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Enhancement of polyphenols and antioxidant and inhibitory properties of *Corchorus olitorius* and *Amaranthus hybridus* leaves by the Ultraviolet-C treatment

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ABSTRACT

The effect of the postharvest (Ultraviolet-C) treatment on total phenolics, total flavonoids, total antioxidant capacity, 1,1-diphenyl-2-picrylhydrazyl scavenging activity (DPPH), and reducing power of *Corchorus olitorius* (CO) and *Amaranthus hybridus* (AH) was investigated. Their abilities to inhibit induced lipid peroxidation, α-amylase, and α-glucosidase after the ultraviolet-C treatment were also evaluated. The leaves were exposed to ultraviolet-C radiation for 10 min. Total phenolics, total flavonoids, total antioxidant capacity, DPPH scavenging ability, and reducing power were significantly ($p < 0.05$) increased after the ultraviolet-C treatment. Also, their abilities to inhibit SNP-induced lipid peroxidation, α-amylase and α-glucosidase were also enhanced significantly ($p < 0.05$). Therefore, apart from using ultraviolet-C radiation in water purification, it can be used to enhance health-promoting components in fruit and vegetables.

Introduction

In recent years, regular consumption of functional foods, especially vegetables and fruit, has been encouraged due to their role in human health and disease prevention. This is due to the presence of health-promoting polyphenols and antioxidants they possessed (Gardner et al., 2000). High intake of these nutritional and health-promoting components of food has been associated with a low incidence of cancers, arthritis, and other terminal diseases (Leong and Shui, 2002). However, these health-promoting components of vegetables and fruit are susceptible to depletion or degradation due to poor handling, microbial action, irradiation, and several environmental stresses. These conditions can reduce the overall nutritional quality of fresh vegetables and fruit, including their antioxidant and health functionality after harvest. Numerous postharvest treatments have been used to preserve or enhance the quality of vegetables and fruit and an important example is the ultraviolet radiation treatment (Adetuyi et al., 2020; Olaiya et al., 2016a).

Corchorus olitorius (CO) and *Amaranthus hybridus* (AH) are examples of functional vegetables that are commonly eaten in the tropics. AH belongs to the family Amaranthus and the leaves are eaten as vegetables. In some parts of Nigeria, its leaves combined with some condiments are used to prepare soup (Oke, 1983). The aqueous extract of leaves of AH has shown to have antimicrobial (Maiyo et al., 2010) and hepatoprotective abilities (Adewale and Olorunju, 2013). *Corchorus olitorius* belongs to the family Malvaceae. It is native to the tropical and sub-tropical region of the world. Some biological activities of different parts of CO have been reported (Khan et al., 2006; Oboh et al., 2009).

Ultraviolet radiation (UV) is one of the portions of the electromagnetic spectrum and it has three different types, namely, UV-A, UV-B, and UV-C. The UV-C range (200–280 nm) is called the germicidal range, because of its ability to effectively inactivate microorganisms such as bacteria and viruses (Ribeiro et al., 2012). Also, low doses of UV-C irradiation have been proven to stimulate beneficial reactions in biological organs, a phenomenon known as hormesis (Shama, 2007). However, advances in ultraviolet (UV) light technology have further demonstrated the importance of the UV treatment in food processing by extension of shelf-life of fresh fruit and

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vegetables (Ribeiro et al., 2012), enhancement of bioactive compounds, and induction of defence mechanisms via the antioxidant capacity of fruit and vegetables (Erkan et al., 2008; Alotman et al., 2009; Olaiya et al., 2016a; Choi et al., 2015). An important thing about this non-thermal physical processing method is that it does not alter the organoleptic properties, it is cost-effective, and it does not form any undesirable chemical and waste effluents (Koutchma et al., 2018). Recent studies using different fruit and vegetables have proven the reliability of UV light as a good hormetic agent (Alothman et al., 2009; Olaiya et al., 2016a; Adetuyi et al., 2020). Therefore, the objective of the study is to evaluate the effect of the UV-C postharvest treatment on bioactive components and the antioxidant and health functionality of *Corchorus olitorius* and *Amaranthus hybridus.*

