis of phenylalanine ammonia lyase (PAL). After irradiation, PAL content increases (Charles *et al*., 2008 and Pombo *et al.*, 2011) and this in turn increases the biosynthesis of flavonoids (González-Aguilar, 2007 and Alothman *et al*., 2009). The reason that different result obtained in this study as compared to those in the literature was not determined, but the possible reason might be that UV dosage applied in this study was lower than authors discussed above. The reduction of some phenolics member (e.g. flavonoids) in UV-treated pineapple juice after storage was reported by other authors (Caro *et al*., 2004 and Goh *et al*., 2012).

The effect of UV treatments on the total carotenoids levels (TC) of leafy vegetables throughout refrigerated storage

Data in Table (2) and Figure (2) show the effect of UV treatments on the total carotenoid levels (TC) of leafy vegetables throughout refrigerated storage. The TC of tested leafy vegetables i.e. spinach, lettuce and chicory decreased during storage. The analysis of variance for the TC data indicates that the TC values were significantly affected (P ≤ 0.05) by both the UV-C radiation and the storage period. Initial TC

Table 1. The effect of UV treatments on the total phenolic levels (TP, mg GAE/100g FW) of leafy vegetables throughout refrigerated storage*

Leafy vegetables	Storage period (days)				
	0	2	4	6	8
Spinach	462.56± 23.80^{a}	444.83± 23.52^{ab}	435.69± 10.45^{ab}	420.75± 35.22^{ab}	398.45± 22.45^{c}
Lettuce	286.40± 11.80^{a}	269.78± 13.83^{b}	264.71± 11.56^{b}	249.34± 11.71^{bc}	231.38± 34.61^{bc}
Chicory	405.92± 22.45^{a}	392.26± 12.66^{a}	387.28± 10.58^{ab}	379.20± 10.61^{ab}	377.54± 6.36^{ab}
Spinach-UV	462.56± 23.80^{a}	429.38± 64.15^{b}	410.73± 30.21^{b}	380.04± 40.39^{c}	323.10± 21.63^{d}
Lettuce-UV	286.40± 11.80^{a}	264.54± 20.83^{b}	258.53± 21.34^{b}	225.05± 44.77^{c}	212.17± 26.78^{c}
Chicory-UV	405.92± 22.45^{a}	373.78± 25.65^{b}	364.50± 40.11^{bc}	341.80± 32.93^{bc}	308.41± 51.00^{d}

* Each value represents the mean of three replicates ±SD. Mean values with the different superscript letters in the same raw mean significantly different at level p=0.05.

Table 2. The effect of UV treatments on the total carotenoid levels (mg/100 g FW) of leafy vegetables throughout refrigerated storage*

Leafy vegetables	Storage period (days)				
	0	2	4	6	8
Spinach	41.73± 4.23^{a}	40.17± 3.23^{a}	38.71± 1.87^{a}	35.15± 2.98^{ab}	34.71± 5.98^{ab}
Lettuce	19.82± 1.11^{a}	19.01± 3.76^{a}	18.25± 5.23^{a}	16.34± 2.08^{ab}	15.32± 1.10^{ab}
Chicory	7.98± 1.22^{a}	7.65± 2.87^{a}	7.41± 0.87^{a}	7.29± 1.67^{a}	7.10± 2.14^{a}
Spinach-UV	41.73± 3.22^{a}	36.09± 2.18^{ab}	33.51± 2.11^{ab}	29.20± 3.17^{c}	27.14± 4.18^{c}
Lettuce-UV	19.82± 2.15^{a}	16.01± 3.15^{b}	15.25± 1.66^{bc}	13.72± 5.55^{bc}	9.39± 2.98^{c}
Chicory-UV	7.98± 0.98^{a}	6.54± 2.11^{a}	5.67± 2.09^{ab}	5.17± 1.87^{ab}	4.91± 2.67^{ab}

* Each value represents the mean of three replicates ±SD. Mean values with the different superscript letters in the same raw mean significantly different at level p=0.05.

