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Ultraviolet-c Radiation Induced Changes on Bioactive Compounds Content, Antioxidant Capacity and Microbial Quality of Minimally Processed Molokhia (*Corchorus Olitorius* L.) Leaves

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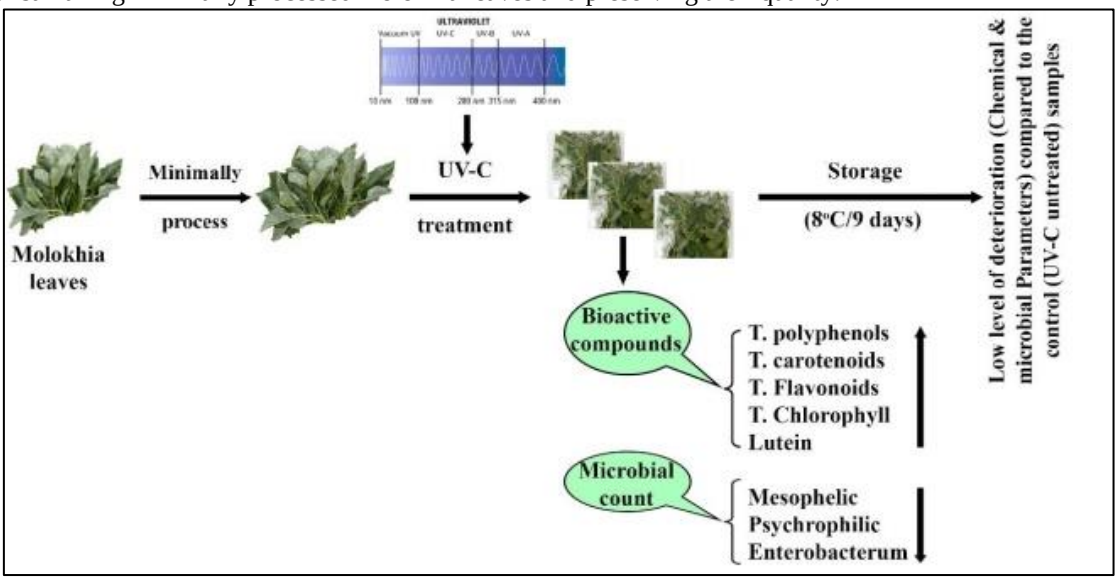
Abstract

Minimally processed or fresh-cut leafy vegetables are very popular for consumption due to their convenience and ready-to-use properties, but they provide an ideal medium for microbial development and degradation of functional properties. Thus, this industry commonly uses chemical agents for disinfection. However, certain problems with the disinfectant agents' usage have been raised and led to the investigation of alternative sanitization treatments. In this respect, ultraviolet (UV-C) radiation could be of interest because no residues are released and the cost is relatively low. The present study was carried out to investigate the influence of UV-C treatment on bioactive compounds content, antioxidant capacity and microbial quality of minimally processed molokhia (*Corchorus olitorius* L.) leaves. Data indicated that UV-C irradiation dose (11.35 kJ m^{-2}) of molokhia leaves led to an initial increasing in bioactive compounds including total polyphenols (41.20%), carotenoids (37.41%), flavonoids (26.43%), chlorophyll (11.93%) and lutein (51.73%) in accompanying with slightly decreasing in antioxidant capacity (-11.78%) on processing day. Also, reduction in mesophilic (-11.50), psychrophilic (-18.90) and enterobacteria (-17.10%) counts was also recorded. The initial bioactive compounds and total antioxidant capacity content decreased as well as the bacterium count gradually increased during the storage period in UV-C treated samples for 9 days at 8 ^{0}C, especially after 6 days. In conclusion, data of the present study should be taken in our consideration when the UV-C radiation used as alternative to chemical agents for sanitizing minimally processed molokhia leaves and preserving their quality.



Keywords: Polyphenols; Carotenoids; Flavonoids; Chlorophyll; Lutein; Mesophilic; Psychrophilic; Enterobacteria.

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1. Introduction

The dietary plant plays an important character in healthcare management system and substantially, in the weight and feed efficiency due to its palatability and it is nutritional endowments [1]**.** For example, vegetables play an important role in human diets, as they support the normal functioning of the different body systems. Among vegetables, the green leafy vegetables occupy an important position. They are rich source of macro and micro nutrients, such as proteins, dietary fibers, minerals, vitamins, as well as bioactive compounds as polyphenols, flavonoids, carotenoids and pigments, which offer many biological roles for health benefits [2]. Fiber has been reported to have beneficial effects on blood cholesterol, help to provide bulk to stool and aid in the movement through the digestive tract, prevention of bowel diseases and improve the glucose tolerance [3]. Phenolic compound, carotenes, flavonoids and vitamins help in the destruction of free radical and other toxic compounds in human body. Vitamins and minerals are essential for life because we need them for good health and for growth including help keep nerve and blood cells healthy, make DNA, prevent diseases (anemia, Alzheimer, neurological and heart diseases, and some cancers), reduce birth defects, can strengthen bones, and prevent cell damage [4-8]. So, World Health Organization (WHO) and Food and Agriculture Organization (FAO) reported that a minimum of 400g of fruits and vegetables per day, excluding starchy tubers, are recommended for the prevention of chronic diseases such as heart disease, cancer, diabetes and obesity, as well as for the prevention and release of several micronutrients [9].

Among the leafy vegetables, molokhia/Jew's mallow (*Corchorus olitorius* L.; Family, *Malvaceae*) comes to form a distinct center among these plants, which are widely cultivated in by international countries. In Egypt, Molokhia is one of the popular summer leafy vegetables, which is grown on an area of 18,053 feddans with a total production of 157,241 tons, then harvested, processed and exported to various countries of the world [10]. It is also widely consumed in those countries as a healthy food prepared in different ways as salads, soups, spices, flavouring agent and granishes, [11]. Molokhia leaves are rich in, fiber, vitamin C, folic acid, β-carotene, iron, potassium, calcium, magnesium and more than 32 vitamins, minerals and trace elements [12]. The plant has strong antioxidant activity with vitamin E equivalent to α-tocopherol. Consumption of molokhia leaves is reported to be demulcent, deobstruent, diuretic, lactagogue, purgative, and tonic as well as a folk remedy for aches and pains, dysentery, enteritis, fever, pectoral pains, and tumors [13]. Also, they are used for cystitis, dysuria, fever, and gonorrhea [14]. Furthermore, it can act anti-inflammatory and has gastroprotective properties [15]. Finally, every 100 grams of Molokhia contains about 26 calories, and thus it is considered low in calories and rich in fiber, which makes it a suitable dish for those looking for low-calorie recipes that help in losing weight and cubing appetite [12].