Materials and methods

Plant Materials

Corchorus olitorius and *Amaranthus hybridus* used in the study were acquired from the local market (Bodija), Ibadan, Nigeria and identified in the Department of Botany, University of Ibadan. The vegetables were of eating quality, with no blemishes, damage, or infections. They were carefully selected to be identical in terms of shape and size. The leaves were sorted out to eliminate damaged and shrivelled leaves. The selected leaves were washed with distilled water to remove dirt. Samples were divided into two, one was irradiated with ultraviolet-C for 10 min and the other was not irradiated. After treatment, the vegetables were air-dried and milled with an electric blender into powdery form.

Ultraviolet C (UV-C) radiation treatment

The UV-C radiation treatment was done according to the method described by (Alothman et al., 2009). The UV-C lamps were turned on for 5 min prior to use for stabilization. Three replicates of vegetable samples were placed on a polypropylene tray at the lower surface of the radiation chamber. Radiation was done in the dark at room temperature, with the samples receiving a UV radiation dose of 2.217 J/m^2 on average. The wavelength of the used UV-C lamp was 210 nm and the tray was rotated four times during the treatment to ensure uniform exposure.

Extraction of Sample

Twenty grams (20 g) of the powered vegetables were extracted with 200 ml of distilled water. After 24 hr, the extract was filtered using a muslin cloth and the filtrate was concentrated using a rotary evaporator. The extracts were kept in the refrigerator until use.

Total phenolic determination

The total phenolic content (TPC) of irradiated and un-irradiated samples was determined using the method of (Kim et al., 2003) as modified by (Karigidi et al., 2018). After the appropriate reconstitution of the sample, 1 ml of the sample (1 mg/ml) was mixed with 1 ml (10%) of the Folin-Ciocalteu phenol reagent. After 5 min, 5 ml of 7% Na_2CO_3 was added, immediately followed by the addition of 5 ml of distilled water, and the reaction mixture was shaken together. The mixture was kept in the dark for 90 min at room temperature. The absorbance was measured at 750 nm and the TPC was calculated from the gallic acid calibration curve and expressed as gallic acid equivalent (mg GAE/100g).

Total flavonoid determination

The total flavonoid content (TFC) of the irradiated and un-irradiated samples was determined using the method of (Park et al., 2008). After appropriate reconstitution of the sample, 0.3 ml (1 mg/ml) of the sample was mixed with 3.4 ml (30%) of methanol, 0.15 ml (0.5 M) of $NaNO_2$ and 0.15 ml (0.3 M) of $AlCl_36H_20$, consecutively. After 5 min, 1 ml of 1 M NaOH was added and mixed well. The absorbance was measured at 506 nm and the flavonoid content was evaluated from the quercetin calibration curve and expressed as quercetin equivalent (mg QUE/100g).

Total antioxidant capacity (Phosphomolybdate assay)

The total antioxidant capacity (TAC) of irradiated and un-irradiated samples was determined using the method of Prieto et al. (1999). After the appropriate reconstitution of the sample, 0.1 ml (1 mg/ml) of the sample solution was mixed with 1.0 ml of the phosphomolybdum reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The sample was capped and incubated in boiling water at 95 °C for 90 min. The sample was allowed to cool at room temperature and the absorbance taken at 695 nm against a sample blank and the total antioxidant capacity was calculated from the ascorbic acid calibration curve and expressed as ascorbic acid equivalent (mg AAE/100g).

DPPH scavenging activity determination

DPPH radical scavenging activity of irradiated and un-irradiated samples was determined using the method of Gyamfi et al. (1999) using minor modification. After appropriate dilution, 1 ml (0.4-1.0 mg/ml) of sample was

added to 4 ml of the DPPH solution (30 mg/l) prepared in methanol. The samples were shaken and allowed to stand in the dark for 30 min. The absorbance was measured at 520 nm. The inhibition percentage was calculated as:

