International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2021; 9(1): 2308-2314

Received: 09-10-2020
Accepted: 24-12-2020

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Comparative study of UV-C and Titanium dioxide/UV-C Photo catalytic washing disinfection in fresh cut cabbage

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DOI: https://doi.org/10.22271/chemi.2021.v9.i1af.11573

Abstract
Growing incidents of foodborne disease outbreaks linked to fruits and vegetables have drawn concern on efficient cleaning of leafy vegetables. A photocatalytic reaction under UV radiation with titanium dioxide (TiO_2) gives a good disinfection efficiency. Our aims were to set up a photocatalytic system for fresh cabbage. In addition, we evaluated the cleaning disinfection efficacy of Titanium-dioxide (TiO_2) illuminated with UV-C for 20 mins on fresh-cut white cabbage against surface microbes. The photocatalytic experiments were compared with tap water washing for 2 mins which is a common practice for home. The effects UV-C lights (20 mins) and TiO_2 photocatalyst (5 min) alone were also studied. The overall quality parameters were compared before and after treatments. Photocatalyst TiO_2 illuminated under UV-C showed significant reduction in total aerobic bacteria (2.67 log cfu/g) and yeast mold count (2.93 log cfu/g) followed by UV-C (TAC-2.25 log cfu/g; TYC-2.23 log cfu/g). The effects of TiO_2 alone and tap water exhibited same effect as control. The pH of fresh cut cabbage remained same when treated with UV-C with or without photocatalysts. However, Vitamin-C and phenolic content of fresh cut cabbage were reduced when treated with UV-C without photocatalysts by 18.42 mg/100g. TiO_2 with UV-C retained the health components like ascorbic acid (28.63±0.77 mg/100g) and TPC(128.54±0.63 mg GAE/100g) showed better disinfection efficacy against natural surface pathogens on cabbage than UV-C alone, can be established as better washing alternative at home and in industry.

Keywords: Photo catalyst, titanium-dioxide, disinfection, UV-C, fruits and vegetables, washing

Introduction
Access to sufficient amounts of safe and nutritious food is the key to sustaining life and promoting health. Zemichael Gizaw (2019) highlighted microbial contamination as a noteworthy public health concern in developing countries than developed countries (Henson, *et al.* 2006; Dharod, *et al.* 2009; Baluka, *et al.* 2015; Paudyal, *et al.* 2017) [11, 4, 1]. lacking proper hygiene and sanitation due to weak safety regulatory standards enforced in developing countries (Rhodehamel, *et al.* 1992; Henson, *et al.* 2008; Grace, *et al.* 2015) [27, 12, 8]. Furthermore, Bacteria and fungi were identified as the most prevalent diseases causing pathogens (Hunter, *et al.* 1994; CDC, 2012) [13]. The incidence of recorded fresh-produced food-borne illnesses has increased markedly, which may be due to rising fresh-produced intake (Meireles, *et al.* 2016) [21]. An illustration of such a minimally processed category of foods is leafy greens. There is no pathogen kill stage in the green leafy food chain, such as boiling, before consumption. This offers an incentive for pathogenic microbes to multiply and results in threats to food safety if consumed (Mogren, *et al.* 1965) [22]. The demand of fresh fruits and vegetables grown organically or conventionally is inescapable of surface pathogens from farm to fork due to improper handling and storage, posing a risk to public health that are associated with consumption of poorly washed and disinfected fresh products (Kuan, *et al.* 2017) [20]. In recent years, Photocatalysis is gaining attention over traditional washing technologies for fruits and vegetables such as chlorination, Ozonation, UV-C, Chemical treatments (Selma, *et al.* 2008; Ramesh, *et al.* 2016; Ramesh, *et al.* 2018; Zhu, *et al.* 2018; Calvo, *et al.* 2019) [29, 25, 26, 33, 2]. When lighted with UV light, the TiO2 photocatalyst reaction produces powerful oxidising ability. It is a promising hurdle approach of combining photoactive element under the illumination of ultraviolet range resulting in ROS production that helps in surface disinfection against natural micro flora present on vegetables.