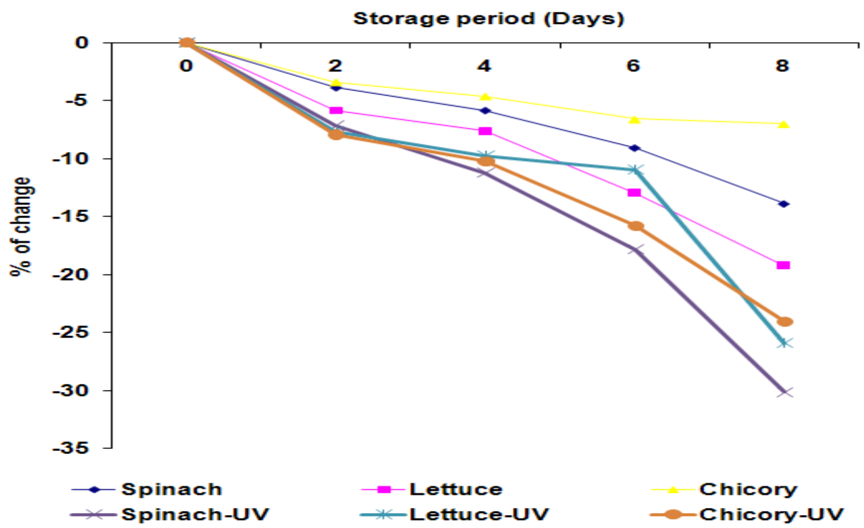

Figure 1. The effect of UV treatments on total phenolic levels (% of change) of leafy vegetables throughout refrigerated storage

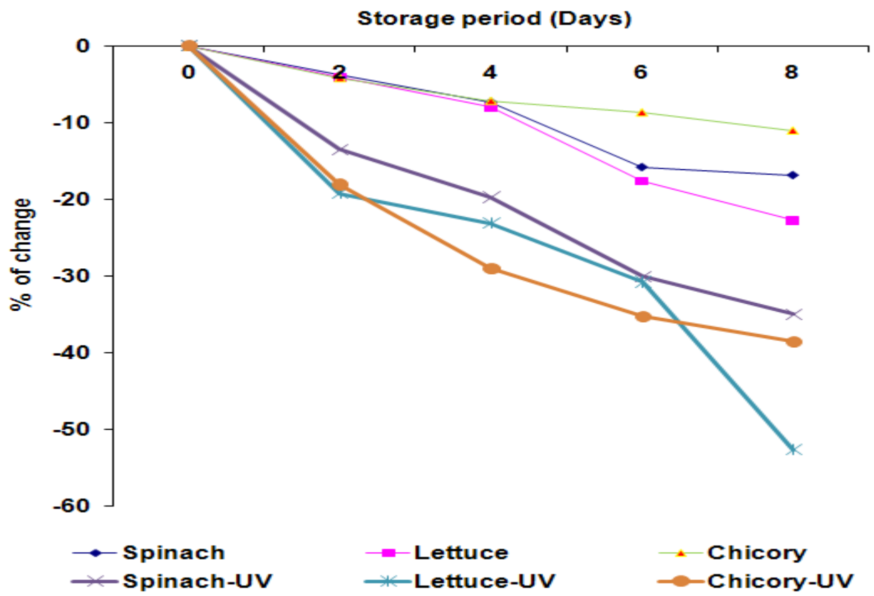

Figure 2. The effect of UV treatments on the total carotenoids levels (% of change) of leafy vegetables throughout refrigerated storage

values for all UV-treated samples were significantly lower than those for the control ($P < 0.05$). For the control samples, the TC of spinach, lettuce and chicory was recorded 41.73, 19.82 and 7.98 mg /100 g FW, which decreased by -16.82, -22.70 and -11.03 % (a percent of control samples) at the end of storage period (8 days at 4 ^{0}C), respectively. All the UV- treatment was effective in significant decreasing ($P \leq 0.05$) the level of the TC in vegetable samples after storage periods. The decreasing rates for the all tested vegetables i.e. spinach, lettuce and chicory were -34.96, -52.62 and-38.47 % (as a percent of control samples). In similar study, Dragan *et al.,* (2011) reported that a major contributor to the total carotene content of vegetables was b-carotene which is the main carotenoid with pro-vitamin A activity. Regarding the concentration of this pigment Podsedek (2007) also reported that leafy vegetables are indeed a richer source of β-carotene than other crops. Bhaskarachary *et al.,* (2008) also demonstrated similar domination of β-carotene in 17 species of leafy vegetables.Significant reduction of TC in pineapple juice after UV treatment was also reported by Goh *et al.,* (2012). The lower TC in UV treated juice can be explained by the light sensitive nature of carotenoids. This might be due to the nature of double bonds in carotenoids which easily absorbed UV and then undergo the process of UV photolysis. Also, Goh *et al.,* (2012) reported that the greater reduction of TC in UV-treated pineapple juice might be due to the oxidation enzyme which cannot be inactivated by UV treatment.

