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Preservation of Leafy Vegetables by Co-treatment with Refrigeration Process and ultraviolet radiation (UV-c) and its potential effects on bioactive compounds content and antioxidant activity

By

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Abstract:

The present study was carried out to investigate the influence of ultraviolet radiation (**UV-c**) treatment on the stability of bioactive compounds and antioxidant activity in leafy vegetables (Molokhia, Spinach and lettuce) throughout refrigerated storage. The analysis of variance for the total phenolics, carotenoids and chlorophyll levels indicates that their values were significantly ($P \leq 0.05$) affected by both the UV-C radiation and the refrigeration storage period. Initial total phenolics, carotenoids and chlorophyll levels for all UV-treated samples were significantly lower ($P < 0.05$) than those for the control samples. When the all selected leafy vegetables were included in the statistical analysis, there was a positive significant relationship between total phenolics ($p \leq 0.05$, $r^2 = 0.5182$), total carotenoids ($P \leq 0.01$, $r^2 = 0.6827$), total chlorophyll ($P \leq 0.05$, $r^2 = 0.6715$) and antioxidant activity in control and UV-treated samples throughout refrigerated storage. Also, bacteriological analysis indicated that total

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aerobic bacterial growth (TABG) of molokhia, spinach and lettuce was recorded 4.21, 4.65 and 4.43 $\log_{10}$ cfu/g which increased by rates of 51.98, 43.92 and 54.34% for the untreated samples and 41.73, 33.32 and 42.45% 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 (Molokhia, Spinach and lettuce) 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: ultraviolet radiation, molokhia, spinach, lettuce, phenolics, carotenoids, chlorophyll**,** , total aerobic bacterial growth

Introduction

Food preservation refers to a variety of techniques for keeping food from spoiling once it has been harvested. Such techniques reach back to the Stone Age. Drying, chilling, and fermentation are the oldest means of food preservation, whereas canning, pasteurisation, freezing, irradiation, the addition of chemicals/preservatives, and advancements in packing materials are the most contemporary. Refrigeration is the process of transferring heat from one location to another. Heat transport work is typically powered by mechanical effort, but it can also be powered by heat, magnetism, electricity, lasers, or other sources. Many foods' shelf lives can be extended by storing them at temperatures below 4° C (40° F). Fresh fruits and vegetables, eggs, dairy products, and meats are all often chilled goods. Low temperatures can destroy some foods, such as tropical fruits (such as bananas). Furthermore, cooling cannot improve the quality of spoiled food; it can only slow it down. Dehydration of foods owing to moisture condensation is a concern with modern mechanical refrigeration, which has been solved using humidity control devices within the storage chamber and proper packing techniques.

The refrigeration technique made it possible to handle and store perishables in a sanitary manner, which boosted output, consumption, and nutrition. We shifted away from salts and toward a more manageable sodium level as a result of a change in our food preservation technology. After the 1890s, the capacity to transfer and store perishables like meat and dairy resulted in a 1.7 percent rise in dairy consumption and a 1.25 percent increase in overall protein intake in the United States (Craig et al., 2004). People were eating these perishables not just because it was easier for them to preserve them, but also because advances in refrigerated shipping and storage reduced spoilage and waste, lowering the cost of these items. Refrigeration is responsible for at least 5.1 percent of the increase in adult stature (in the United States) due to increased nutrition. When indirect impacts such as higher nutritional quality and reduced disease are factored in, the entire impact is much larger. Recent research has also found a link between the number of refrigerators in a home and the likelihood of stomach cancer death (Park et al., 2011).

Due to its palatability and nutritional endowments, the dietary plant plays an essential role in the healthcare management system, particularly in weight and feed efficiency (Kunle et al., 2017). Vegetables, for example, are crucial in human diets because they support the correct functioning of several body systems. They deliver vitamins, minerals, fibre, essential oils, and phytonutrients to our cells. Vegetables have a low fat and calorie content (Banerjee *et al.,* 2012). Molokhia, spinach, and lettuce are examples of leaf vegetables that come from a wide range of plants with edible leaves. We've all heard of lettuce, spinach, and mustard, but nettles in the early springtime are also a good source of vitamin C. Leafy green vegetables are commonly eaten because they are high in -carotene, ascorbic acid, minerals, and dietary fibre. Spinach, lettuce, and chicory are some of the most popular vegetables. They are grown all over the world and are among the most popular raw green leafy vegetables due to their delicious taste and great nutritional content. They are also a good source of phytochemicals, such as carotenoids (Chang *et al.*, 2013).

Increased customer demand for fresh, minimally processed veggies has resulted in a rise in the quantity and diversity of produce offered at marketplaces. Spinach, lettuce, chicory, and other similar vegetables have remained the most popular fresh processed vegetables for the past decade (Nguyen-the and Carlin, 1994). Because of the natural diversity in the material, the limited shelf-life of fresh processed green vegetables is one of the most serious issues faced by commercial marketers. To ensure the required post-harvest lifespan, all fresh-cut items must be handled in modified atmosphere packaging (MAP) (Brecht et al., 2003). With present approaches and methodologies, however, identifying an ideal storage environment is extremely difficult. As a result, efforts are made to optimise processing in order to prevent microbial spoilage losses, as well as the development of moderate but effective alternative preservation treatments. The reduction or eradication of external contamination utilising surface decontamination techniques is a good process for reducing the microbiological risk associated with the intake of fresh fruits and vegetables (Yaun *et al.*, 2004).