Minimally processed or fresh-cut vegetables such as Molokhia are very popular for consumption due to their convenience and ready-to-use properties, but they provide an ideal medium for microbial development. Steps such as slicing, shredding and improper refrigeration during shelf-life have been associated with an increase in foodborne pathogens such as *Salmonella*, *Shigella* spp., *Campylobacter*, *Escherichia coli*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Clostridium* spp., *Bacillus cereus* [16]. Also, decrease in bioactive compounds and antioxidant capacities of the plant were reported. The risk involved with the consumption of minimally processed or fresh-cut vegetables could be minimized either reducing or eliminating external surface contamination [17]. Simply washing of fresh vegetables including Molokhia with water may not remove pathogens and other spoilage microorganisms, therefore, other alternative processes were researched [18]. Some traditional disinfectants (chlorine, bromine, iodine, trisodium phosphate, sodium chlorite, sodium hypochlorite, acids, hydrogen peroxide, ozone, permanganate salts etc.) commonly uses in a washing step as sanitization treatment [19]. However, certain by-products of this protocol, such as chloroform, haloacetic acids and other trihalomethanes, are potentially harmful to humans [20]. So, there were other attempts to reduce the number of microorganisms on the surface of vegetables and maintain their functional properties as well as extend the shelf life by modifying the packaging atmosphere, reducing storage temperatures and using edible films. These treatments are selective in reducing the number of pathogens and preserving the functional properties on minimally processed or fresh-cut vegetables [21-23]. Therefore, alternative disinfectant treatment needs to be investigated. Among the possibilities, disinfection by non-ionizing UV-C radiation is interesting, because it 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 subsequently the cost is relatively low [24, 25]. Several studies have demonstrated the efficiency of UV-C radiation on microbial inhibition growth [26-30]. To the best of our knowledge, only a few studies have focused on the effect of UV-C radiation on minimally processed vegetables, while there are no studies specifically on molokhia leaves. Therefore, the purpose of this study was to evaluate the effect of UV-Con the microbial quality of minimally processed molokhia leaves. Changes in bioactive compounds and antioxidant capacity during shelf-life were also studied.

2. Material and Methods

2.1. Materials

Molokhia (*Corchorus olitorius* L.) leaves grown in the open air were manually harvested on 25 May 2022 in local farm of Shebin El-Kom, Minoufiya Governorate, Egypt. Immediately after harvest the leaves were transported to Egyptian Saudi Food Industries Company, Sadat City, Egypt, where they were precooled to 5^0C with forced air. After cooling, the leaves were transported in a portable ice box at 5^0C over 50 km to the lab, and stored at 5^0C. The next day the leaves were minimally processed in a clean room at 8 ^{0}C as described below.

Chemicals: Gallic acid (GA), catechine (CA), α-tocopherol, β-carotene and Butylated hydroxytoluene (BHT), DDPH (2,2-diphenyl-1-picrylhydrazyl), solvents [methanol (MeOH), acetonitrile (ACN) and tetrahydrofuran (THF)]

were purchased from Sigma-Aldrich Chemical Co., St. Louis, MO. Tryptone phosphate, plate count modified agar and violet red bile dextrose agar were obtained from BioScience Diagnostics Pte Ltd, Singapore. All other chemicals (Except as otherwise stated), reagents and solvents were of analytical grade were purchased from El-Ghomhorya Company for Trading Drug, Chemicals and Medical Instruments, Cairo, Egypt.

2.2. Methods

2.2.1. Sample Preparation, Treatments and Storage Conditions

Samples are of separate leaves with petioles. The process of manual sorting of samples is carried out to discard of leaves with defects such as yellowing, decay, cuts and bruising. The following treatments were applied to sound leaves according to washing method and UV-C radiation. Leaves were washed for 2 min in tap water at 4^0C and excess surface water was removed using a handheld salad spinner (Flora-Pyramids Paper Mills S.A.E., 6th October City, Giza, Egypt) for 45 s. This treatment was employed as control since it is commonly followed in minimally processed leafy vegetables. For UV-C treatment samples, this treatment was the same as control samples but leaves were subjected to 11.35 kJ m^{-2} UV-C. For every treatment and immediately after the last step in each case, 120 g of leaves were aseptically placed in polypropylene baskets of approximately 2000 mL (22 cm×20 cm×11 cm) capacity and thermally sealed at the top with a biaxially oriented polypropylene film (BOPP) (INEOS Koeln GmbH, Germany) to constitute a passive modified atmosphere. The oxygen and carbon dioxide permeability of BOPP film at 23 ^{o}C and 0% relative humidity was provided by the supplier as 900 cm^3 m^{-2} day^{-1} atm^{-1} and 1100 cm^3 m^{-2} day^{-1} atm^{-1}, respectively. Six replicates of one basket per processing treatment and storage duration (0, 3, 6 and 9 days) were prepared and stored in a clean cold room (80% relative humidity) at 8 ^{0}C.

2.2.2. UV-C radiation

The UV-C radiation doses applied were selected based on previous studies on fresh leafy vegetables [2, 31]. 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 254 nm) 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. Molokhia leaves samples were then placed over a tray (50 x 40 cm, LxW) for the UV-C treatments. The tray consisted in a polystyrene net that minimized blockage of the UV-C radiation. The UV-C radiation dose selected for these experiments was 11.35 kJ m^{-2}. 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.

2.2.3. Biochemical Analysis

2.2.3.1. Total Phenolics Content

Total phenolics in molokhia leaves were determined using Folin-Ciocalteu reagent such as described by Singleton and Rossi [32]. In brief, 200 mg 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. A 100 µl 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 (UV–visible spectrophotometer, Shimadzu Corporation, Tokyo, Japan). Results are expressed as chlorogenic acid equivalents (CAE) kg^{-1} fresh weight (FW).