The effect of UV treatments on the total chlorophyll levels (TCP) of leafy vegetables throughout refrigerated storage

Data in Table (3) and Figure (3) show the effect of UV treatments on the total chlorophyll levels (TCP) of leafy vegetables throughout refrigerated storage. The TCP of tested leafy vegetables i.e. spinach, lettuce and chicory decreased during storage. The analysis of variance for the TCP data indicates that the TCP values were significantly affected ($P \leq 0.05$) by both the UV-C radiation and the storage period. Initial TCP values for all UV-treated samples were significantly lower than those for the control ($P < 0.05$). For the control samples, the TCP of spinach, lettuce and chicory was recorded 179.90, 91.17 and 308.00 mg /100 g FW, which decreased by-26.45, -29.87 and -18.51% (a percent of control samples) at the end of storage period (8 days at 4 ^{0}C), respectively. All the UV- treatment was effective in significant decreasing ($P \leq 0.05$) the level of the TC in vegetable samples after storage periods. The decreasing rates for the all tested vegetables i.e. spinach, lettuce and chicory were -32.85, -37.03 and -35.68% (as a percent of control samples). Although UV-C radiation prolonged shelf life of fresh-cut spinach, lettuce and chicory based on total microbial counts some reducing in an important bioactive compounds i.e. phenolics have been reduced. Chlorophyll refers to the phytochemical that gives plants their green color and pigmentation. This chemical is responsible for absorbing solar energy to facilitate photosynthesis, a process in which plants convert energy from sunlight into sugars. Chlorophyll can get from green vegetables or through liquid supplementation purchased from vitamin stores. Chlorophyll provides nutritional benefits to the body and helps keep you healthy including healthy bones, strong muscles, maintaining normal blood pressure and needs for the blood to clot properly. (Niizu and Rodriguez-Amaya, 2005and Liu *et al.,* 2007). In similar study, Dragan *et al.,* (2011) reported that chlorophyll was the most abundant pigment observed among species/cultivars including the tested leafy vegetables. Species/cultivars with high levels of chlorophylls also had relatively high amount of lutein and total carotenes. The positive correlation between the contents of chlorophyll and carotenoids have been also reported for other leafy crop species, like as kale (Kopsell et al., 2004), Swiss chard (Ihl et al., 2006) and lettuce (Caldwell and Britz, 2006).

The effect of UV treatments on the antioxidant activity (AA) of leafy vegetables throughout refrigerated storage

Data in Table (4) and Figure (4) show the effect of UV treatments on the AA of leafy vegetables throughout refrigerated storage. The AA of tested leafy vegetables i.e. spinach, lettuce and chicory decreased during storage. The analysis of variance for the AA data indicates that the TPC values were significantly affected ($P < 0.05$) by both the UV-C radiation and the storage period. Initial AA values for all UV-treated samples were significantly higher than those for the control ($P \leq 0.05$). For the control samples, the AA of spinach, lettuce and chicory was recorded 64.45, 57.72 and 62.64% , which decreased to -19.35, -23.92 and -13.25% (a percent of control samples) at the end of storage period (8 days at 4 ^{0}C), respectively. All the UV- treatment was effective in

Table 3. The effect of UV treatments on the total chlorophyll levels (mg/100 g FW) of leafy vegetables throughout refrigerated storage*

Leafy vegetables	Storage period (days)				
	0	2	4	6	8
Spinach	179.90± 11.21 ^{a}	161.12± 10.65 ^{b}	151.10± 11.87 ^{bc}	142.34± 5.78 ^{bc}	132.32± 9.65 ^{d}
Lettuce	91.17± 9.34 ^{a}	81.12± 5.33 ^{b}	74.04± 8.76 ^{b}	71.12± 3.98 ^{bc}	63.94± 7.43 ^{c}
Chicory	308.00± 12.67 ^{a}	290.00± 10.67 ^{ab}	280.00± 33.98 ^{b}	265.00± 20.65 ^{b}	251.00± 23.89 ^{bc}
Spinach-UV	179.90± 11.21 ^{a}	155.90± 17.87 ^{b}	143.20± 12.87 ^{bc}	131.52± 17.98 ^{c}	120.80± 20.67 ^{cd}
Lettuce-UV	91.17± 9.34 ^{a}	76.21± 21.87 ^{b}	70.32± 21.77 ^{b}	65.41± 13.76 ^{bc}	57.41± 20.51 ^{bc}
Chicory-UV	308.00± 12.67 ^{a}	281.00± 31.87 ^{b}	265.80± 34.65 ^{b}	239.60± 21.76 ^{c}	198.10± 15.65 ^{d}

* Each value represents the mean of three replicates ±SD. Mean values with the different superscript letters in the same raw mean significantly different at level p=0.05.