The use of nonionizing, germicidal, artificial ultraviolet (UV) light is a superficial postharvest treatment that can be used in conjunction with chilling to preserve fruit and vegetables (Constantin and Manuela, 2010). This treatment has certain advantages for the fresh cut industry, as it is allowed for use on food products by the Food and Drug Administration (FDA) in the United States, does not leave a residue, and does not necessitate expensive safety equipment (Yaun et al., 2004). UV radiation has been shown to be effective in inhibiting microbial growth in several investigations (Abshire and Dunton, 1981; Sumner *et al.,* 1995; Bintsis *et al.,* 2000; Gardner and Shama, 2000; Yaun *et al.,* (2004). Only a few research, however, have looked at UV radiation in minimally processed fruits and vegetables.

The goal of this study is to see how effective UV radiation is at reducing the microbial load of the resident microflora of some of the most common green vegetables, such as molokhia, spinach, and lettuce, before they are refrigerated. The effects of UV treatment on the content of bioactive chemicals and biological activity of such green vegetables will also be investigated.

Materials and Methods

Materials

Leafy vegetables, Molokhia (*Corchorus olitorius*) , Spinach *(Spinacia oleracea)* and lettuce *(Lactuca sativa)* were obtained as a donation from Egyptian Saudi Food Industries Company, p3 El Motwrien Area, Sadat City, Egypt. The samples were collected by special arrangement and transported refrigerated directly to the laboratory.

Methods

UV-C radiation treatments

The UV-C radiation device was made up of one bank of three stainless-steel reflectors with unfiltered germicidal emitting bulbs (Atlanta Light Bulbs Inc., Tucker, Georgia) positioned 15 cm above the radiation vessel. The light released was in the UV-C range (220–290 nm, with peak emission around 254 nm). All of the Occupational Safety Procedures for users were taken into account by enclosing the UV-C lamps, reflectors, and treatment area in a wooden box with a metal frame and a stainless steel top. The UV lamps were set at 30 minutes to allow them to stabilise. After that, leafy vegetables such as molokhia, spinach, and lettuce were placed on a tray (50 x 40 cm, LxW) for UV-C treatments. A polystyrene net made up the tray. The UV-C radiation dose selected for these experiments was: 8.0 kJm^2. Non-radiated molokhia, spinach and lettuce 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-Ctreatment. After radiation, 100 g of molokhia, spinach and lettuce were packaged and sealed in polypropylene bags and stored at 4 ^{0}C for 8 days.

Biochemical analysis

Total phenolics

Folin-Ciocalteu reagent was used to measure total phenolics in plant parts extracts (Singleton and Rossi, 1965). On an orbital shaker set to 200 rpm, 200 milligrammes of sample were extracted for 2 hours at room temperature with 2 ml of 80 percent MeOH containing 1% hydrochloric acid. The supernatant was decanted into 4 ml vials after centrifugation at 1000g for 15 minutes. The pellets were mixed together and tested for total phenolics. After 5 minutes at 22 0C, 0.75 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) was added to the mixture; after 90 minutes at 22 0C, 0.75 ml of sodium bicarbonate (60 g/l) solution was added to the mixture, and absorbance was measured at 725 nm. Results are expressed as gallic acid equivalents (GAE/100g fresh weight).

Determination of total carotenoids content

Total carotenoids content 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

The total chlorophyll content of vegetables leaves was determined using a portable chlorophyll metre, a hand-held plant nutrition metre, and a hand-held chlorophyll analyzer from Sasha, China.

Antioxidant activity

Antioxidant activity (AA) of leafy vegetables and standard (BHT; Sigma Chemical Co., St. Louis, Mo) was determined according to the β-carotene bleaching (BCB) method following a modification of the procedure described by Marco (1968). 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.

Total bacterial counts

According to the American Public Association (1978), total bacterial counts of beverages were determined by plating adequate dilutions in duplicates on nutrient agar medium (Difco Manual 1966). This medium is made up of beef extract (3 g/L), bacto peptone (5 g/L), agar (15 g/L), sodium chloride (5 g/L), 1000 mL distilled water, and a pH of 7. Plates were incubated for 3 days at 32°C before being counted and recorded.

Statistical analysis

All analyses were carried out three times, with the results expressed as an average standard deviation (SD). The Student t-test and the MINITAB application were used for statistical analysis (Minitab Inc., State College, PA). Microsoft Excel was used to conduct the correlation analysis (Excel 2013 , v15.0).