2.2.3.2. Total Carotenoids Content

Total carotenoids (TC) content analysis was performed according to Dóka , *et al.* [33]. The total carotenoids content was determined from the absorbance measured at 450 nm (UV–visible spectrophotometer, Shimadzu Corporation, Tokyo, Japan). The samples extracted with ACN: MeOH: THF (50:45:5, by volume) and the standard diluted in the same solvent mixture were measured and the TC content was expressed as β-carotene equivalents (BCE) kg^{-1} fresh weight (FW).

2.2.3.3. Total Flavonoids Content

Total flavonoids contents in molokhia leaves were estimated using colorimetric assay described by Zhishen, *et al.* [34]. To aliquot (0.05 mL) of the extract /standard (catechin, CA), 150 µL of sodium nitrate (5 %) and 2.5 mL of distilled water were added. After 5 min, 0.3 mL of aluminum chloride (10 %) was added. At 6 min, 1 mL of NaOH (0.001 M) and 0.55 mL distilled water was added to the mixture and left at room temperature for 15 min. Absorbance of the mixtures was measured at 510 nm (UV–visible spectrophotometer, Shimadzu Corporation, Tokyo, Japan). Results are expressed as catechin equivalents (CE) kg^{-1} fresh weight (FW).

2.2.3.4. Lutein Content

Chromatographic analysis was performed on an SP Thermo Separation Products Liquid Chromatography (Thermo Separation products, San Jose, CA, USA) with a Consta Metvic 4100 pump, a Spectra Series AS100, Spectra System UV 1000 UV/Visible Spectrophotometer Detector, Spectra System FL 3000 and a PC 1000 system software. The column used (Alltech, Deerfield, IL, USA) was Adsorbosil C_{18} (5 µm, 100 mm × 4.6 mm I.d.). Lutein

was extracted from the molokhia leaves according to the methods described by Bangbang, *et al.* [35] and analyzed by HPLC techniques. Under the chromatographic conditions used in that method (injection volume, 50 µL; flow rate, 1 mL/min; wave length, 450 nm; mobile phases were acetonitrile (phase A), methanol (phase B), and n-hexane (phase C); gradient was programmed as follows: initial conditions of 76% solution A, 21.5% solution B, 2.5% solution C; the proportion of mobile phase A decreased linearly from 76% to 70% over 20-22 min and B decreased linearly from 21.5% to 20% over 20-22 min; in the following 28-30 min, phase A increased to 76% and phase B increased to 21.5%) mean values ±SD of lutein recovery was 86.84±2.64%.

2.2.3.5. Total Chlorophyll Content

Total chlorophyll content was determined directly in molokhia leaves by using portable chlorophyll meter, hand-held chlorophyll analyzer, Model, Chlorophyll meter SPAD-502Plus, Sasha, China. Results are expressed as mg kg^{-1} fresh weight (FW).

2.2.3.6. Total Antioxidant Activity

The antioxidant activity of leaves was evaluated as the free radical-scavenging capacity according to the method described by Brand-Williams, *et al.* [36]. A solution of 0.1 mmol L^{-1} DPPH in methanol was prepared. A 100 µL aliquot of extract (described above) was added to 900 µL of this solution. The mixture was incubated for 30 min at room temperature in darkness. The antioxidant activity was measured by the decrease in absorbance at 515 nm (UV–visible spectrophotometer, Shimadzu Corporation, Tokyo, Japan). The results were expressed as g ascorbate equivalent antioxidant capacity (AEAC) kg^{-1} FW.

2.2.3.7. Microbial Analyses

To determine microbial growth throughout shelf-life, three random samples from each treatment were analyzed on initial, processing Day, and after 3, 6 and 9 days of storage such as described by Difco [37]. In brief, 10 g of molokhia leaves sample was mixed with 90 mL of sterile tryptone phosphate water (pH 7) for 1 min in a sterile stomacher bag then. Serial dilutions were prepared in 9 mL of tryptone phosphate water. From each dilution, 1 mL aliquots were aseptically pipetted for bacterial microflora. The following media and incubation conditions were used: plate count modified agar for mesophilic and psychrophilic aerobic bacteria, incubated at 30 ^{0}C for 48 h and at 7 ^{0}C for 7 days, respectively; violet red bile dextrose agar (pH 7.2) for enterobacteria, incubated at 37 ◦C for 48 h. All microbial counts were expressed as log colony-forming units (cfu) g-1 sample.

2.3. Statistical Analysis

All analyses were performed three times, and the results were expressed as a mean ± standard deviation (SD). Data were subjected to analysis of variance (ANOVA) using MINITAB program (Minitab Inc., State College, PA). Correlation analysis was performed by using Microsoft Excel program (Excel 2013, v15.0).

3. Results and Discussion

3.1. Total Polyphenols, Carotenoids and Flavonoids of Minimally Processed Molokhia after UV-C Radiation Treatment and MAP Storage