Table 4. The effect of UV treatments on the antioxidant activity (AA, %) of leafy vegetables throughout refrigerated storage*

Leafy vegetables	Storage period (days)				
	0	2	4	6	8
Spinach	64.45± 5.56 ^{a}	62.40± 4.88 ^{a}	60.07± 3.04 ^{a}	55.63± 9.22 ^{ab}	51.98± 7.71 ^{ab}
Lettuce	57.72± 4.20 ^{a}	54.31± 2.79 ^{a}	51.81± 4.02 ^{a}	47.62± 3.37 ^{ab}	43.92± 5.93 ^{ab}
Chicory	62.64± 4.17 ^{a}	60.11± 7.17 ^{a}	58.53± 2.63 ^{a}	55.11± 4.22 ^{a}	54.34± 4.16 ^{a}
Spinach-UV	64.45± 8.26 ^{a}	56.07± 5.61 ^{ab}	51.32± 1.92 ^{ab}	48.41± 3.91 ^{ab}	41.73± 2.77 ^{c}
Lettuce-UV	57.72± 5.26 ^{a}	51.64± 3.11 ^{b}	46.62± 6.33 ^{bc}	40.33± 3.70 ^{c}	33.32± 2.02 ^{c}
Chicory-UV	62.64± 2.37 ^{a}	56.03± 8.06 ^{ab}	52.03± 4.02 ^{ab}	48.13± 2.95 ^{ab}	42.45± 5.77 ^{c}

* Each value represents the mean of three replicates ±SD. Mean values with the different superscript letters in the same raw mean significantly different at level p=0.05.

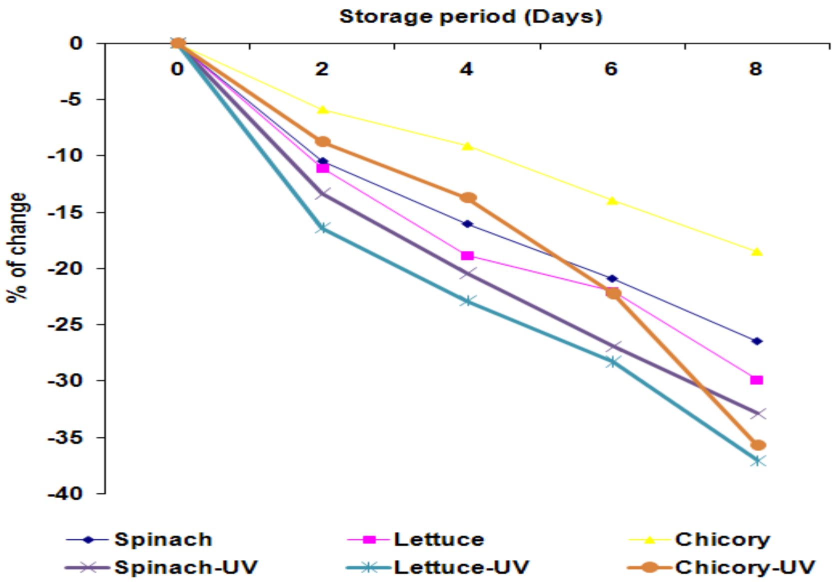

Figure 3. The effect of UV treatments on the total chlorophyll levels (% of change) of leafy vegetables throughout refrigerated storage