Results and Discussion

The effect of UV-treatment on the total phenolics (TP) content of leafy vegetables in refrigerated storage

Data in Figures (1 and 2) shows the effect of UV-treatments on the total phenolic (TP) content of leafy vegetables in refrigerated storage. The TP content of the selected leafy vegetables i.e. molokhia, spinach and lettuce decreased during refrigerated storage. The analysis of variance for the TP data indicated that the TP values were significantly affected ($P \leq 0.05$) by both the UV and the days of storage period. Initial TP values content for all UV-treated samples were significantly ($P \leq 0.05$) lower than those for the control samples. For the control samples, the TP of molokhia, spinach and lettuce was recorded 501.67, 437.91 and 271.13 µg GAE/100 g FW, which decreased to 413.48, 348.40 and 219.29 µg GAE/100 g FW (-17.58, -20.44 and -19.12% as a percent of control samples) at the end of storage period (8 days at 4 ^{0}C), respectively. On the other side, UV- treatment was more significant ($P \leq 0.05$) effective in decreasing the level of the TP in selected vegetable samples after refrigerated storage periods. The decreasing rates for Molokhia, spinach and lettuce were -24.46, -28.01 and -21.84% (as a percent of control samples), respectively. These findings are consistent with those of Caro et al., (2004) and Goh et al., (2012), who found a decrease in flavonoids (phenolics) in UV-treated pineapple juice following storage. Flavonoids in UV-treated fruit juices such as starfruit, citrus, and pineapple, on the other hand, were shown to move in the reverse way (Arcas et al., 2000; Bhat et al., 2011 and Goh et al., 2012). The increase in production of phenylalanine ammonia lyase was used to explain the increase in total flavonoids in UV-treated samples (PAL). The production of flavonoids is increased after UV irradiation because the PAL content increases (Charles et al., 2008, Alothman et al., 2009 and Pombo et al., 2011) The reason(s) 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.

Several authors reported that phenolic compounds are found throughout the plant kingdom including those selected in the presemt study (Aly et al., 2017; Marzouk et al., 2020 and Elhassaneen et al., 2021-a). Velioglu et al. (1998) investigated the antioxidant activity and total phenolics of 28 plant products, including various by-products, and discovered that total phenolic content ranged from 169 to 10548 mg. Dry product, 100 g-1 Furthermore, several studies have revealed significant differences between different vegetable plant sections, which are attributable to the type, variety, and colour of vegetable fruits (Kumar *et al*., 1991and Onyeneho and Hettiarachchy, 1993). Many studies reported that phenolic compounds exhibited their different biological activities including antioxidant, scavenging and antimicrobial activities (Abd Aly et al., 2017 and Elalal et al., 2021), Such biological activities play important roles in prevention/treatment of different diseases including diabetes, obesity, liver disorders and cardiovascular diseases (Sayed Ahmed e al., 2016; Aly et al., 2017; Elhassaneen et al., 2020; Elhassaneen et al., $2021_{b\text{-}d}$; Mehram et al., 2021).

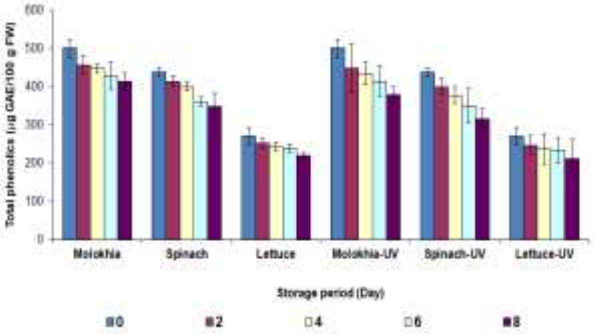

Figure 1. The effect of UV- treatments on the total phenolic content (TP, µg GAE/100g FW) of leafy vegetables in refrigerated storage

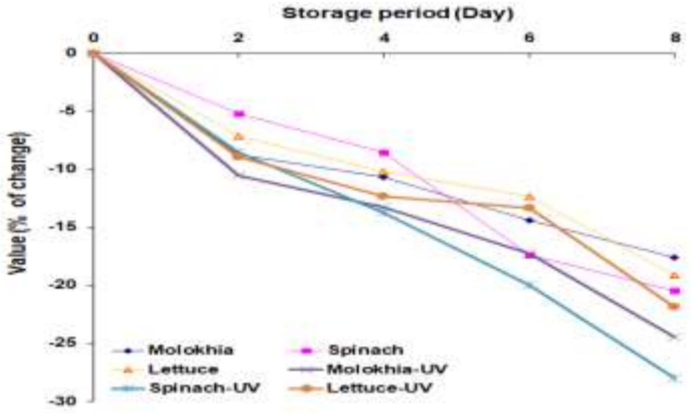

Figure 2. The effect of UV- treatments on the total phenolic content (TP, % of change) of leafy vegetables in refrigerated storage

The effect of UV-treatment on the total carotenoids (TC) content of leafy vegetables in refrigerated storage