(TiO_2) is commonly used in food as an additive approved by the US FDA and known as photoactive elements that can inactivate bacteria in an aqueous solution. However, Numerous studies were focused on UV-C disinfection of fruits and vegetables. The UV-C based treatments reported the degradation of Vitamin-C and total phenolic components which contribute to the antioxidant of leafy vegetables (Guneser, *et al.* 2012; Falguera, *et al.* 2013) [9, 5].

In this study we used the colloidal solution of Titanium dioxide as a washing solution and discarded after single use. The primary project's objective was to set up a photocatalytic reactor to investigate the disinfection effects of UV-C radiation alone and in combination with photocatalyst (TiO_2) on the shredded white cabbage. The photocatalysts alone and tap water washing were also studied as a comparative study. The secondary objective of this study was measure the effects of various treatments on pH, Vitamin-C and total phenolic components of fresh cut white cabbage just after the experiment.

2. Materials and Method

2.1 Fresh cabbage sample

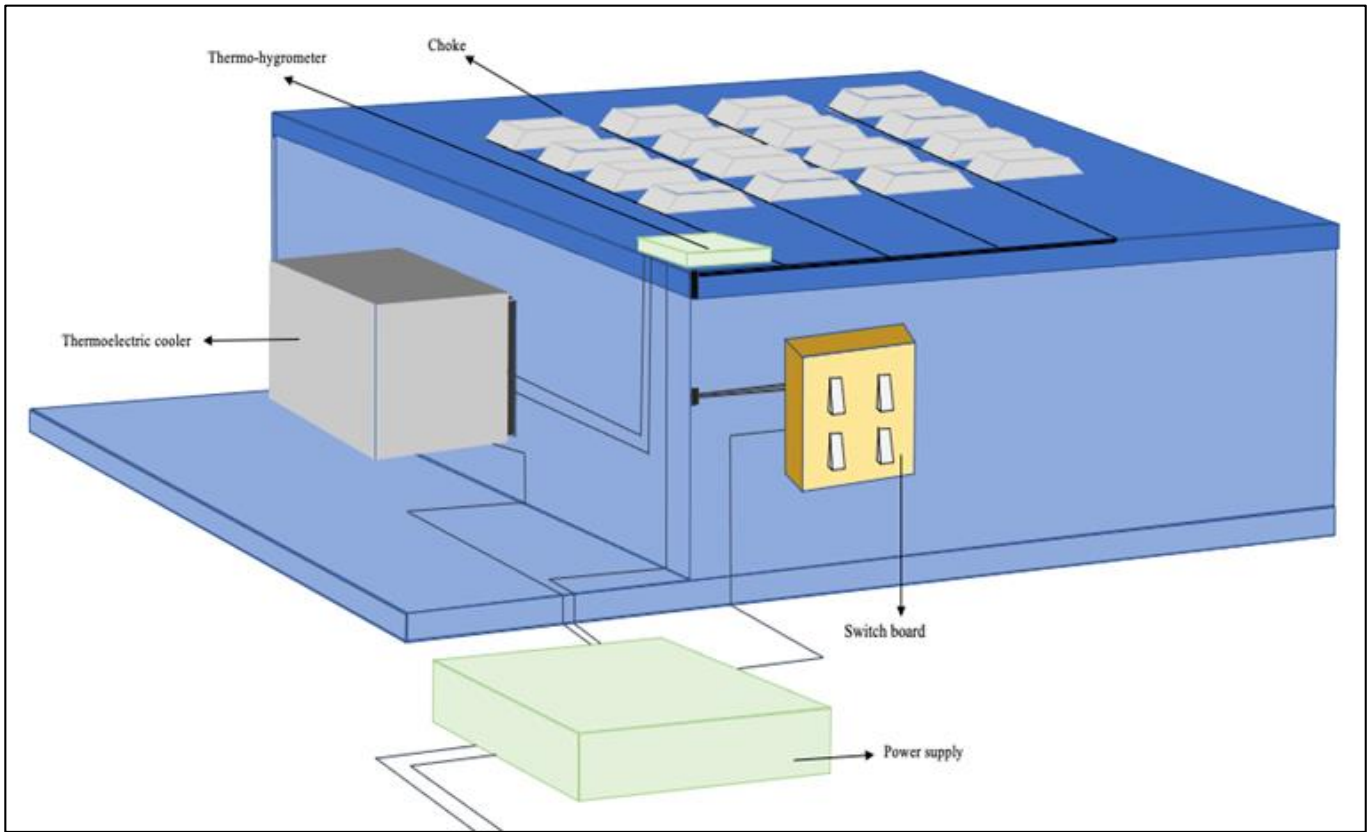
Fresh white cabbage was procured from a local market of Thanjavur in Tamil Nadu, India. The core and outermost leaves were stripped and discarded followed by shredding of leaves (3mm width) with an alcohol-sterilized knife.