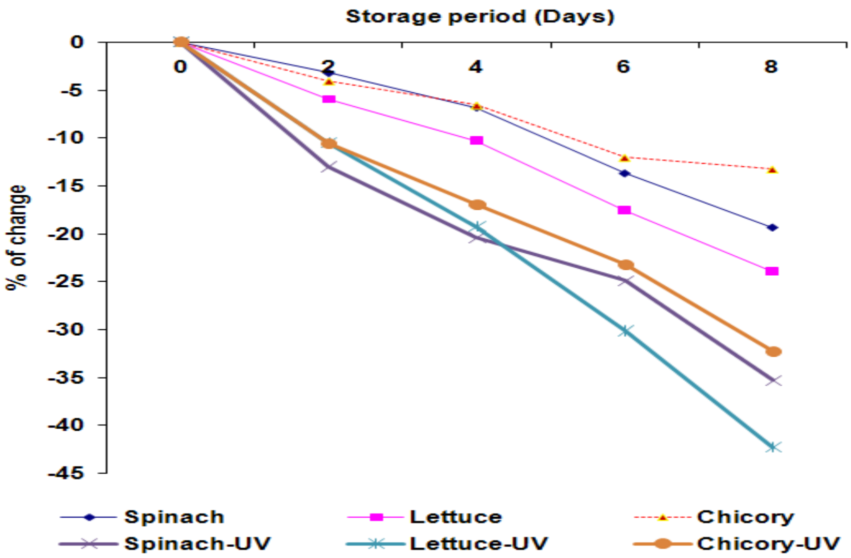

Figure 4. The effect of UV treatments on the antioxidant activity (% of change) of leafy vegetables throughout refrigerated storage

decreasing the level of the AA in vegetable samples after storage periods. The decreasing rates for the all tested vegetables i.e. spinach, lettuce and chicory were -35.25, -42.27 and -32.23% (as a percent of control samples).

The effect of UV treatments on the total aerobic bacterial growth (TABG) of leafy vegetables throughout refrigerated storage

Data in Table (5) and Figure (5) show the effect of UV treatments on the TABG of leafy vegetables throughout refrigerated storage. The microflora of tested leafy vegetables i.e. spinach, lettuce and chicory increased during storage. The analysis of variance for the TABG data indicates that the TCP values were significantly affected ($P \leq 0.05$) by both the UV-C radiation and the storage period. For the control samples, the TABG of spinach, lettuce and chicory was recorded 5.51, 4.88 and 4.56 $\log_{10}$cfu/g which increased to 8.13, 7.88 and 7.72 $\log_{10}$cfu/g (58.11, 61.47 and 69.30% as a percent of control) at the end of storage period (8 days at 4 ^{0}C), respectively. All the UV- treatment was effective in reducing growth the TABG in vegetable samples after storage periods in comparing with the untreated samples. The increasing effect rates for the all tested vegetables were closed to each other ranged 31.80-34.93% (as a percent of control samples). Therefore, UV-C radiation prolonged shelflife of fresh-cut spinach, lettuce and chicory based on total microbial counts. Actually, in compliance with the recommended microbial limit of total plate counts for ready-to-eat vegetables established by the Spanish legislation (Boletı´n Oficial del Estado, BOE, 2001) (7 log cfu g$^-$1), the untreated control of spinach, lettuce and chicory had a shelf-life that was at least 4, 6 and 6 days shorter than UV-C treated samples, respectively. All of the present data are in accordance with that obtained by Allende *et al.*, (2006). They reported that there were different growth behaviors among bacterial groups but they all responded similarly towards UV-C radiation treatment. UV-C radiation was effective in reducing growth of most of the tested micro-organisms. Maximum growth reductions were observed between 2 and 6 days of storage for the higher radiation doses (2.37 and 7.11 kJm_2). Also, Allende and Artes (2003a, b) found similar results when minimally processed lollo rosso and red oak leaf lettuces were treated with 0.4, 0.81, 2.44, 4.07, and 8.14 kJm^{-2} on only

Table 5. The effect of UV treatments on the total aerobic bacterial growth (log_{10}cfu/g) of leafy vegetables throughout refrigerated storage*

Leafy vegetables	Storage period (days)				
	0	2	4	6	8
Spinach	5.15± 0.56 [a]	6.03± 0.75 [a]	7.37± 0.53 [b]	6.93± 1.02 [b]	8.14± 0.56 [c]
Lettuce	4.88± 0.83 [a]	6.16± 0.26 [b]	6.39± 0.32 [b]	6.75± 1.07 [bc]	7.88± 1.16 [bc]
Chicory	4.56± 0.97 [a]	4.95± 0.95 [a]	5.56± 0.83 [ab]	6.54± 1.60 [b]	7.72± 0.62 [c]
Spinach-UV	5.21± 2.12 [a]	5.36± 0.33 [a]	5.61± 0.52 [a]	6.30± 0.62 [ab]	6.87± 0.19 [ab]
Lettuce-UV	4.97± 0.12 [a]	5.17± 1.49 [a]	5.48± 0.73 [ab]	5.92± 0.41 [ab]	6.66± 0.11 [ab]
Chicory-UV	4.46± 0.14 [a]	4.48± 1.06 [a]	4.89± 1.05 [a]	5.66± 1.12 [ab]	6.02± 1.34 [ab]

* Each value represents the mean of three replicates ±SD. Mean values with the different superscript letters in the same raw mean significantly different at level p=0.05.