The total polyphenols, carotenoids and flavonoids content on fresh molokhia samples were recorded 426.63 mg CAE kg^{-1}, 42.65 mg BCE kg^{-1} and 46.68 mg CE kg^{-1} in fresh weigh basis, respectively (Table 1). Just after processing, the control samples were recorded slightly and no significantly decreasing for the all previous compounds by the rate of -0.93, -3.66 and -4.93 %, respectively. While treatment of the samples with UV-C led to a significant ($p \leq 0.05$) increase in total polyphenols, carotenoids and flavonoids content with rates of 41.20, 37.41 and 26.43%, respectively (Figure 1). On the other hand, storage at 8 ^{0}C for 9 days led to a gradual decrease in all those bioactive compounds as a general trend with respect to the initial content, especially after 6 days. Such data are in accordance with that observed by Ahmed [2], Essa [31], Caro, *et al.* [38] and Goh, *et al.* [39] who reported reduction in total phenolics, carotenoids and flavonoids in UV-C treated leafy vegetables and pineapple juice after storage. Such studies with the others have been suggested that the enzyme of phenylalanine ammonia-lyase activity in minimally processed leafy vegetables is stimulated, which controls the metabolism of phenols, as well as polyphenol oxidase and peroxidase, and these results in the synthesis of brown pigments from the oxidation of phenols [40, 41]. Also, Kulkarni and Aradhya [42] noticed that a decrease in phenolic compounds with advanced ripening banana, guava and pomegranate. Furthermore, Perkins-Veazie, *et al.* [43] found that no clear UV-C pre-treatment effect on the total phenolic content of blueberries after 7 days at 5 ^{0}C plus 2 days at 20 ^{0}C. On the other side, the opposite direction was recorded for flavonoids in UV-C pre-treatment fruit juices including starfruit, citrus and pineapple [39, 44, 45]. Also, Erkan, *et al.* [46] reported that an increasing in the phenolic content of strawberries as a result of UV-C pre-treatment. Although the reason(s) for the difference in the results in this study compared to those in some other studies has not been determined, the possible reason may be due to the dose of ultraviolet radiation applied and the exposure conditions (temperature, storage period, etc.) were different from the authors discussed above. For total carotenoids, significant ($p \leq 0.05$) reduction in pineapple juice after UV-pretreatment was reported which can be explained by the light sensitive nature of such compounds [39]. This might be due to the nature of double bonds in carotenoids which easily absorbed UV and then undergo the process of UV photolysis. In this context, Sofia et al., [47] studied the photochemical (UV–vis/H_2O_2) degradation of carotenoids, kinetics and molecular end products and found that all such compounds degraded under the combined influence of photolysis and ·OH scavenging, with

fucoxanthin exhibiting the fastest degradation kinetics and *meso*-zeaxanthin the slowest. The major degradation products were apo-aldehydes and apo-ketones, with the latter tending to accumulate, but epoxidation of the carotenoids also took place, and longer irradiation times resulted in lower molecular weight products. Also, Goh, *et al.* [39] reported that the greater reduction of total carotenoids in UV-treated pineapple juice might be due to the oxidation enzyme which cannot be inactivated by UV-C pre-treatment. Recently, Aslam, *et al.* [48] illustrated the mechanism of carotenoid oxidation and their initial products which reported that carotenoids are sensitive to thermal, light/UV and oxidation. With this context, Cisneros-Zevallos [22] proposed that the oxidative stress induced by UV-C radiation would affect the secondary metabolism of fresh product and could increase the synthesis of phytochemicals with nutraceutical activity.

In general, several studies reported that polyphenols, carotenoids and flavonoids are found throughout the plant kingdom including those selected in the present study [49-53]. Also, Velioglu, *et al.* [54] determined the total phenolics in several plant parts and found that the total phenolics content varied from 169 to 10548 mg.100 g^{-1} of dry product. Such studies indicated that big differentiations have been recorded amongst different vegetables plant parts which due to the type, variety and color of vegetable fruits. On the other side, many studies reported that such bioactive compounds exhibited that their different biological activities including antioxidant, scavenging and antimicrobial activities [49, 55]. Such biological activities play important roles in prevention/treatment of different diseases including diabetes, obesity, liver disorders and cardiovascular diseases [39, 56-61]. Also, flavonoids are a group of bioactive compounds that are extensively found in foodstuffs of plant origin which their regular consumption is associated with reduced risk of a number of chronic diseases, including cancer, cardiovascular disease and neurodegenerative disorders [62]. Their actions at the molecular level include antioxidant effects, as well the ability to modulate several key enzymatic pathways. Additionally, carotenoids are natural lipid-soluble (lipophilic) pigments synthesized by plants which play an important role in the process of photosynthesis by protecting the chlorophyll against near-UV or visible light and also stabilizes the cell membrane by binding with free radicals [63]. They have been proven health-promoting effects like role as vitamin A precursor. Also, carotenoids showed potent therapeutic potential like antioxidant, anti-inflammatory, antitumour, anticardiac, and anti-ageing activities [49, 55, 64, 65]. Keeping in view its potential properties and diverse functions especially in food and pharmaceutical industries; many researchers focused on processing of carotenoids in vegetables and fruits. Others do all the best to serve those compounds from degradation in their different sources.

3.2. Total Chlorophylls and Lutein of Minimally Processed Molokhia after UV-C Radiation Treatment and MAP Storage

Data in Table 2 and fig.2 shows the total chlorophylls and lutein of minimally processed molokhia after UV-C radiation treatment and MAP storage at 8 ^{0}C for up to 9 days. From such date it could be noticed that the total chlorophylls and lutein content on fresh molokhia samples were recorded 2057.56 ± 98.04 and 76.33 ± 5.87 mg kg^{-1} in fresh weigh basis, respectively. Just after processing, the control samples were recorded slightly and no significantly decreasing for the all previous compounds by the rate of -0.93, -3.66 and -4.93 %, respectively. While treatment of the samples with UV-C led to a significant ($p \leq 0.05$) increase in total chlorophylls and lutein content with rates of 11.93 and 51.37%, respectively. On the other hand, storage at 8 ^{0}C for 9 days led to a gradual decrease in all those compounds as a general trend with respect to the initial content, especially after 6 days.

Chlorophyll refers to the phytochemical that gives plants their green color and pigmentation. This compound is responsible for absorbing solar energy to facilitate photosynthesis, a process in which plants convert energy from sunlight into sugars. Such as reviewed by Artes, *et al.* [66] when the chloroplast is disorganized during leaf senescence, chlorophyll become very susceptible to structural modifications, which occur as a result of almost any postharvest operation, leading to the formation of several derivatives. The main changes include the formation of pheophythin (by interchange of Mg^{++} with protons from the medium produces a severe colour change from bright green to olive-brown) and chlorophyllide (by hydrolysis of the phytol ester caused by chlorophyllase). With this context, Abe and Watada [21] reported that ethylene produced during minimal processing of leafy vegetables accelerates the loss of chlorophyll. Chlorophyll provides nutritional benefits to the body and helps keep the healthy including right bones, strong muscles, maintaining normal blood pressure and needs for the blood to clot properly [67, 68].