2.2 Design of reactor used in the treatment

A bench-scale reactor (0.4x0.32x0.15m) was fabricated and installed at Indian Institute of Food Processing Technology, Thanjavur, Tamil Nadu. The reactor was split into two different Poly Vinyl Chloride (PVC) foam board treatment chambers and coated internally with aluminium foil (0.13 mm thickness) for efficient UV photon reflection, adequate insulation and better reactor cooling (Figure 1). Twelve UV lamps (15 cm long) were placed in tube holders, four of each lamp were mounted at the top and two were fixed separately at each side of the treatment chambers, such that the sample perceived the major amount of lamp light.

Each type of lamp was fixed at a distance of 12 cm (top side) and 6 cm (Lateral sides) from the sample, respectively (Fig.1). The fluence of four UV-C (8W, TL Philips), four UV-B (8W, Philips TUV) and four UV-A (8W, Philips TL BLB) was measured using a radiometer (Lutron UV 340A) on the reactor surface as 4.2 ± 0.12,1.8 ± 0.70, 1.2 ± 0.65 m W/ cm^2. A thermoelectric cooler was inserted to maintain the temperature of 23± 2º C during the experiment and relative humidity was recorded as 80±5% (HTC-1Thermo-Hygrometer). Titanium dioxide (TiO_2) colloidal solution (Concentration 1% w/v, purity 99.9%, Average particle size 25 nm, Specific surface area TiO_2 200-220 m^2/g) was used as photocatalysts and purchased from Nano Research Lab Jamshedpur, Jharkhand, India.

In this study, UV-C light was only used for disinfection purpose while other lights will be used in future experiments. The UV-C light was turned on 30 minutes before the experiment and Colloidal solutions were pre-exposed to UV light for 5 min with Reactive oxygen species to ensure a steady-state and enhance the photocatalytic activity (Ryu, *et al.* 2008) [28].