Data in Figures (3 and 4) shows the effect of UV-treatments on the total carotenoids (TC) content of leafy vegetables in refrigerated storage. The TC content of the selected leafy vegetables i.e. molokhia, spinach and lettuce decreased during refrigerated storage. The analysis of variance for the TC data indicated that the TC values were significantly affected ($P \leq 0.05$) by both the UV and the days of storage period. Initial TC values content for all UV-treated samples were significantly ($P \leq 0.05$) lower than those for the control samples. For the control samples, the TC of molokhia, spinach and lettuce was recorded 187.13, 125.19 and 59.46 µg /100 g FW, which decreased to 90.89, 57.15 and 22.71 µg/100 g FW (-51.43, -54.35 and -61.81% as a percent of control samples) at the end of storage period (8 days at 4 ^{0}C), respectively. On the other side, UV- treatment was more significant ($P \leq 0.05$) effective in decreasing the level of the TC in selected vegetable samples after refrigerated storage periods. The decreasing rates for Molokhia, spinach and lettuce were -59.95, -71.34 and -66.04% (as a percent of control samples), respectively.

Dragan et al. (2011) found that -carotene, the principal carotenoid with pro-vitamin A activity, was a considerable contribution to the TC content of vegetables in a comparable study. In terms of -carotene content, Podsedek (2007) found that leafy vegetables are definitely a richer source of this pigment than other crops. Bhaskarachary et al., (2008) showed that -carotene predominated in 17 species of green vegetables, including the ones included in this study. Goh et al. also reported a substantial (p0.05) reduction of TC in pineapple juice after UV treatment (2012). The light sensitivity of carotenoids accounts for the decreased TC in UV treated juice. This could be related to the nature of carotenoids' double bonds, which absorb UV readily and then undergo UV photolysis. Sofia Semitsoglou et al. (2022) investigated the photochemical (UV–vis/H2O2) degradation of carotenoids, as well as the kinetics and molecular end products, in this context (Figure 1). They discovered that all carotenoids deteriorated when photolysis and OH scavenging were coupled, with fucoxanthin having the fastest degradation rates and meso-zeaxanthin having the slowest. The main degradation products detected by electrospray ionisation tandem mass spectrometry (ESI) tandem mass spectrometry (MS/MS) were apo-aldehydes and apo-ketones, with the latter tending to accumulate. However, epoxidation of the carotenoids occurred as well, and longer irradiation times resulted in lower molecular weight products. Furthermore, Goh et al. (2012) suggested that the higher TC reduction in UV-treated pineapple juice could be attributable to an oxidation enzyme that is not inactivated by

UV treatment. Finally, Aslam et al., (2021) described the mechanism of carotenoid oxidation and their beginning products, stating that carotenoids are thermally, light/UV, and oxidation sensitive (Figures 8 and 9).

Carotenoids are lipid-soluble (lipophilic) pigments that are produced by plants and some microbes and play a significant function in the photosynthetic machinery (Huang et al., 2017). They are primarily found in the chloroplasts in binding form. Carotenoids play a vital role in photosynthesis by protecting chlorophyll from UV and visible light, as well as stabilising the cell membrane by binding with free radicals (Solovchenko and Merzlyak, 2008). They have been shown to have health-promoting properties, such as serving as a vitamin A precursor. Carotenoids also have medicinal properties such as antioxidant, anti-inflammatory, anti-tumor, anti-cardiac, and anti-aging (Neville et al., 2013; Aly et al., 2017 ; Saini et al., 2018 and Zeng et al., 2019). Many researchers focused on the processing of carotenoids in vegetables and fruits because of their potential qualities and different applications, particularly in the food and pharmaceutical industries. Others make every effort to protect those substances from deterioration in their various sources.

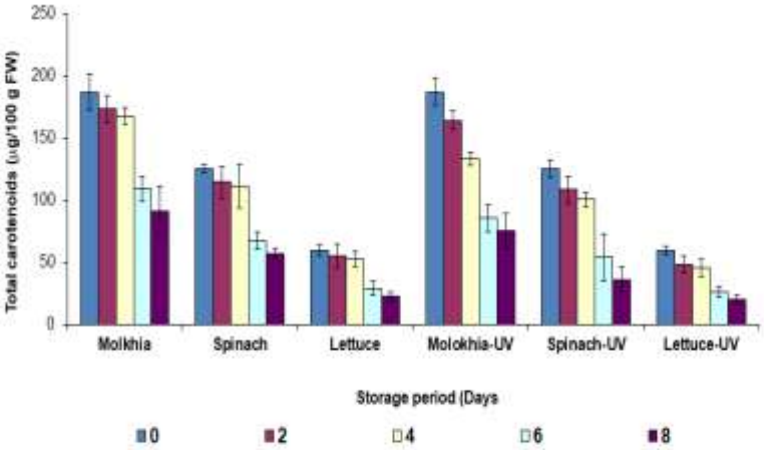

Figure 3. The effect of UV treatments on the total carotenoid levels (µg/100 g FW) of leafy vegetables throughout refrigerated storage.