Inhibition percentage of DPPH = {(Abs control- Abs Sample)/ (Abs Control)} × 100

Amylase inhibition assay

The amylase inhibition assay of the irradiated and un-irradiated samples was determined using the method of Worthington (1993). After appropriate dilution, 400 µl (0.1-0.4 mg/ml) of sample and 500 µl (0.02 M) of sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 0.5 mg/ml α-amylase solution were incubated at room temperature for 10 min. Thereafter, 500µl (1%) of a starch solution prepared with 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added. The reaction mixtures were then incubated at 25 °C for 10 min. The reaction was halted with 1.0 ml (96 mM) of dinitrosalicylic acid. The test tubes were then incubated in a boiling water bath for 5 min and allowed to cool. The absorbance was read at 540 nm. The percentage of inhibition was calculated:

Inhibition percentage = {(Abs Control- Abs Sample)/ (Abs Control)} × 100

Glucosidase inhibition assay

Glucosidase inhibition assay of the irradiated and un-irradiated was done using the method of Apostolidis et al. (2007). After appropriate dilution, 500 µl (0.1 mg/ml-0.4 mg/ml) of sample was suspended in 1000 µl α-glucosidase solution (1.0 U/L) prepared in of 0.1 M phosphate buffer (pH 6.9) and pre-incubated for 10 min at 25 °C. After pre-incubation, 500 µl of 5 mM nitrophenyl-glucopyranoside solution prepared in 0.1 M phosphate buffer (pH 6.9) was added. The reaction mixtures were incubated at 25 °C for 5 min. The absorbance of the reaction mixture was measured at 405 nm. The percentage of inhibition was calculated:

Inhibition percentage = {(Abs Control- Abs Sample)/ (Abs Control)} × 100

Lipid peroxidation inhibition

Preparation of kidney homogenate: The preparation of kidney homogenate was done by the method of Akinyemi et al. (2013). The rats were sacrificed under sodium pentobarbitone anaesthesia and the kidney was removed, placed on ice, and weighed. The kidney was homogenized immediately in cold normal saline water (1:4 w/v of fresh weight). The kidney homogenate was centrifuged at 3000 rpm for 10 min. The lipid-rich supernatant obtained was used for the lipid peroxidation assay.

Lipid peroxidation assay: The lipid peroxidation inhibition assay of irradiated and un-irradiated samples was determined using the method of Ohkawa et al. (1973) with minor modification. The reaction mixture was made up of 200 µl of the tissue homogenate, 30 µl of 0.1M Tris-HCl buffer (pH 7.4), 100 µl (0.1-0.4 mg/ml) of sample, and 30 µl of the freshly prepared pro-oxidant solution (5 mM Sodium nitroprusside). The reaction mixture was incubated at 37 °C for 2 h. The chromophore was developed by adding 300 µl (8.1%) of Sodium dodecyl sulphate, 600 µl (pH 3.4) of acetic acid, and 600 µl (0.8%) of TBA, consecutively to the reaction mixture, followed by incubation for 1h at 100 °C. The absorbance of the formed TBA-adduct was monitored at 532 nm. The amount of TBARS produced was calculated by the method of (Banerjee, 2005).

Statistical analysis

The data is expressed as the mean ± SD of three measurements. The significance of the differences between the means of the samples was established by the analysis of variance using the least significant difference (LSD) $p < 0.05$, charts were drawn on the graph pad prism 5. The Pearson correlation test was conducted to determine the correlation among total phenolics, total flavonoids, antioxidant activities, and enzyme inhibition. Significant levels were established using $p < 0.05$.

Results and discussion

Total phenolics, total flavonoid, and total antioxidant capacity

The effect of the ultraviolet-C treatment on TP, TF, and TAC of the vegetables (AH and CO) are presented in Table 1. The UV-C treatment was able to significantly increase the content of total phenolics from 12.83 (mg GAE/100 g) to 14.40 (mg GAE/100 g) in AH and 14.87 (mg GAE/100 g) to 16.58 (mg GAE/100 g) in *Corchorus olitorius*. Also, total flavonoids were significantly increased from 17.03 (mg QUE/100 g) to 19.70 (mg QUE/100 g) in AH and 20.08 (mg QUE/100 g) to 23.00 (mg QUE/100 g) in *Corchorus olitorius*. The TAC was slightly increased but there was no significant difference in the treated and untreated samples. The enhancement of the polyphenolics (TP and TF) in irradiated vegetables, according to previous research, is as a result of their accumulation as a defence mechanism against irradiation-induced stress (Alothman et al., 2009; Gitz et