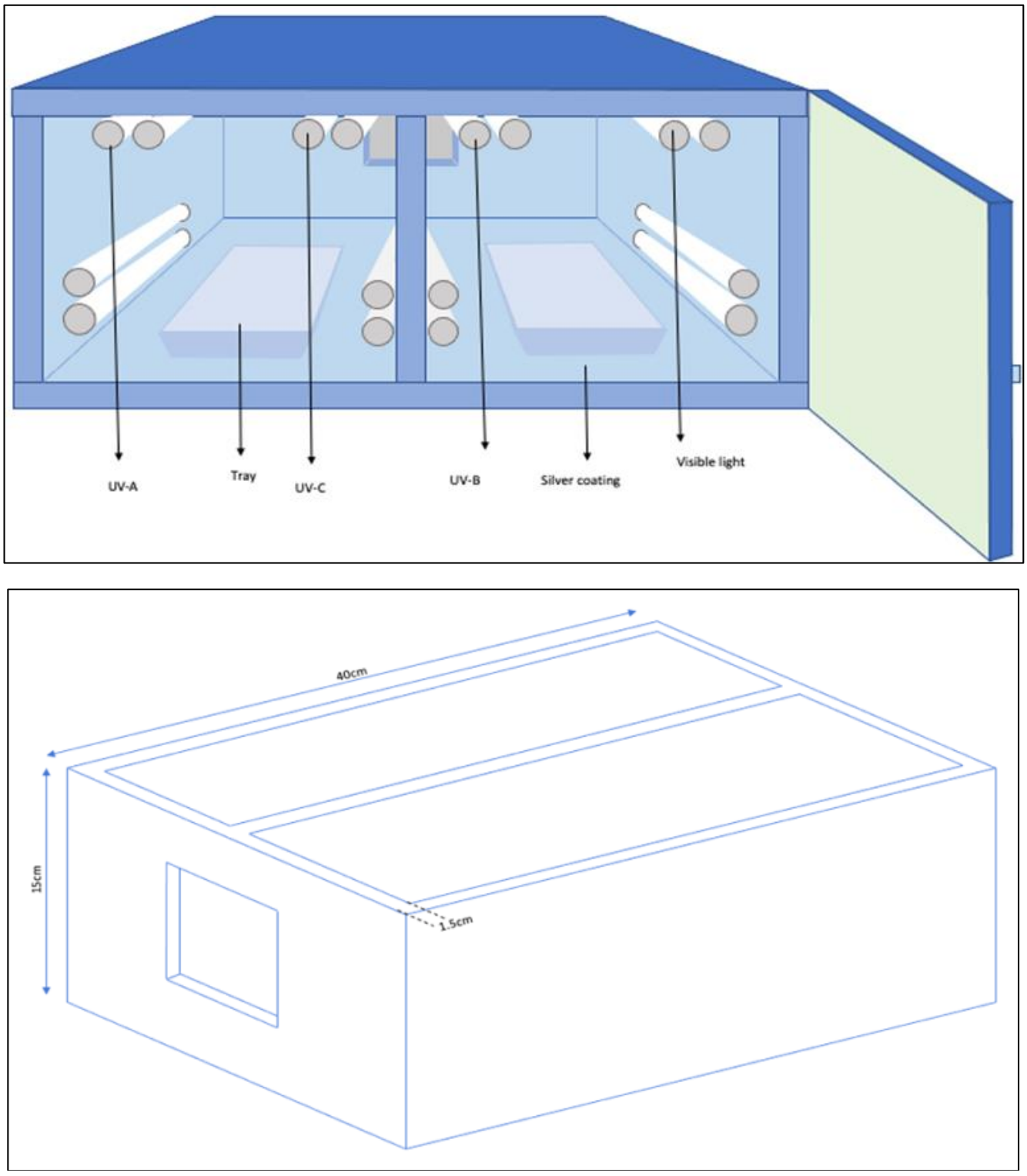


Fig 1: Design of Photocatalytic reactor with various UV lamps fitted inside and other parts with dimensions

Experimental settings for Disinfection of freshly cut cabbage

50g of shredded white cabbage were kept in sanitized trays filled with water for disinfecting process except for control and then treatments were performed as follows:

2.4 Sample analysis

2.4.1 Microbial study

Total aerobic bacteria and yeast and mould present on the surface of fresh-cut white cabbage were enumerated by spread plate method. In our study, 1:10 dilutions of blended white cabbage in 0.1% sterile peptone water were used to measure the microbial load using the spread plate method.

Sterile plate count agar and Rose Bengal agar were used followed by incubation at 35 ± 2ºC for 48 h and 25 ± 2ºC for 5 days to determine total aerobic bacteria and yeast and mould survival counts respectively.

Manual colony counter was used to count survival colonies and expressed as the log colony-forming units (CFU) per gram of sample.

2.4.2 Estimation of pH

The change in hydrogen ion concentration was noted using the electrometric method by placing filtered 10g of homogenized treated samples in 40ml of deionized water below the calibrated pH meter and identifying them against the pH scale (0-14) at room temperature.

2.4.3 Measurement of Total phenolic content
The total phenolic content was spectrophotometrically determined according to the Folin-Ciocalteu method**,** with gallic acid as the standard. A standard curve with varied gallic acid concentrations ranging from 0.2 to 1 mg / L was used to extract the sample TPC. The outcomes were reported as Gallic acid equivalents (GAE) in mg per 100 ml of sample.

2.4.4 Measurement of Ascorbic acid
The analyte concentration of Ascorbic acid content was determined using the titration method as reported by Harris and Ray, 1935, with slight modifications. 5 ml working standard was pipetted into 100mL conical flask. 10ml 4% oxalic acid was added and titrated against the dye (V_1 mL). End point was appearance of pink colour persisting for a few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid. 0.5g of white cabbage was extracted in 4% oxalic acid and made up-to 100mL and centrifuged. 5mL of the supernatant was pipetted out and 10mL 4% oxalic acid was added and titrated against the dye (V_2 mL).
The titrant values were noted and the ascorbic acid content per ml was estimated using the following equation:

$$\text{Amount of ascorbic acid}\,\frac{\text{mg}}{100\text{g}}\,\text{sample} = \frac{0.5\text{ mg}}{v_1\text{ mL}} \times \frac{V_2}{5\text{mL}} \times \frac{100\text{mL}}{\text{Wt.of the sample}} \times 100$$

2.4.5 Statistical analysis
All tests were carried out in triplicates. A one-way ANOVA and Student-Newman–Keuls test was performed using Statistical Package for the Social Sciences software (SPSS ver. 26; IBM Corp). For all experimental tests, ANOVA test was performed to assess significance using a 95% confidence level. Results at $p< 0.05$ were regarded as significantly different.