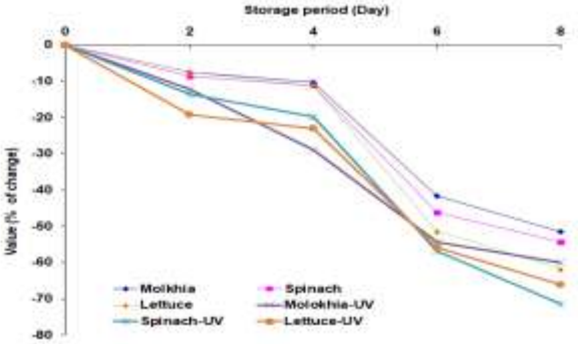

Figure 4. The effect of UV treatments on the total carotenoids levels (% of change) of leafy vegetables throughout refrigerated storage

The effect of UV-treatment on the total chlorophyll content of leafy vegetables in refrigerated storage

The effect of UV treatments on the total chlorophyll content of green vegetables in refrigerated storage is shown in Figures 5 and 6. During refrigerated storage, the total chlorophyll content of selected green vegetables such as molokhia, spinach, and lettuce decreased. The total chlorophyll values were significantly affected ($P \leq 0.05$) by both the UV and the days of storage period, according to the analysis of variance for total chlorophyll data. Initial total chlorophyll values content for all UV-treated samples were significantly ($P \leq 0.05$) lower than those for the control samples. For the control samples, the total chlorophyll of molokhia, spinach and lettuce was recorded 412.50, 240.94 and 122.10 µg /100 g FW, which decreased 127.21, 57.61 and 26.73 µg/100 g FW (-69.16, -76.09 and -78.11% as a percent of control samples) at the end of storage period (8 days at 4 ^{0}C), respectively. On the other side, UV- treatment was more significant ($P \leq 0.05$) effective in decreasing the level of the total chlorophyll in selected vegetable samples after refrigerated storage periods. The decreasing rates for Molokhia, spinach and lettuce were -73.07, -78.80 and -83.99 (as a percent of control samples), respectively.

The phytochemical chlorophyll is responsible for plants' green hue and pigmentation. This chemical absorbs solar energy to aid photosynthesis, which is the process by which plants transform sunlight energy into carbohydrates. Chlorophyll can be obtained via green vegetables or by taking a liquid supplement from a vitamin store. It provides nutritional advantages to the body and aids in keeping you healthy, such as healthy bones, strong muscles, normal blood pressure, and blood clotting requirements (Niizu and Rodriguez-Amaya, 2005and Liu *et al.*, 2007) (See Figure 10). Dragan et al. (2011) found that chlorophyll was the most prevalent pigment among the species/cultivars studied, including the green vegetables. The results of this investigation showed that vegetables with high chlorophyll levels also have comparatively high total carotenes. Other leafy species, such as kale (Kopsell et al., 2004) and Swiss chard, have also shown a favourable association between chlorophyll and carotenoids (Ihl et al., 2006).

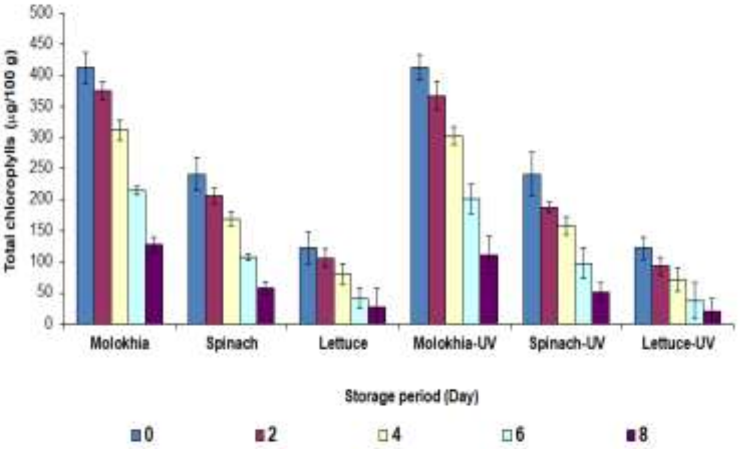

Figure 5. The effect of UV treatments on the total chlorophyll levels (µg/100 g FW) of leafy vegetables throughout refrigerated storage

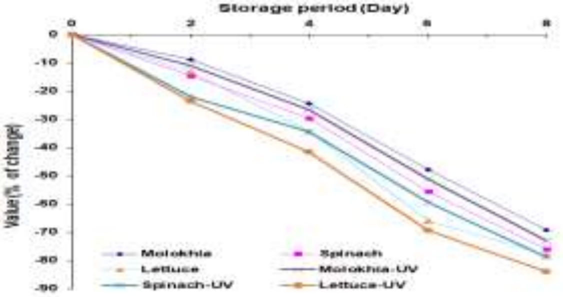

Figure 6. The effect of UV treatments on the total chlorophyll levels (% of change) of leafy vegetables throughout refrigerated storage