al., 2004). Also, the increase in the phenolic and flavonoid content of the UV-C treated vegetable extracts could be attributed to the activation of phenylalanine ammonia lyase (PAL) and chalcone synthase, the enzymes that are responsible for phenolic and flavonoid biosynthesis in plant tissues by ultraviolet radiation (Solovchenko and Merzlyak, 2008; Alothman et al., 2009; Papoutsis et al., 2016). The activation of PAL activity has been strongly correlated with the increase of phenolic content in mango fruits (González-Aguilar et al., 2007) treated with UV-C radiation. This result is in agreement with the work of (Alothman et al., 2009; Severo et al., 2015; Olaiya et al., 2016a; and Adetuyi et al., 2020) who reported an increase in phenolics and flavonoids of fruit and vegetables upon treatment with UV-C radiation.

DPPH scavenging ability

DPPH (1, 1 Diphenyl-2-picrylhydrazyl) is a stable free radical which dissolves in methanol to give a characteristic absorption band at 517-520 nm. Its assay measures the ability of the extracts to convert the DPPH to its reduced form Diphenylpicrylhydrazine with the loss of its violet colour (Alam et al., 2013; Olaiya et al., 2016b). The result of the DPPH scavenging ability of irradiated and un-irradiated samples was presented in Figure 1. The results showed that both the UV-C irradiated and un-irradiated vegetable leaf extracts can scavenge DPPH in a concentration dependent manner (Fig. 1) and the IC_{50}, which is the concentration of the extracts required to achieve 50% inhibition, was calculated (Table 2). The treatment with UV-C was able to increase the ability of the samples to scavenge DPPH as shown by the IC_{50}. The observed increment in the DPPH radical scavenging ability of the samples upon postharvest UV-C treatment may be attributed to the enhanced content of phenols and flavonoids, as the correlation analysis (Table 3) shows a very strong positive relationship between the polyphenolic (0.92 for TPC and 0.88 for TFC) and DPPH. The result in this study is in agreement with the work of Adetuyi et al., (2018) and Choi et al. (2015), where UV-C radiation was able to enhance the DPPH scavenging activity.

Table 1. The effect of ultraviolet-C postharvest irradiation on TPC, TFC, and TAC of AH and CO

	AH		CO	
	Unirradiated	**Irradiated**	**Unirradiated**	**Irradiated**
TPC	12.83 ± 0.56^a	14.40 ± 0.89^b	14.87 ± 0.57^a	16.58 ± 0.40^b
TFC	17.03 ± 0.50^a	19.70 ± 0.30^b	20.08 ± 0.33^a	23.00 ± 0.55^b
TAC	2.43 ± 0.10^a	2.65 ± 0.10^a	2.57 ± 0.10^a	2.83 ± 0.10^a

Values were represented as mean ± SD of triplicate reading. Values with a similar letter across the row are not different significantly ($p < 0.05$). TPC: Total phenolic (mg GAE/100g), TFC: Total flavonoid (mg QUE/100g), TAC: Total antioxidant capacity (mg AAE/100g)