Lutein is an oxygenated carotenoid found naturally in high quantities in green leafy vegetables such as spinach, kale and yellow carrots [69]. It is synthesized only by plants and like other xanthophylls and animals obtain lutein by ingesting plants. In human, it is also naturally present in a concentrated area of the macula of retina, a small area of the retina responsible for central vision, and in macular pigment [69]. Therefore, many studies have shown that higher dietary intake of lutein is associated with a reduced risk of age-related cataract, especially nuclear cataract [70, 71]. A lower risk of coronary heart disease, stroke, and metabolic syndrome, possibly through less atherosclerosis and lower inflammatory activity as dietary intake of lutein were also recorded. Such physiological roles of lutein due to its acts as an antioxidant, protecting cells against the damaging effects of free radicals such as inhibit peroxidation of membrane phospholipids and reduce lipofuscin formation [69, 72]. Therefore, the results of the current study, the use of UV-C in post-harvest treatments in leafy vegetables to maintain the concentration levels of chlorophyll and lutein and protection from cracking, is very important from a nutritional and health point of view.

3.3. Antioxidant Capacity of Minimally Processed Molokhia after UV-C Radiation Treatment and MAP Storage

Antioxidant capacity (AC) of minimally processed molokhia after UV-C pre-treatment and MAP storage at 8 ^{0}C for up to 9 days were shown in Table 3 and Fig.3. Such data indicated that the AC of fresh molokhia sample was recorded 9.42 ± 0.88 g AEAC kg^{-1} in fresh weigh basis. Just after processing, the control samples were recorded slightly and no significantly decreasing (-0.42%) and the UV-C pretreated samples recorded significantly ($p\leq0.05$) decreasing (-11.78%) in AC. On the other hand, storage at 8 ^{0}C for 9 days led to a gradual decrease in antioxidant activity as a general trend with respect to the initial content, especially after 6 days. Such data are in accordance with that observed by Ahmed [2] and Essa [31] who reported decreasing in AC in UV-C treated leafy vegetables and after storage at 4 ^{0}C for 12 days. Also, Vicente, *et al.* [73] reported that after 18 days storage at 10 ^{0}C the AC decreased in all UV-C treated and untreated red peppers. Furthermore, Costa, *et al.* [74] noticed that the AC of UV-C treated broccoli florets stilled constant during 6 days at 20 ^{0}C with respect to control samples, where a decrease occurred after 4 days. The present data with the others reported that such decreases in AC could be explained by minimally processed tissues being primarily submitted to oxidative stress, perhaps causing cells membrane damage and changing the composition/profile and content of antioxidant compounds, leading to changes in the AC of the tissue, which also can be due to UV-C damage [2, 31, 73]. In general, Velioglu, *et al.* [54] reported a good relationship between the antioxidant activities and total phenolics (antioxidants) of 28 plant products including vegetables. The antioxidants in plants can act by various mechanisms, such as inhibiting the generation and/or scavenging of reactive species, raising the level of endogenous antioxidant defenses by up-regulating gene expression, inhibiting the lipid peroxidation [49, 75-77]. Molokhia is one of the leafy vegetables with higher antioxidant capacity due to its high total phenolics, carotenoids, flavonoids, pigments etc. content, suggesting that its regular consumption may be of interest for disease prevention.

3.4. Microbial Analysis of Minimally Processed Molokhia after UV-C Radiation Treatment and MAP Storage

The microbial load including mesophilic, psychrophilic and enterobacteria, on fresh molokhia samples were recorded 5.2, 5.3 and 4.1 log cfu g^{-1} in fresh weigh basis, respectively (Table 3). After processing, the control samples were recorded slightly and no significantly increasing for the all microbes by the rate of 3.8, 3.8 and 2.4%, respectively. While treatment of the samples with UV-C led to a significant ($p\leq0.05$) decrease in mesophilic, psychrophilic and enterobacteria microbes with rates of -11.5, -18.9 and -17.1%, respectively (Fig. 4). On the other hand, storage at 8 ^{0}C for 9 days led to a gradual significant ($p\leq0.05$) increase in all those microbes as a general trend with respect to the initial content, especially after 6 days. Such data are in accordance with that observed by Ahmed [2] and Essa [31] who reported reduction in total aerobic bacterial growth in UV-C treated leafy vegetables. Also, Nguyen-The and Carlin [78] have reported similar mesophilic bacteria counts in minimally processed vegetables, ranging from 3 to 6 log cfu g^{-1} just after processing and from 3 to 9 log cfu g^{-1} after commercial cold storage. In similar study, Allende, *et al.* [79] reported that there were different growth behaviors among bacterial groups but they all responded similarly towards UV- radiation treatment i.e. UV- 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}). Furthermore, Allende and Artes [80, 81] found similar results with the present study when minimally processed lollo rosso and red oak leaf lettuces were pretreated with 0.4, 0.81, 2.44, 4.07, and 8.14 kJm^{-2} UV-C doses on only one side of the tissue. The effect of UV-treatment as follow could be interpreted as follow:, UV waves penetrate 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/UVTechnology/ UVScience.aspx]. Also, Artes, *et al.* [24] reported that UV-C radiation inhibit the microbial growth through inducing the formation of pyrimidine dimers that distort the DNA helix and block microbial cell replication. The cells unable to repair their radiation-damaged DNA die. Moreover, Bintsis, *et al.* [27] reviewed that UV light acts indirectly against microorganisms by stimulating defense mechanisms in the processed products, which delays decomposition and aging. Data of the current study with the others proved that the effectiveness of UV-C depends on the treatment temperature, the applied irradiation dose, which is determined by the structure, surface and topography of the treated product [27, 28]. Therefore, the increase in the count of tested microbes for samples treated with UV-C during the storage period may be explained by higher doses of UV-C would help the growth of some bacteria, probably owing to an increase in superficial damage on molokhia leaves that makes nutrients available for microbial growth. In similar study, Nigro, *et al.* [82] reported that UV-C can change the cell permeability in leafy vegetables, increasing the leakage of electrolytes, amino acids and carbohydrates subsequently can stimulate bacterial growth and lead to shortened shelf-life of minimally processed products.

3.5. Correlation Analysis between Antioxidant Capacity (AC), Bioactive Compounds and Microbiological Parameters in UV-C Pretreated Molokhia and MAP Storage.