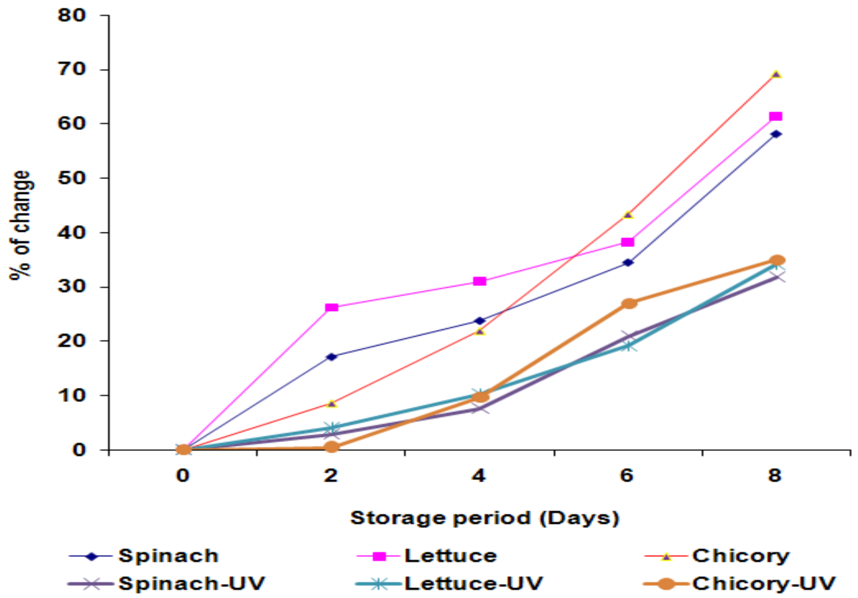

Figure 5. The effect of UV treatments on the total aerobic bacterial growth (TABG) of leafy vegetables throughout refrigerated storage

one side of the tissue. Data of previous studies mentioned that studies illustrated that the UV light penetrates the outer cell wall of the microorganism, passes through the cell body, reaches the DNA and alters the genetic material. The microorganisms are thereby destroyed in a non-chemical manner. [http://www.aquafineuv.com/ UV Technology/UV Science.aspx]

Correlation analysis

In the correlation analysis, important differences were found between phenolics, carotenoids, chlorophyll and antioxidant activity of control and UV-treated leafy vegetables throughout refrigerated storage (Figures 6). When all leafy vegetables were included in the statistical analysis, there was a positive significant ($p \leq 0.05$) relationship between total phenolics (r^2 = 0.584), total carotenoids (r^2 = 0.385), total chlorophyll (r^2 = 0.312) and antioxidant activity in control and UV-treated leafy vegetables throughout refrigerated storage. These