3. Results and Discussion
3.1 Effect on Microbial count
The microbial inhibition (log CFU/g) achieved before (control) and after washing with tap water, photocatalyst (TiO_2,), lights (UV-C) and combined effects (TiO_2 /UV-C) is depicted in Table 2. Microbial inactivation by UV-C and TiO_2 alone were also evaluated as a comparative study. The initial total aerobic bacterial (TAB) and Yeast & mould counts (TYC) of freshly cut cabbage were 5.34 and 4.63 log cfu/g. Based on the result obtained, washing with the tap water for 5 mins (T_1) was not significantly different from the control (T_0) sample in inhibiting both total aerobic bacteria (TAB) and total yeast & mould counts (TYC). A 0.56 log reduction (TAB) and 0.29 log reduction (TYC) were obtained by dipping in TiO_2 solution followed by washing with tap water but not significantly different from Tap water washing. This result shows that TiO_2 is not very effective as an antibacterial solution without light. However, TiO_2/UV-C exhibited appreciable disinfection efficiency than UV-C alone by reducing TAB 2.67 log cfu/g (TAB) and TYC by 2.93 log cfu/g (Figure 2). The efficacy of photocatalyst assisted UV-C inhibition was better, apparently due to the excitation of TiO_2 when exposed to UV light that results in production of reactive oxygen species such as hydroxyl, superoxide anion radical and hydrogen peroxide. These reactive oxygen species can inhibit the activity of enzymes in cell of microbes and induce an oxidative damage (Ireland, *et al.* 1993; Kim, *et al.* 2017) [17]. The similar result was observed when OH radical concentration induced by photocatalysis of UV-C/TiO_2 was substantially greater than that generated by UV-C only (Park *et al*. 2016). Furthermore, Kim *et al*. (2009) [18] found that the natural microbes and inoculated colonies of *E.coli, L.monocytogenes, S.aureus, and S.typhimurium* on iceberg lettuce were effectively reduced by UV-TiO_2 photoactivity than UV alone. In another experiment, surface disinfection of orange surface inoculated with *E. coli O157:H7* population by washing with tap water, UV-C alone and TiO_2-UV. This result was strongly in correlation with (Yoo, *et al*. 2015b) [31, 32] that showed better disinfection with photocatalysis treatment than UV-C alone.

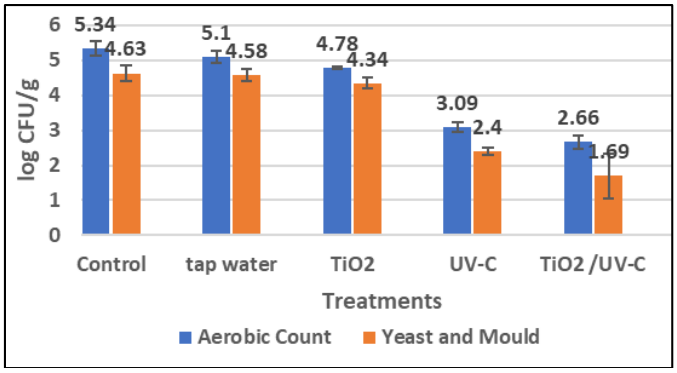


Fig 2: Effect of various treatments on total aerobic count and total yeast and mold count of fresh cut white cabbage

3.2.2 Change in pH
It is necessary to ascertain the pH shift of the treated sample to make sure that optimal conditions are not provided for the growth of microbes. The study reported no significant change in pH among any treatments in accordance with the control sample (Table 3). Regardless of treatment conditions, all samples showed a decrease in pH level after the experiments ranges from 5.50-5.65 (Figure 3). These result was constantly in agreement with photocatalysis effect combined with high hydrostatic pressure on orange fruit surfaces (Yoo, *et al.* 2015) [31, 32].