Correlation analysis between AC, bioactive compounds and microbiological parameters in UV-C pretreated molokhia samples was tabulated in Table 5. When all UV-C treated molokhia samples were included in the statistical analysis, there was a slightly negative non-significant relationship between total phenolics (r^2 = -0.1192), total carotenoids (r^2 = - 0.1509), total flavonoids (r^2 = -0.1765), total chlorophyll (r^2 = -0.1224), lutein (r^2 = -0.1365) and AC. Also, there was a negative non-significant relationship between all tested microbiological parameters

including mesophilic (r^2 = -0.2745), psychrophilic (r^2 = -0.3321), enterobacteria (r^2 = -0.3158) and AC in the same molokhia samples. These correlations confirmed that those bioactive compounds (total phenolics, total carotenoids, total flavonoids, total chlorophyll and lutein) partially minimally responsible for the AC of the UV-C treated molokhia samples. Also, these data indicates that many other bioactive compoundsand nutrients beside those compounds such vitamins, phytochemicals, minerals etc., probably contribute in the AC of UV-C treated molokhia samples. Such data confirmed by Cisneros-Zevallos [22] who found that oxidative stress induced by UV-C radiation would affect the secondary metabolism of fresh molokhia samples and could increase the synthesis of phytochemicals with nutraceutical activity. Also, storage molokhia samples treated with UV-C radiation at 8 ^{0}C for a period of 9 days led to a significant ($p \leq 0.05$) decrease in biologically active compounds, and a significant ($p \leq 0.05$) increase in the microbial count, which was correlated with a decrease in antioxidant capacity. With this context, Nigro, *et al.* [82] found that UV-C treated can change the cell permeability in molokhia samples, increasing the leakage of nutrients and phytochemicals, which can stimulate bacterial growth and decrease the AC. This information was confirmed by several authors who reported that antioxidant properties of plant parts have 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 [2, 49, 83]. On the other side, positive significant relationships between total phenolics (r^2 = 0.5207), total carotenoids (r^2 = 0.7715), total flavonoids (r^2 = 0.5742), lutein (r^2 = 0.6159) and total chlorophyll were recorded in all UV-C treated molokhia samples. With this context, the positive correlation between the contents of chlorophyll and carotenoids has been also reported for other leafy species, like as spinach [2], Essa [31], Kopsell, *et al.* [84] and Ihl, *et al.* [85].

4. Conclusion

The fresh-cut vegetable industry commonly uses disinfectant agents as sanitization treatment but certain by-products of this procedure are potentially harmful to humans. Therefore, the present study used UV-C irradiation as alternative agents because no residues are released and the cost is relatively low. Data indicated that UV-C irradiation led to an initial increasing in bioactive compounds including total polyphenols, carotenoids, flavonoids, chlorophyll and lutein in accompanying with antioxidant capacity on processing day. Also, reduction in mesophilic, psychrophilic and enterobacteria counts was also recorded. The initial bioactive compounds and total antioxidant capacity content decreased as well as the bacterium count gradually during the storage period in UV-C treated samples for 9 days at 8 ^{0}C, especially after 6 days. Such present data should be taken in our consideration when the UV-C radiation used as alternative to chemical agents for sanitizing minimally processed molokhia leaves and preserving their quality.

Ethical Approval

All experiments of the study were ethically approved by the Scientific Research Ethics Committee, Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval no. 15- SREC- 12-2021).

Authors' Contributions

Prof. Dr. Yousif Elhassaneen, proposed the research problem, reviewed the experimental design, supervised the practical experiments, assisted in discussing and interpreting the results, and assisted in writing and final reviewing the original draft. Associate Prof. Dr. Amal Nasef, assisted in experimental design, practical experiments, discussion and interpretation of the results, and contributed in writing the original draft. Researcher Areeg Nour El-Deen, collected the review of literatures, conducted the practical experiments, arranged and tabulated the results, and wrote the paper draft**.**

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Conflicts of Interest

No potential conflict of interest was reported by the authors.

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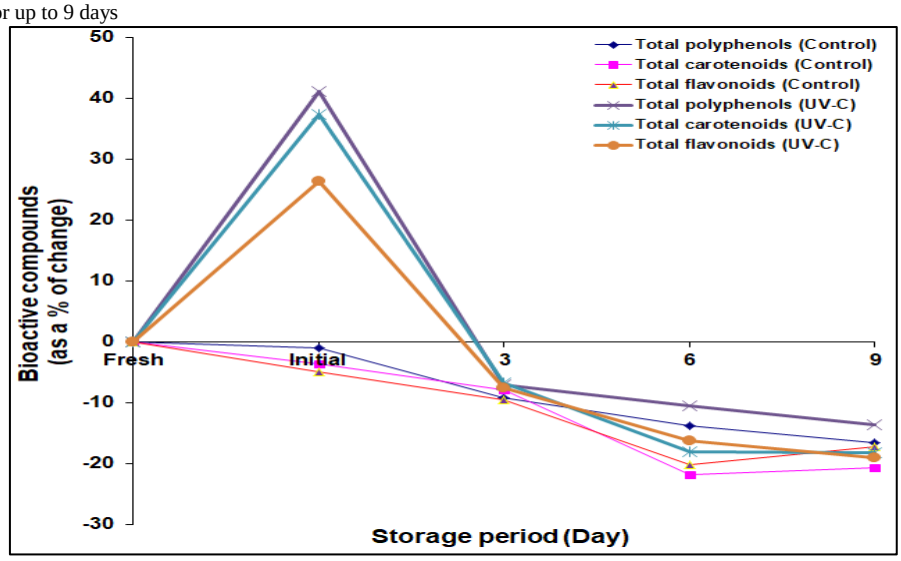
Table-1. Bioactive compounds (total polyphenols, carotenoids and flavonoids) of minimally processed molokhia after UV-C radiation treatment and MAP storage at 8 ^{0}C for up to 9 days*