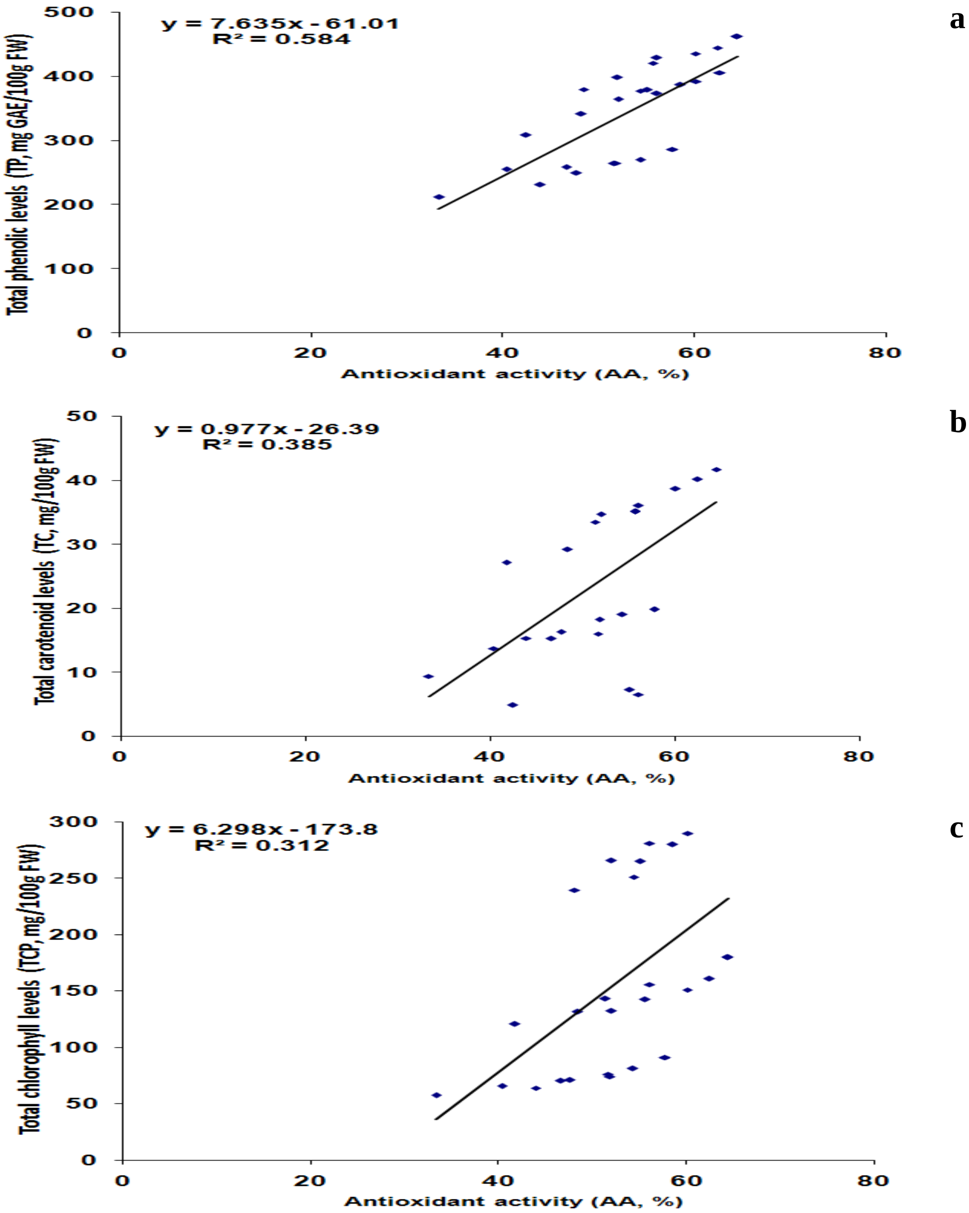

Figure 6. Correlation between antioxidant activity (AA) and bioactive compounds in control and UV-treated leafy vegetables throughout refrigerated storage: (a) AA vs. total phenolics, (b) AA vs. carotenoids, and (c) AA vs. chlorophyll.

correlations confirm that phenolic compounds mainly, carotenoids and chlorophyll partially responsible for the antioxidant activity of the control and UV-treated leafy vegetables throughout refrigerated storage. Also, these data indicates that many other bioactive compounds beside phenolics, carotenoids and chlorophyll such vitamins, fibers, minerals etc probably contribute in the antioxidant activity of the tested leafy vegetables. This information was confirmed by Krishnaswamy and Raghuramulu (1998) who reported that antioxidant properties, one medicinal plant that has been proposed having interesting antioxidant activity and protective capacities due to the presence of components such as vitamins C and E, and other non-nutrient substances is dietary practices including leafy vegetables. Also, Velioglu *et al*., (1998) reported that the correlation coefficient between total phenolics and antioxidative activities of 28 plant products and by-products, including sunflower seeds, flaxseeds, wheat germ, buckwheat, several fruits, vegetables, and medicinal plants was statistically significant. Also, Elhassaneen and Abd Elhady (2014) reported relationship between antioxidant activity and total phenolics in selected vegetable commonly consumed in Egypt.

Conclusion

Irradiation is a non-thermal preservation method which involves the exposure of food materials or products to radiation, for example ultraviolet (UV-C) rays. This treatment brings some benefits to the fresh cut industry of leafy vegetables (spinach, lettuce and chicory) including prolonged their shelf life based on total microbial counts but some reducing in an important bioactive compounds i.e. phenolics,

carotenoids and chlorophyll have been reduced. Such notice should be taken in our consideration when the UV-treated leafy vegetables will be used as functional foods and/or in diets planning.