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

Data in Figures (7 and 8) shows the effect of UV-treatments on the antioxidant activity (AA) of leafy vegetables in refrigerated storage. The AA in the selected leafy vegetables i.e. molokhia, spinach and lettuce decreased during refrigerated storage. The analysis of variance for the AA data indicated that the AA values were significantly affected ($P \leq 0.05$) by both the UV and the days of storage period. Initial AA values content for all UV-treated samples were significantly ($P \leq 0.05$) lower than those for the control samples. For the control samples, the AA of molokhia, spinach and lettuce was recorded 66.18, 63.13 and 56.54 µg /100 g FW, which decreased 66.18, 63.13 and 56.54 µg/100 g FW (-28.94, -30.08 and -34.04% (as a percent of control samples) at the end of storage period (8 days at 4 ^{0}C), respectively. On the other side, UV- treatment was more significant ($P \leq 0.05$) effective in decreasing the level of the AA in selected vegetable samples after refrigerated storage periods. The decreasing rates for Molokhia, spinach and lettuce were -30.91, -43.87 and -49.96% (as a percent of control samples), respectively. The current findings contradict those of Dyshlyuk et al., (2020), who found that the total content of phenolic compounds, carotenoids, and flavonoids increases in tomatoes at all wavelengths investigated (353 nm, 365 nm, and 400 nm), while the content of chlorophylls reacts ambiguously: it increases at some wavelengths and decreases at others. In comparison to untreated samples, tomatoe samples irradiated for 360 minutes within the region of 365 nm show the greatest increase in antioxidant activity. From the original, the increase in phenolic compounds is 42.9–55.0 percent, carotenoids 24.0–56.0 percent, flavonoids 28.0–33.0 percent, -carotene 70.9–71.6 percent, lycopene 62.6–69.0 percent, and lutein 64.8–72.0 percent for various types of tomatoes.

In general, such AA (β -Carotene Bleaching, BCB) assay based on measured the ability of an antioxidant (plant parts and standard) to inhibit lipid peroxidation (Marco, 1968). Such data in Tabel (3) and Figure (4) indicated that selected leafy vegetables i.e. molokhia, spinach and lettuce recorded moderate AA. Comparing with the antioxidant standard used (α-tocopherol, the AA of that selected leafy vegetables are coming well with the value 50 mg/L of BHT standards. Such AA recorded by selected leafy vegetables i.e. molokhia, spinach and lettuce could be attributed to their good content of different bioactive compounds categories. Some of those compounds measured in the present study including total phenolics and carotenoids Known for its high antioxidant effect (Moreira et al., 2018; Marzouk et al., 2020 and Elhassaneen et al., 2021-a).

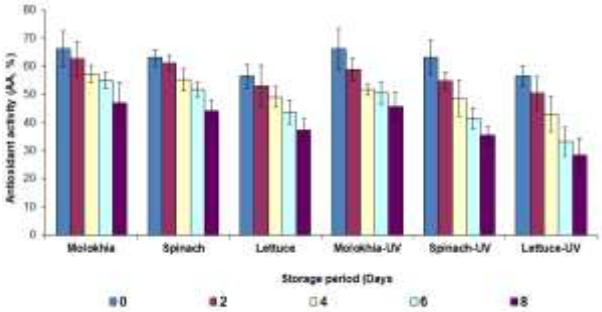

Figure 7. The effect of UV treatments on the antioxidant activity (AA, %) of leafy vegetables throughout refrigerated storage

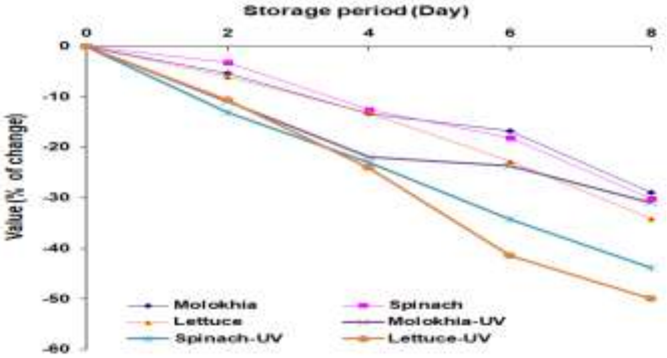

Figure 8. The effect of UV treatments on the antioxidant activity (% of change) of leafy vegetables throughout refrigerated storage

The effect of UV-treatment on the total aerobic bacterial growth (TABG) level of leafy vegetables in refrigerated storage