Sample	Fresh	Initial (on processing day)	Storage period (day)		
			3	6	9
Total polyphenols (mg CAE kg^{-1} FW)					
Molokhia (control)	426.63 ± 29.17^a	422.65 ± 29.32 ^{b}	383.89 ± 19.10 ^{b}	364.67 ± 45.83 ^{b}	352.65 ± 19.71 ^{b}
Molokhia (UV-C)	426.63 ± 29.17^a	602.40 ± 43.21 ^{a}	559.89 ± 24.11 ^{a}	539.64 ± 39.62 ^{a}	520.87 ± 33.53 ^{a}
Total carotenoids (mg BCE kg-1 FW)					
Molokhia (control)	42.65 ± 3.61 ^{a}	41.09 ± 2.49 ^{b}	37.84 ± 1.90 ^{b}	32.14 ± 3.01 ^{b}	32.60 ± 1.09 ^{b}
Molokhia (UV-C)	42.65 ± 3.61 ^{a}	58.61 ± 6.17 ^{a}	54.67 ± 3.51 ^{a}	48.01 ± 2.96 ^{a}	47.96 ± 4.23 ^{a}
Total flavonoids (mg CE .kg-1 FW)					
Molokhia (control)	46.68 ± 2.78 ^{a}	44.38 ± 3.01 ^{b}	40.15 ± 4.04 ^{b}	35.45 ± 3.43 ^{b}	36.76 ± 2.42 ^{b}
Molokhia (UV-C)	46.68 ± 2.78 ^{a}	59.02 ± 1.99 ^{a}	54.61 ± 5.87 ^{a}	49.40 ± 1.87 ^{a}	47.83 ± 4.03 ^{a}

Each value represents mean of three replicates ±SD. Means in the same column with different superscript letters were significantly different at $P \leq 0.05$. MAP, modified atmosphere packaging, CAE, chlorogenic acid equivalents, BCE, β-carotene equivalents, CE, catechin equivalents , FW, fresh weight.

Fig-1. Bioactive compounds (total polyphenols, carotenoids and flavonoids) of minimally processed molokhia after UV-C radiation treatment and MAP storage at 8 ^{0}C for up to 9 days



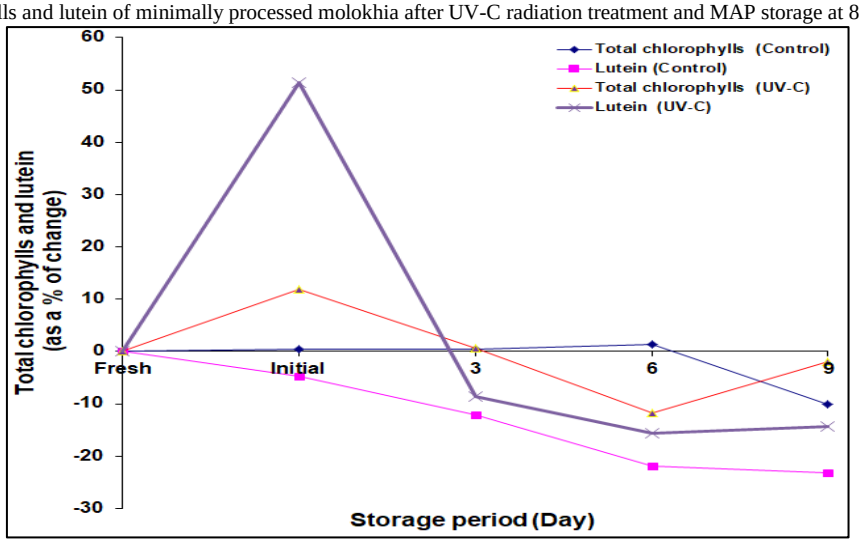
Each value represents mean of three replicates. MAP, modified atmosphere packaging

Table-2. Total chlorophylls and lutein of minimally processed molokhia after UV-C radiation treatment and MAP storage at 8 ^{0}C for up to 9 days*

Sample	Fresh	Initial (on processing day)	Storage period (day)		
			3	6	9
Total chlorophylls (mg kg^{-1} FW)					
Molokhia (control)	2057.56 ± 98.04^a	2065.43 ± 69.31^b	2075.67 ± 64.32^b	2093.67 ± 71.18^a	1856.46 ± 49.42^b
Molokhia (UV-C)	2057.56 ± 98.04^a	2303.03 ± 83.21^a	2317.89 ± 86.27^a	2034.03 ± 97.19^b	2257.20 ± 92.53^a
Lutein (mg kg^{-1} FW)					
Molokhia (control)	76.33 ± 5.87^a	72.72 ± 6.02^b	63.86 ± 4.09^b	56.78 ± 7.01^b	55.89 ± 3.92^b
Molokhia (UV-C)	76.33 ± 5.87^a	115.54 ± 8.02^a	105.63 ± 5.05^a	97.53 ± 3.98^a	99.05 ± 6.87^a

Each value represents mean of three replicates ±SD. Means in the same column with different superscript letters were significantly different at $P \leq 0.05$. MAP, modified atmosphere packaging, CAE, chlorogenic acid equivalents, BCE, β-carotene equivalents, CE, catechin equivalents , FW, fresh weight.

Fig-2. Total chlorophylls and lutein of minimally processed molokhia after UV-C radiation treatment and MAP storage at 8 ^{0}C for up to 9 days



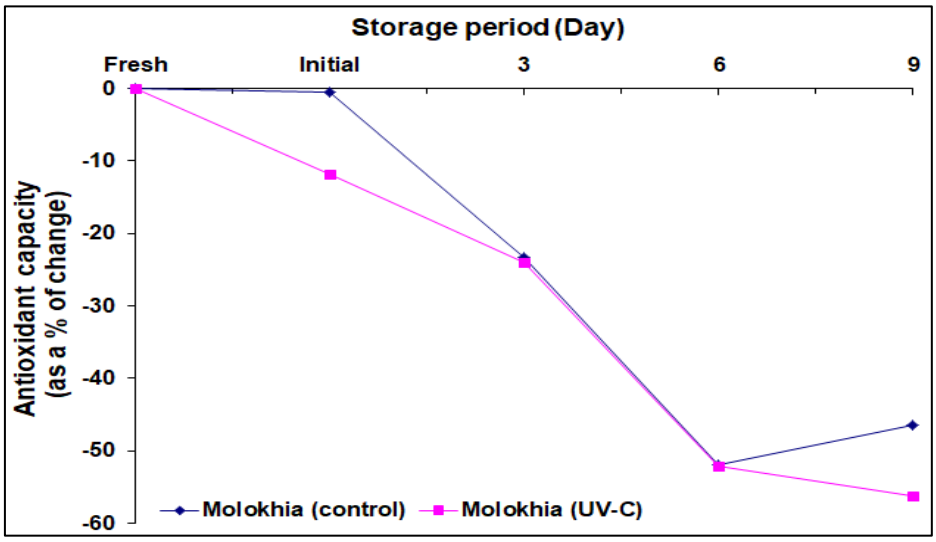
Each value represents mean of three replicates. MAP, modified atmosphere packaging.