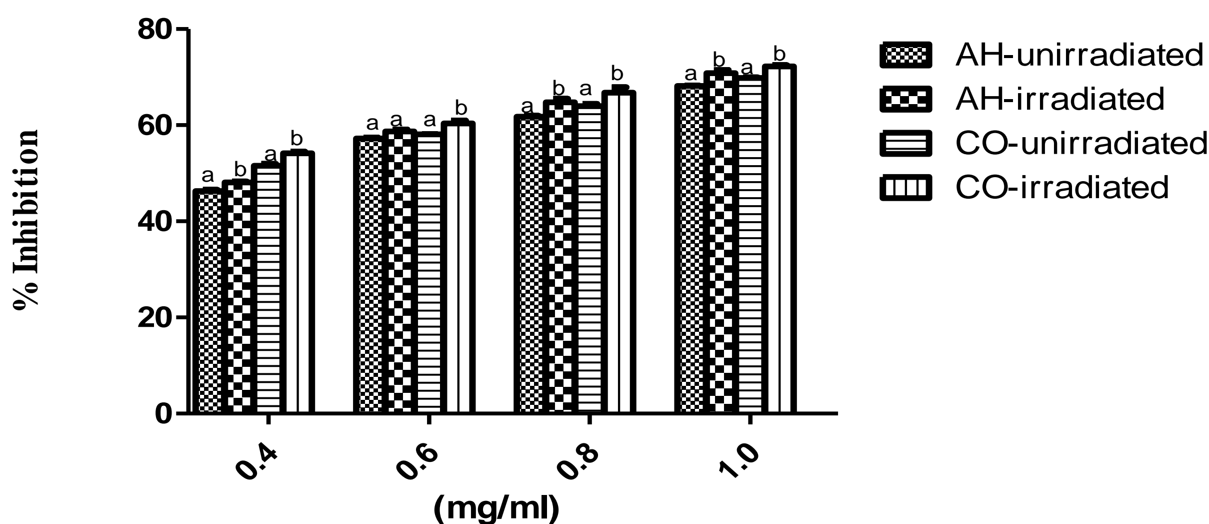

Fig. 1. The effect of ultraviolet C post-harvest treatment on the DPPH scavenging ability of AH and CO. Values with the same superscript letter on grouped bars are not different significantly ($p < 0.05$).

Table 2. IC_{50} (mg/ml) of the DPPH, α-amylase, and α-glucosidase of ultraviolet-C postharvest treated vegetables

	AH			CO
	Unirradiated	**irradiated**	**Unirradiated**	**Irradiated**
DPPH	0.46 ±0.01[a]	0.43 ±0.02[b]	0.38 ±0.03[a]	0.36 ±0.02[a]
α-amylase	0.54 ±0.05[a]	0.40 ±0.02[b]	0.39 ±0.02[a]	0.34 ±0.02[b]
α-glucosidase	0.58 ±0.08[a]	0.52 ±0.04[a]	0.49 ±0.04[a]	0.42 ±0.02[b]

Values were represented as mean ± SD of triplicate reading. Values with similar letter across the row are not different significantly ($p < 0.05$).

Table 3. Correlations among polyphenol (TPC and TFC), antioxidant ability (TAC and DPPH) and carbohydrate metabolizing enzymes (α-amylase and α-glucosidase)

	TPC	TFC	TAC	DPPH	AMY	GLU
TPC	1					
TFC	0.94*	1.00				
TAC	0.74*	0.75*	1.00			
DPPH	0.92*	0.88*	0.78*	1.00		
AMY	0.58*	0.56	0.76*	0.81*	1.00	
GLU	0.97*	0.93*	0.75*	0.91*	0.65*	1.00

*Correlation is significant at 0.05 level

α-amylase and α-glucosidase enzymes

The effect of the postharvest ultraviolet C treatment on α- amylase and α-glucosidase enzymes was shown in Fig. 2 and Fig. 3, respectively. These enzymes, α-glucosidase and α- amylase, are involved in the breakdown of starch into glucose in the body thereby increasing postprandial glucose concentration. The inhibitors of these enzymes are important because they serve to reduce the activity of these enzymes, therefore, playing a prominent role in the regulation of body glucose in the body, especially after a meal intake (Randhir et al., 2008; Karigidi et al., 2019). In this study, the treatment with UV-C radiation led to the significant enhancement of the inhibitory potential of the vegetables to inhibit these enzymes, as shown by the improved IC_{50} (Table 2). The lower the IC_{50} of the extract the better its activity against the enzymes. The increase in the ability of the vegetable extracts to inhibit these enzymes might not be unconnected with the increase in the polyphenol profile, as many researchers have reported the ability of these polyphenols to inhibit these enzymes (Rakumar et al., 2010). Also, the correlation analysis (Table 3) showed a strong positive relationship between the polyphenols and the enzymes in this study. The result obtained in this study is in agreement with other researchers where they have established a strong positive correlation between polyphenols and these digestive enzymes (Adefegha and Oboh, 2016; Adetuyi et al., 2020).