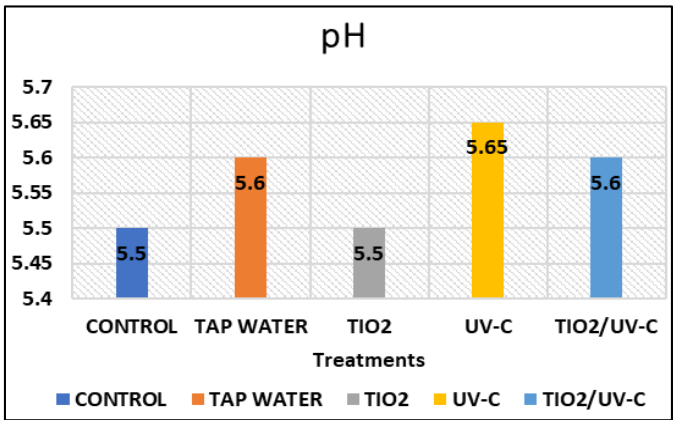


Fig 3: Effect of various treatments on pH of fresh cut cabbage

3.2.3 Effect on Total phenolic content
The phenolic content of cabbage is associated with its antioxidant and antibacterial capacity (Cartea, *et al.* 2011; Pataro, *et al.* 2015). The total phenolic content (TPC) evaluated in the untreated and treated sample is shown in Table 3. The exposure of cabbage with only tap water and

photocatalysts (TiO_2) did not affect the TPC (Figure 4), unlike UV-C which significantly decreased to 122.02±3.77 than control (P>0.05). However, combined effects of TiO_2/UV-C showed similar effects to the previous study on white grape juice (Ramesh, *et al*. 2018) [26].

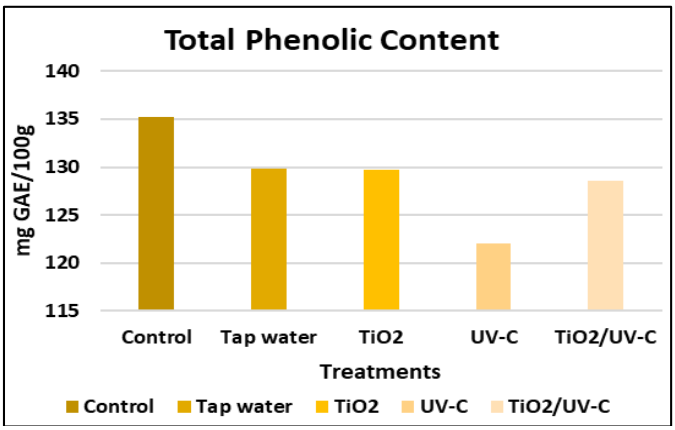


Fig 4: Effect of various treatments of total phenolic content of fresh cut white cabbage

3.2.4 Effect on Ascorbic acid content

Cabbage is one of the oldest vegetables cultivated and known for its rich Vitamin-C as a dietary source (García-Viguera, *et al*. 2014).

In all treatments, Reduction in ascorbic acid content was recorded (Table 3). There were no changes observed after washing with tap water and TiO_2 as compared to control. A significant loss of ascorbic acid was observed after UV-C alone exposure while TiO_2/UV-C retained 28.63±0.77 mg/100g relative to control sample (Figure 5). Various studies have documented degradation of Vitamin C through UV-C on vegetables and its products due to the production of reactive oxygen species (Guneser, *et al.* 2012; Falguera, *et al.* 2013) [9, 5]. Vitamin C and other nutrients are highly responsive to UV-C but unable to absorb light > 300 nm (Koutchma, *et al.* 2009) [19] photocatalytic disinfection based on UV-B and UV-A can be alternative options to avoid degradation of nutrients sensitive to light.