Table-3. Antioxidant capacity (g AEAC kg^{-1} FW) of minimally processed molokhia after UV-C radiation treatment and MAP storage at 8 ^{0}C for up to 9 days*

Sample	Fresh	Initial (on processing day)	Storage period (day)		
			3	6	9
Molokhia (control)	9.42 ± 0.88^a	9.38 ± 0.94^a	7.19 ± 0.31^a	4.51 ± 0.57^a	5.02 ± 0.76^a
Molokhia (UV-C)	9.42 ± 0.88^a	8.31 ± 0.97^b	6.31 ± 0.48^a	3.98 ± 0.62^a	3.64 ± 0.55^b

Each value represents mean of three replicates ±SD. Means in the same column with different superscript letters were significantly different at $P \leq 0.05$. MAP, modified atmosphere packaging, AEAC, ascorbate equivalent antioxidant capacity, FW, fresh weight.

Fig-3. Antioxidant capacity (as a % of change) of minimally processed molokhia after UV-C radiation treatment and MAP storage at 8 ^{0}C for up to 9 days



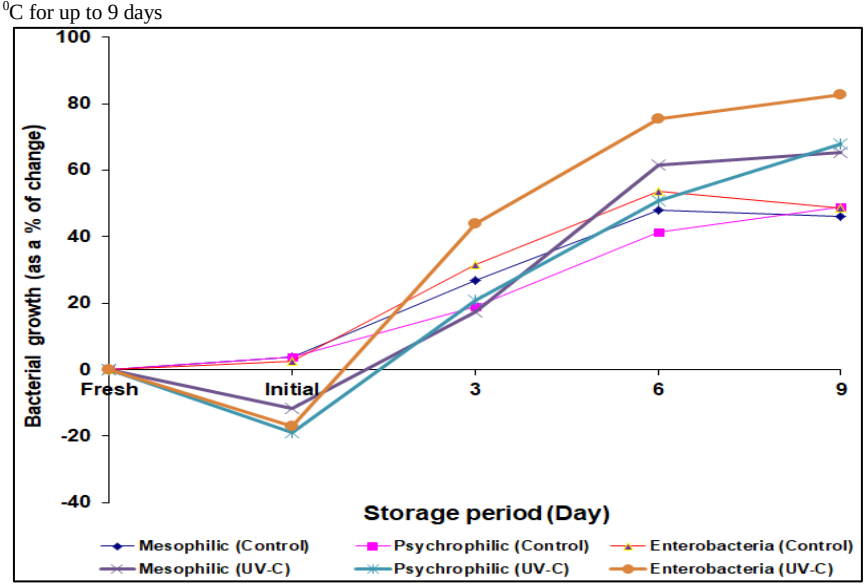
Each value represents mean of three replicates. MAP, modified atmosphere packaging.

Table-4. Microbial analysis (mesophilic, psychrophilic and enterobacteria counts) of minimally processed molokhia after UV-C radiation treatment and MAP storage at 8 ^{0}C for up to 9 days*

Sample	Fresh	Initial (on processing day)	Storage period (day)		
			3	6	9
Mesophilic bacteria count (log cfu g-1)					
Molokhia (control)	5.2 ± 0.48 ^{a}	5.4 ± 0.69 ^{a}	6.6 ± 0.56 ^{a}	7.7 ± 0.93 ^{a}	7.6 ± 0.45 ^{b}
Molokhia (UV-C)	5.2 ± 0.48 ^{a}	4.6 ± 0.27 ^{b}	6.1 ± 0.54 ^{a}	8.4 ± 0.76 ^{a}	8.6 ± 0.63 ^{a}
Psychrophilic bacteria count (log cfu g-1)					
Molokhia (control)	5.3 ± 0.77 ^{a}	5.5 ± 0.87 ^{a}	6.3 ± 0.77 ^{a}	7.5 ± 0.74 ^{a}	7.9 ± 0.57 ^{b}
Molokhia (UV-C)	5.3 ± 0.77 ^{a}	4.3 ± 0.31 ^{b}	6.4 ± 0.47 ^{a}	8.0 ± 0.57 ^{a}	8.9 ± 0.20 ^{a}
Enterobacteria count (log cfu g-1)					
Molokhia (control)	4.1 ± 0.20 ^{a}	4.2 ± 0.67 ^{a}	5.4 ± 0.67 ^{a}	6.3 ± 0.65 ^{b}	6.1 ± 0.43 ^{b}
Molokhia (UV-C)	4.1 ± 0.20 ^{a}	3.4 ± 0.50 ^{b}	5.9 ± 0.65 ^{a}	7.2 ± 0.74 ^{a}	7.5 ± 0.55 ^{a}

Each value represents mean of three replicates ±SD. Means in the same column with different superscript letters were significantly different at $P \leq 0.05$.MAP, modified atmosphere packaging.

Fig-4. Microbial analysis (mesophilic, psychrophilic and enterobacteria counts) of minimally processed molokhia after UV-C radiation treatment and MAP storage at 8 ^{0}C for up to 9 days



Each value represents mean of three replicates. MAP, modified atmosphere packaging

Table-5. Correlation analysis between antioxidant capacity (AC), bioactive compounds and microbiological parameters in UV-C pretreated molokhia and MAP storage

Correlation	r^2	Correlation	r^2
AC vs. total phenolics	- 0.1192	AC vs. psychrophilic	- 0.3321
AC vs. carotenoids	- 0.1509	AC vs. enterobacteria	- 0.3158
AC vs. flavonoids	- 0.1765	Chlorophyll vs. carotenoids	0.7715**
AC vs. chlorophyll	- 0.1224	Chlorophyll vs. total phenolics	0.5207*
AC vs. lutein	- 0.1365	Chlorophyll vs. flavonoids	0.5742*
AC vs. mesophilic	- 0.2745	Chlorophyll vs. lutein	0.6159*

***** P≤ 0.05; ** P≤ 0.01