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تأثير المعاملة بالأشعة فوق البنفسجية على ثبات المركبات النشطة بيولوجيا والنشاط المضاد للأكسدة في الخضروات الورقية أثناء التخزين المبرد

ناهد شحاته[١] ، إيمان نجم [٢]

[١] قسم الاقتصاد المنزلى- كلية التربية النوعية- جامعة الزقازيق - الزقازيق- مصر
[٢]قسم الصناعات الغذائية - كلية الزراعة- جامعة القاهرة - القاهرة- مصر

تعتبر الخضروات المختلفة مصدرا للمكونات التي تعزز الصحة البشرية، كما تستخدم الخضروات الورقية على نطاق واسع في النظام الغذائي البشري لأسباب عديدة بما في ذلك إرتفاع محتواها من المركبات النشطة بيولوجيا ذات الأدوار الفسيولوجية الهامة. كما يعد التشعيع هو طريقة حفظ غير حرارية التي يتم من خلالها تعرض المواد الغذائية أو منتجاتها للإشعاع، على سبيل المثال الأشعة فوق البنفسجية ذات المنطقة جـ (UV-C). وقد أجرى هذا البحث لدراسة تأثير المعاملة بالأشعة فوق البنفسجية على ثبات المركبات النشطة بيولوجيا والنشاط المضاد للأكسدة في الخضروات الورقية أثناء التخزين المبرد. ويشير تحليل التباين لمجموع الفينولات والكاروتينات والكلوروفيلات الكلية فى عينات الخضروات الورقية المختبرة إلى أن قيمها كانت معنوية ($p\leq0.05$) وتأثرت بدرجة كبيرة بالمعاملة بالأشعة فوق البنفسجية والتخزين بالتبريد، حيث أدت المعاملة بالأشعة فوق البنفسجية الى حدوث إنخفاض معنوى ($p\leq0.05$) لمجموع الفينولات والكاروتينات والكلوروفيلات الكلية في الخضروات الورقية مقارنة بالعينات الضابطة. عندما تم تضمين جميع الخضروات الورقية في التحليل الإحصائي، كانت هناك علاقة معنوية ($p\leq0.05$) بين الفينوليات الكلية ($r2 = 0.584$)، الكاروتينات الكلية ($r2 = 0.385$)، مجموع الكلوروفيلات ($r2 = 0.312$) والنشاط المضاد للأكسدة في العينات الضابطة والعينات المعاملة بالأشعة فوق البنفسجية أثناء التخزين المبرد. كما أظهر التحليل البكتريولوجي أن إجمالي نمو البكتيريا الهوائية فى السبانخ والخس والسريس قد سجل ٥.٥١ ، ٤.٨٨ ، ٤.٥٦ (log_{10} cfu/g) التي زادت بمعدلات ٥٨.١١ و ٦١.٤٧ و ٦٩.٣٠٪ لعينات الخضروات الغير معالجة و ٣١.٨٠ ، ٣٤.١٥ ، ٣٤.٩٣٪ للعينات المعاملة بالأشعة فوق البنفسجية بعد ٨ أيام من التبريد على ٤ درجة مئوية على التوالى وفي النهاية، تجلب المعاملى بالأشعة فوق البنفسجية بعض الفوائد لصناعة قطع الخضار الورقية الطازجة مثل السبانخ والخس والسريس مثل طول مدة صلاحيتها والتى تم إختباره من خلال مجموع التعداد الميكروبي، ولكن على الجانب الآخر فإن تلك المعاملة تؤدى الى حدوث إنخفاض في المحتوى من المركبات الهامة النشطة بيولوجيا مثل الفينولات والكاروتينات والكلوروفيلات. لذلك ينبغي أخذ النتائج في الاعتبار عند استخدام الخضروات الورقية المعاملة بالأشعة فوق البنفسجية كأغذية وظيفية أو إدخالها في تخطيط الوجبات الغذائية.

الكلمات المفتاحية: الأشعة فوق البنفسجية ذات المنطقة جـ، الفينولات،الكاروتينات، الكلوروفيلات، السبانخ، الخس، السريس، العدد الكلى للبكتيريا الهوائية.