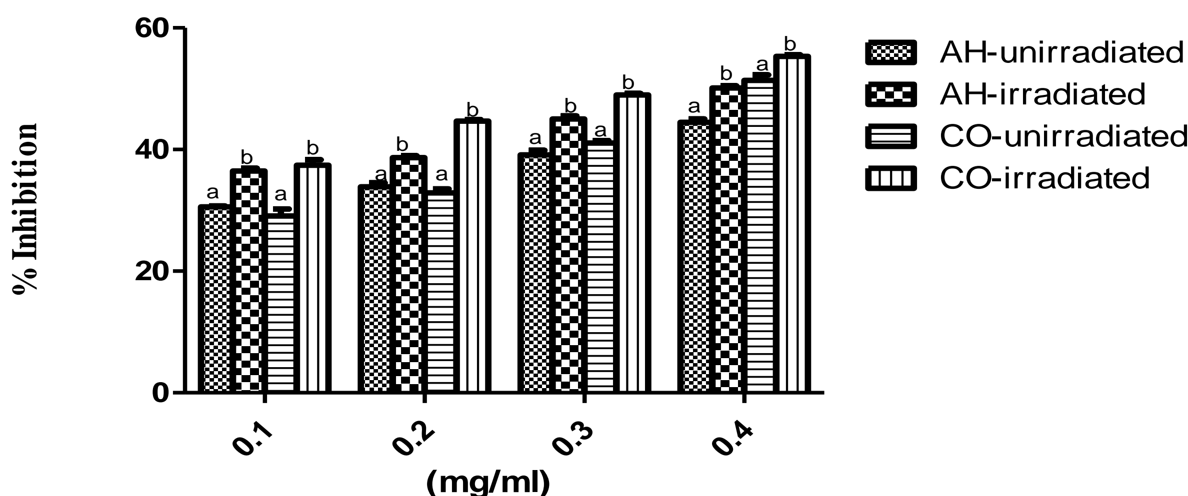

Fig. 2. The effect of ultraviolet C post-harvest treatment on the α-amylase inhibition property of vegetables. Values with the same superscript letter on grouped bars are not different significantly ($p < 0.05$).

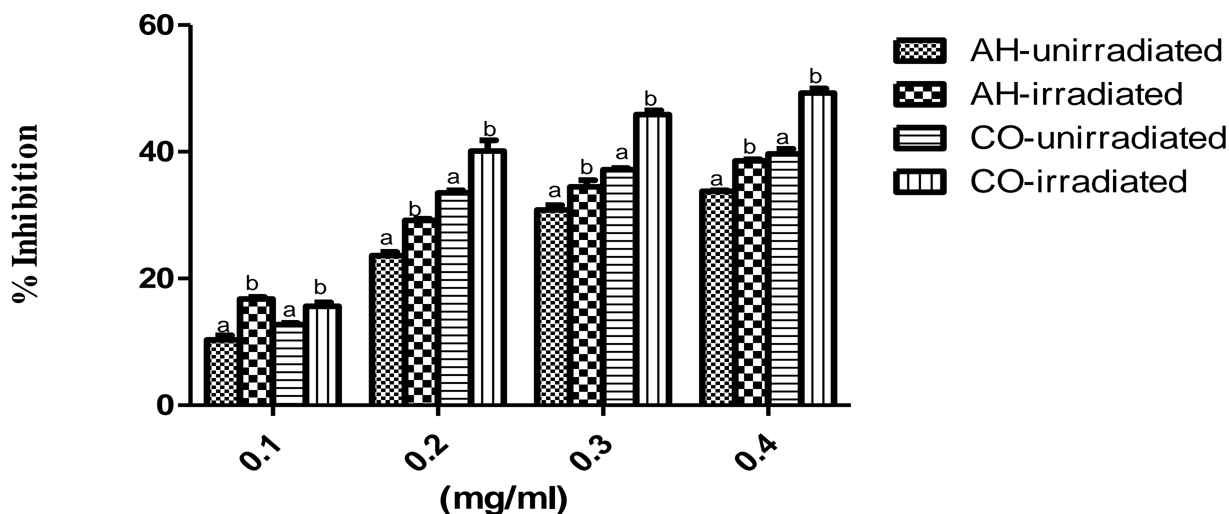

Fig. 3. The effect of the ultraviolet C post-harvest treatment on α-glucosidase inhibition property of AH and CO. Values with the same superscript letter on grouped bars are not different significantly ($p < 0.05$).