Data in Figures (9 and 10) shows the effect of UV-treatments on the **TABG** level of leafy vegetables in refrigerated storage. The **TABG** level in the selected leafy vegetables i.e. molokhia, spinach and lettuce decreased during refrigerated storage. Both the U ($P \leq 0.05$)), according to the analysis of variance for the TABG data**.** For the control samples, the **TAB**G of molokhia, spinach and lettuce was recorded 4.21, 4.65 and 4.43 $\log_{10}$ cfu/g which increased to 7.27, 8.02 and 7.54 $\log_{10}$cfu/g (72.57, 72.47 and 70.19 % 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 selected vegetable samples after storage periods in comparing with the UV untreated samples. For the UV-treated samples, the **TAB**G of molokhia, spinach and lettuce was recorded 6.25, 6.87 and 6.41 $\log_{10}$cfu/g (48.31, 47.79 and 44.58% as a percent of control) at the end of storage period (8 days at 4 ^{0}C), respectively. Based on total microbiological counts, UV-treatment extended the shelf-life of fresh-cut molokhia, spinach, and lettuce. Actually, the untreated control of molokhia, spinach, and lettuce had a shelf-life that was at least 6, 4 and 6 days shorter than UV-treated samples, according to the recommended microbial limit of total plate counts for ready-to-eat vegetables established by Spanish legislation (Bolet'n Oficial del Estado, BOE, 2001) (7 log cfu g-1) or proposed by Cnerna, CNRS (1996) (7.5 log cfu All of the current findings are consistent with those of Allende et al., (2006), who found that whereas different bacterial species had diverse growth patterns, they all responded similarly to UV-radiation treatment. The majority of the microorganisms studied grew slower when exposed to UV rays. For the higher radiation doses (2.37 and 7.11 kJm 2), maximum growth decreases were found between 2 and 6 days of storage. Also, 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 one side of the tissue, Allende and Artes (2003a, b) reported similar effects. The effect of UV-treatment is interpreted as follows using data from prior studies: UV radiation penetrates the microorganism's outer cell wall, passes through the cell body, and reaches the DNA, changing the genetic material. The germs are therefore killed without using chemicals. [http://www.aquafineuv.com/UVTechnology/UVScience.aspx].

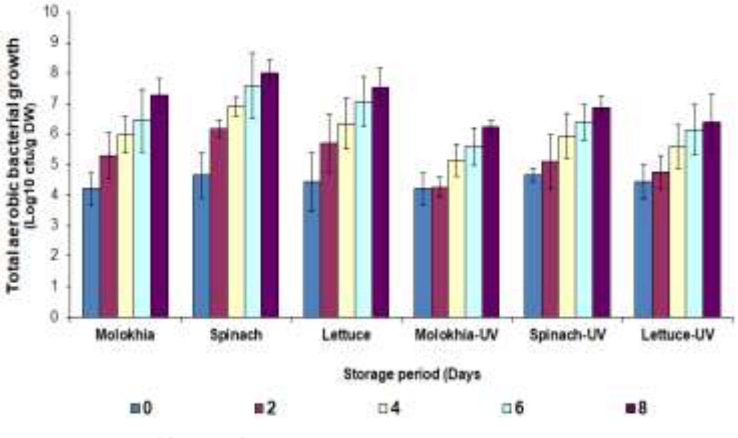

Figure 9. The effect of UV treatments on the total aerobic bacterial growth ($\log_{10}$cfu/g) of leafy vegetables throughout refrigerated storage

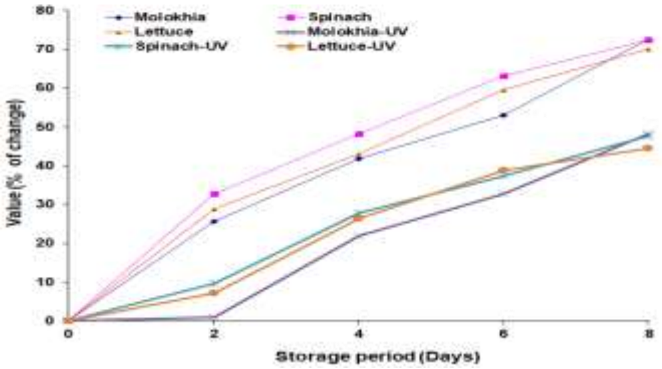

Figure 10. The effect of UV treatments on the total aerobic bacterial growth (% of change) of leafy vegetables throughout refrigerated storage

Correlation analysis between antioxidant activity (AA) and bioactive compounds in control and UV-treated leafy vegetables in refrigerated storage

Correlation analysis between AA and bioactive compounds in control and UV-treated leafy vegetables (molokhia, spinach and lettuce) in refrigerated storage was tabulated in Table (1). There was a positive significant ($p \leq 0.05$) relationship between total phenolics ($r^2 = 0.5182$), total carotenoids ($r^2 = 0.6827$), total chlorophyll ($r^2 = 0.6715$) and AA in control and UV-treated leafy vegetables in refrigerated storage when all leafy vegetables (molokhia, spinach, and lettuce) were included in the statistical analysis. These findings show that phenolic chemicals, primarily carotenoids and chlorophyll, are partially responsible for the AA of control and UV-treated green vegetables stored in refrigerated storage. Furthermore, these findings suggest that many additional bioactive substances, including as vitamins, fibres, minerals, and others, contribute to the AA of the selected green vegetables in addition to phenolics, carotenoids, and chlorophyll.