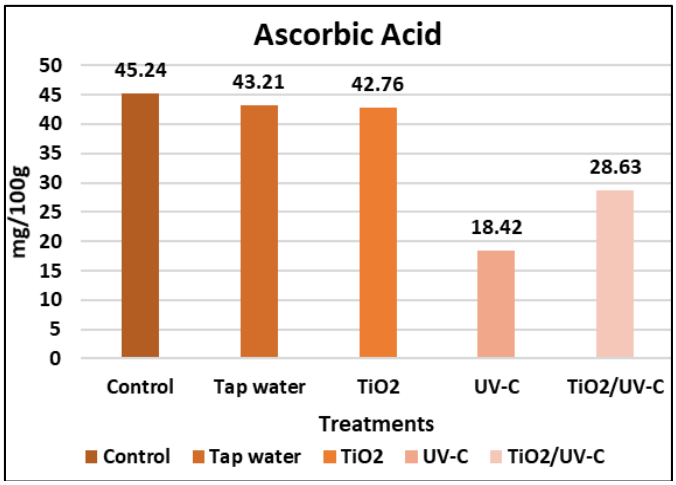


Fig 5: Effect of various treatments of Ascorbic content of fresh cut white cabbage

Table 1: Experimental design

Treatment	Description
Control	The fresh cut leaves without cleaning and any treatments. This treatment was used as control
Tap water	The fresh cut cabbage was washed with tap water for 2 minutes
TiO_2	The sample was dipped in water containing 1% conc. TiO_2 (2g/L) for 10 minutes
UV-C	The sample was dipped in 1L water illuminated with UV-C for 20 minutes
TiO2/UV-C	The sample was dipped in water containing 1% conc. TiO_2 (2g/L) and illuminated with UV-C for 20 minutes

Table 2: Effect of different washing treatments on microbial inactivation on fresh cut white cabbage

Treatment	Total aerobic count		Total Yeast & Mold count	
	Treated* (Log cfu/g)	Reduction# (Log cfu/g)	Treated* (Log cfu/g)	Reduction# (Log cfu/g)
Control	5.34± 0.21 ^{g}	0.00	4.63±0.20 ^{e}	0.00
Tap water	5.15±0.17 ^{fg}	0.19	4.58±0.17 ^{e}	0.05
TiO_2	4.78±0.04 ^{ef}	0.56	4.34±0.16 ^{de}	0.29
UV-C	3.09±0.13 ^{c}	2.25	2.40±0.10 ^{b}	2.23
TiO_2/UV-C	2.67±0.31 ^{b}	2.67	1.70±0.65 ^{a}	2.93

Values with different letters show statistical significance (a = 0.05)

*mean ± SD

Control (log cfu/g) - treated (log cfu/g

Table 3: Effect of washing treatments on pH, Total Phenolic content and ascorbic acid content

Treatment	pH	Total Phenolic content (mg GAE/100g)	Ascorbic acid content(mg/100g)
Control	5.50 ± 0.05^{a}	135.26 ± 0.95^{a}	45.24 ± 1.97^{a}
Tap water	5.60 ± 0.05^{a}	129.80 ± 3.77^{a}	43.21 ± 0.65^{a}
TiO_2	5.50 ± 0.05^{a}	129.75 ± 3.78^{a}	42.76 ± 0.31^{a}
UV-C	5.65 ± 0.05^{a}	122.02 ± 3.77^{c}	18.42 ± 0.78^{c}
TiO_2/UV-C	5.60 ± 0.05^{a}	128.54 ± 0.63^{b}	28.63 ± 0.77^{b}

4. Conclusion

The results obtained herein demonstrate that washing with TiO_2 illuminated under UV-C (Photocatalysis) is an eco-friendly and non-thermal technique for disinfection of fresh fruits and vegetables with minimal changes in health-related components like phenolic content and ascorbic acid of cabbage than UV-C and tap water alone.

In the industrial washing process, treatment for 20 minutes is perhaps too prolonged. In this experiment, TiO_2/UV-C photocatalytic disinfection could be more likely to produce

better result in lesser treatment times. A little more research is recommended into methods to improve the generation of free radical species by coating TiO_2, applying various wavelength of the UV light, providing the reactor with oxygen gas or altering the pH of the washing water.

We demonstrate that photocatalytic treatment with TiO_2/UV-C is a viable solution to traditional disinfection for the prevention of microbes in fresh fruit and vegetables.

To more study to improve the effectiveness of the procedure, the TiO_2/UV-C photocatalytic system can be integrated into an efficient washing process. The TiO_2/UV-C photocatalytic system can be integrated into an efficient washing method through further study to improve the effectiveness of the treatment.

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