Lipid peroxidation

Lipid peroxidation is a process whereby free radicals abstract electrons from lipids in the cell, thereby leading to the oxidative degradation of the cell and it is usually a chain-propagated reaction (Yin et al., 2011). The complex mechanisms of the lipid peroxidation process and its deleterious effects have attracted scientific investigations across many disciplines. The lipid peroxidation process in the study was induced by the pro-oxidant effect of sodium nitroprusside and measured by the rate of thiobarbituric acid reactive substance (TBARS) produced during the reaction. The result of lipid peroxidation inhibition in an incubated kidney homogenate is presented in Fig. 4. This result revealed that the vegetables were able to inhibit the induced lipid peroxidation in a dose dependent manner; also the ability to inhibit lipid peroxidation was enhanced upon treatment with UV-C radiation. This enhancement might not be unconnected with increased polyphenols and antioxidant capacity of the treated vegetables, since many studies have established a synergy between polyphenols, antioxidants, and the inhibition of lipid peroxidation (Adetuyi et al., 2020).

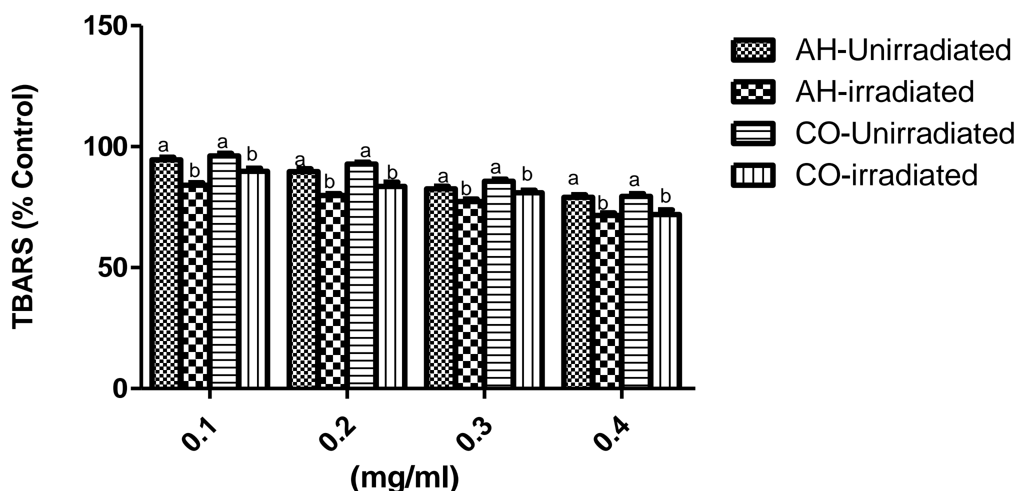

Fig. 4. The effect of the ultraviolet C post-harvest treatment on *in vitro* lipid peroxidation's inhibition property of AH and CO. Values with the same superscript letter on grouped bars are not different significantly ($p < 0.05$).

Conclusion

This present study has shown that *Amaranthus hybridus* and *Corchorus olitorius* irradiated with UV-C light consistently possessed higher total phenolics and total flavonoid contents, which culminated into improved antioxidants. Also, there was a significant improvement of the inhibitory properties of the vegetables on digestive enzymes linked to diabetes mellitus upon UV-C treatment. The production of TBARS, an end product of lipid peroxidation, was also reduced in kidney homogenate treated with irradiated samples. Therefore, this non-thermal physical processing method may serve as an important tool in food industries to improve the health functionality of food.

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