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 practises including leafy vegetables, according to Krishnaswamy and Raghuramulu (1998). Velioglu et al. (1998) also found a statistically significant connection coefficient between total phenolics and AA in 28 plant items, including leafy vegetables. Also, Elhassaneen and Abd Elhady (2014) reported relationship between AA and total phenolics in selected vegetable commonly consumed in Egypt. Finally, Aly et al., (2017), Moreira et al.,(2019); Marzouk et al., (2020) and Elhassaneen et al., (2021-a) reported a correlation coefficient between total phenolics and AA was statistically significant in different parts not leafy vegetables such algae, food processing by-products, herbs and medical plants.

Table (1): Correlation analysis between antioxidant activity (AA) and bioactive compounds in control and UV-treated leafy vegetables (molokhia, spinach and lettuce) in refrigerated storage

Correlation	Equation	r^2
AA vs. total phenolics	Total phenolic levels (TP, mg GAE/100 g FW)= 6.7347(AA, %) + 12.849	0.5182 *
AA vs. carotenoids	Total carotenoid levels (TC, mg/100g FW)= 4.2873 (AA, %) - 129.19	0.6827 **
AA vs. chlorophyll	Total chlorophyll levels (TCP, mg/100 g	0.6715*

	FW)= 9.8456 (AA, %) - 333.61	

* $P \leq 0.05$; ** $P \leq 0.01$

Conclusion

UV rays are a non-thermal form of food preservation that involves exposing food ingredients or goods to radiation. This treatment brings some benefits to the fresh of leafy vegetables (molokhia, spinach and lettuce) including prolonged their shelf -life based on reducing the microbial load (total aerobic bacterial growth, TABG) although some reduction in an important bioactive compounds such phenolics, carotenoids and chlorophyll have been observed. Such present data should be taken in our consideration when the UV used as food preservation method (prolonged shelf-life) especially in leafy vegetables.

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الحفاظ على الخضار الورقية عن طريق المعاملة المشتركة بين عمليات الحفظ بالتبريد والأشعة فوق البنفسجية وتأثيراتها المحتملة على محتوى المركبات النشطة بيولوجيًا والنشاط المضاد للأكسدة

أجريت الدراسة الحالية بهدف استكشاف تأثير المعاملة بالأشعة فوق البنفسجية (UV-c)على ثبات المركبات النشطة بيولوجيا والنشاط المضاد للأكسدة في الخضار الورقية (الملوخية، السبانخ، الخس) خلال التخزين بالتبريد. يشير تحليل التباين لمستويات المركبات الفينوليةوالكاروتينات والكلوروفيل إلى أن قيمها تأثرت معنوياً ($P \le 0.05$) بكل من الأشعة فوق البنفسجية و طول مدة التخزين بالتبريد. كانت مستويات الفينولات الكلية والكاروتينات والكلوروفيل لجميع العينات المعاملة بالأشعة فوق البنفسجية أقل بكثير ($P \le 0.05$) من تلك الخاصة بالعينات الضابطة (الغير معاملة). وعندما تم تضمين جميع الخضروات الورقية المختارة في التحليل الإحصائي، كانت هناك علاقة معنوية موجبة بين مستوى الفينولات الكلية ($p \le 0.05, r^2 = 0.5182$)، الكاروتينات الكلية ($P \le 0.01, r^2 = 0.6827$)، ، ، الكلوروفيل الكلي ($P \le 0.05, r^2 = 0.6715$) والنشاط المضاد للأكسدة في العينات الضابطة والمعالجة بالأشعة فوق البنفسجية في جميع فترات التخزين المبرد. كما أشارت التحاليل البكتريولوجية إلى أن النمو الكلي للبكتيريا الهوائية (TABG)للملوخية والسبانخ والخس قد سجل ٤.٢١ و ٤.٦٥ و ٤.٤٣ $\log_{10}$ cfu/g والتي زادت بمعدلات ٥١.٩٨ و ٤٣.٩٢ و ٥٤.٣٤٪ للعينات غير المعاملة و ٤١.٧٣ و ٣٣.٣٢ و. ٤٢.٤٥٪ للعينات المعاملة بالأشعة فوق البنفسجية بعد ٨ أيام من التبريد عند ٤ درجات مئوية على التوالي. وفي النهاية، يجلب المعاملة بالأشعة فوق البنفسجية (UV-C) بعض الفوائد الصحية للخضروات الورقية الطازجة (الملوخية والسبانخ والخس) بما في ذلك إطالة مدة صلاحيتها بناءً على إجمالي عدد الميكروبات ولكن بعضها ينخفض محتواه من المركبات النشطة حيويا مثل الفينولات والكاروتينات والكلوروفيل، ويجب أن يؤخذ هذا الإشعار في الاعتبار عند استخدام الخضروات الورقية المعاملة بالأشعة فوق البنفسجية كأغذية وظيفية و / أو في التخطيط للوجبات الغذائية.

الكلمات المفتاحية: الأشعة فوق البنفسجية، الملوخية، السبانخ، الخس، الفينولات، الكاروتينات، الكلوروفيل، النمو الكلي للبكتيريا الهوائية